

3.2.1 Number of papers published per teacher in the Journals notified on UGC website during the year (session 2022-23)

Title of paper	Name of the author/s	Department of the teacher	Name of journal	Year of publication	ISSN number	Link to the recognition in UGC enlistment of the Journal
Antiobesity activity of dibenzo- α -pyrone derivatives in progesterone-induced obese rats	Dr. Nitin Kumar	Department of Pharmacy, Meerut Institute Technology	High Technology Letters	2022-2023	1006-6748	https://www.scopus.com/sourceid/12667
Marburg Virus- A threat during SARS-CoV-2 era: A Review	Mr. Jonee Panwar and Mr. Soumen Pal	Department of Pharmacy, Meerut Institute Technology	Infect Disord Drug Targets	2022-2023	2212-3989	https://www.scopus.com/sourceid/4600151527
Development and evaluation of <i>Hedyotis corymbosa</i> (L.) extract containing phytosomes: a preclinical approach for treatment of neuropathic pain in rodent model	Dr. Nitin Kumar and Dr. Neeraj Kant Sharma	Department of Pharmacy, Meerut Institute Technology	Journal of Microencapsulation	2022-2023	1464-5246	https://www.tandfonline.com/journals/imnc20
Virosome: An engineered virus for vaccine delivery	Dr. Hasan Ali, Dr. Neeraj Kant Shrama, Dr. Nitin Kumar	Department of Pharmacy, Meerut Institute Technology	Saudi Pharmaceutical Journal	2022-2023	1319-0164	https://www.sciencedirect.com/journal/saudi-pharmaceutical-journal
Nanoencapsulation and characterisation of <i>Hypericum perforatum</i> for the treatment of neuropathic pain	Dr. Nitin Kumar	Department of Pharmacy, Meerut Institute Technology	Journal of Microencapsulation	2022-2023	0265-2048	https://www.tandfonline.com/journals/imnc20

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Hepatoprotective Activity of Lagenaria siceraria Leaf Extract against Carbon Tetrachloride-Induced Damage in Rats	Dr. Neeraj Kant Shrama, Dr. Nitin Kumar, Dr. Hasan Ali	Department of Pharmacy, Meerut Institute Technology	European Chemical Bulletin	2022-2023	2063-5346	https://www.scopus.com/sourceid/21100898023
Heavy Metal - Induced Pollution in the Environment through Waste Disposal	Vikas Kumar, Sandeep Kumar Tyagi, Kuldeep Kumar, Ravindra Singh Parmar	Applied Science (Chemistry)	International Journal of Research Publication and Reviews	July, 2023	2582-7421	www.ijrpr.com
A Case Study on Mini Gas Cylinder Cluster : Evidence from India	Dr. Sandeep Kapoor	Commerce	Prabandhan: Indian Journal of Management	2023	0975-2854	10.17010/pijom/2023/v16i1/172668
A Study of the Impact of Risk Avoidance and Financial Welfare on the intent to invest in the Equity Market	Mr. Abhijit Chatterjee	Commerce	The British Journal of Administrative Management	2022	1746-1278	https://tbjam.org/vol58-special-issue-09/
Effect of exogenous application of odium nitropruide nd GA3 on growth and flowering of dahlia C.V.Knya	Chetan chauhan, Mukh kumar, Sunil malik, Manoj kumar yadav, L.K. gangwar, Akash Tomar, Mohit and varha rani	Agriculture department	Biological forum	2023	0975-1130	https://www.researchtrend.net/bfij/pdf/Effect-of-exogenous-application-of-Sodium-Nitroprusside-(SNP)-and-Gibberellic-Acid-(GA3)-on-growth-and-flowering-of-Dahlia-(Dahlia-variabilis-L.)-CV.-Kenya-Chetan-Chauhan-27.pdf

A Fake News Classification and Identification Model Based on Machine learning approach	Ashish kumar , M.Izharul Hasan Ansari , Kshatrapal Singh	Computer Science & Engineering	Springer Nature Singapore	2022	978-981-19-9304-6	https://link.springer.com/chapter/10.1007/978-981-19-9304-6_44
Identify Fixed American Sign Language by Awareness of Convolutional Neural Network	Mohd.Sadim, Prateek Mishra , Vikas Gupta ,Stuti Saxena , Ranjeet Dubey	Computer Science & Engineering	Scopus	2023	2063-5346	https://www.eurchembull.com/uploads/paper/67b2fa684e2d237e6bd7cfaa2e1102efb.pdf
Sentiment Analysis of Twitter Data Regarding the Agnipath Scheme of the Defense Forces	Vijaylakshmi Sajwan, Monisha Awasthi, Ankur Goel, Priyank Sharma	Indoneshian Journal of Electrical Engineering & Computer Science	Scopus	2023	2502-4752	http://doi.org/10.11591/ijeecs.v30.i3.p1643-1650
Social Media as a Marketing Tool: A Case Study of Nestle India Ltd.	Atul chaudhay, Deepak Sharma, Ankur Goel	International Journal of Research Publication and Reviews	Scopus	2023	2582-7421	https://ijrpr.com/uploads/V4ISSUE5/IJRPR13507.pdf

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Anti-obesity Activity of Dibenzo- α -Pyrone Derivatives in Progesterone-Induced Obese Rats

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ABSTRACT

The recent learning aimed to assess the anti-obesity activity of dibenzo- α -pyrone derivatives in progesterone-Induced Obese Rats. Following the standard Hurler reaction, dibenzo- α -pyrone (DB α P) was prepared. Subsequently, through the Mannich reaction, DB α P derivatives were synthesized. Characterization of these compounds was done by various spectral and analytical methods. From them, three compounds were selected and evaluated for their anti-obesity activity in-vivo model. Female Wistar rats were split into nine sets, which go through normal and negative control (Depo-Medroxyprogesterone Acetate (DPMA), standard drug orlistat, and butylamine, diethylamine and pyrrolidine dibenzo- α -pyrone derivatives (20 and 40mg/kg) daily for 28 days. Parameters like body mass index (BMI), body weight, food consumption pattern, biochemical parameters like glucose, HDL-C, LDL-C, and TG compared to normal control were studied. A remarkable increase in food consumption, BMI, and body weight levels of biochemical parameters glucose, triglyceride, LDL-C & HDL-C were seen. Animals treated with dibenzo- α -pyrone derivatives showed dose-dependent activity. Pyrrolidine dibenzo- α -pyrone derivatives supplementation attenuated all the above alterations. It was concluded that the present findings suggest that dibenzo- α -pyrone derivatives aimed anti-obesity action that proves its ethnomedicinal usage in the care of obesity.

Keywords: anti-obesity; dibenzo- α -pyrone; progesterone; Mannich reaction; orlistat; body mass index



REVIEW ARTICLE

Marburg Virus- A Threat During SARS-CoV-2 Era: A Review

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Abstract: In the German towns of Marburg, Frankfurt, and Belgrade in 1967, this single negative-stranded RNA virus was initially discovered. The importation of infected grivet monkeys from Uganda is what caused this virus-related sickness. As a result of the early link between viruses and non-human primates, this virus is frequently referred to as vervet monkey sickness. This virus causes Marburg hemorrhagic fever in humans and non-human primates. Human endothelial cells serve as the primary vehicle for replication. According to a 2009 report, the virus was being stored in Egyptian fruit bats (*Rousettus aegyptiacus*). Body fluids, unprotected sex, broken or injured skin, and other bodily fluids are the main routes of transmission. After the incubation period, symptoms like chills, headaches, myalgia, and stomach pain start to show up. There is no specific medication for such an infection, only hydration therapy and adequate oxygenation are followed. The following diagnostic techniques can be used to confirm the diagnosis: (i) an antibody-capture enzyme linked immunosorbent assay (ELISA); (ii) an antigen capture ELISA test; (iii) a serum neutralization test; (iv) an RT-PCR assay; (v) electron microscopy; or (vi) virus isolation by cell culture. Because MARV is a risk group 4 infection, laboratory staff must take strict precautions (RG-4).

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1. INTRODUCTION

When diseased African green monkeys were brought to Germany from Uganda and quarantined in the airport for the purpose of utilizing a vaccine, the etiologic agent of Marburg hemorrhagic fever was released in 1967 in Marburg, Frankfurt, and Belgrade [1]. According to the WHO, it is classified as a Grp-4 pathogen and a Category A pathogen by NIH, CDH, depending on the mortality rate and high pathogenicity. The Ravn and Marburg virus strains are the two types [2]. Marburg is divided into two further groups, A and B. During the outbreak, these two distinct strains were isolated from various locations; A strain from the Republic of Congo, B from the Republic of Congo, and C from Kenya. According to a paper, Niemann-Pick C1 (NPC1) is in charge of allowing the virus to enter the host cell. In Angola, the fatality rate during the initial outbreak was 25 percent in 1967 and rose to 80 percent between 1998 and 2000 [3]. This disease can incubate for two to twenty-one days. Epidemiologists have looked into bats, monkeys, and spiders but haven't discovered any definitive information about the

MARV's unique reservoir. However, a large number of cases confirmed that the main host reservoir was a bat from a rural African region, and through bats, it was directly transmitted to humans, or *via* monkeys to humans, and then from an infected person to a healthy person. According to research data connected to the host reservoir, more than 70% of infected cases in Durba/Watsa came from areas with ideal bat habitat, believed to be a zoonosis that occasionally infects humans. Given that primates typically experience a high and swift mortality rate after infection, this population should be taken into account as a potential reservoir [4]. According to reports, a number of bat species act as hosts for filovirus reservoirs, and *Rousettus aegyptiacus* is the primary reservoir for MARV. There are numerous instances where *R. aegyptiacus* was found to be present in MARV-infected areas. This species was found in Uganda's Kitaka Cave, where MARV-infected people had previously worked [5]. It was discovered that the absence of transmission made bats vulnerable when this species cohabitated for up to 42 days with MARV-infected bats.

2. TAXONOMICAL CLASSIFICATION AND STRUCTURE

The Marburg virus belongs to the family of filoviridae and there are different varieties of it. This single-stranded

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RESEARCH ARTICLE



Development and evaluation of *Hedyotis corymbosa* (L.) extract containing phytosomes: a preclinical approach for treatment of neuropathic pain in rodent model

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ABSTRACT

Purpose: The study was aimed to encapsulate *Hedyotis corymbosa* extract (HCE) into phytosomes to improve its therapeutic efficacy in neuropathic pain by enhancing the bioavailability of chief chemical constituent Hedycoyroside -A (HCA).

Methods: For preparing phytosomes complexes (F1, F2, and F3), HCE and phospholipids were reacted in disparate ratio. F2 was chosen to assess its therapeutic efficacy in neuropathic pain induced by partial sciatic nerve ligation. Nociceptive threshold and oral bioavailability were also estimated for F2.

Results: Particle size, zeta potential and entrapment efficiency for F2 were analysed as 298.1 ± 1.1 nm, -3.92 ± 0.41 mV and $72.12 \pm 0.72\%$ respectively. F2 gave enhanced relative bioavailability (158.92%) of HCA along with a greater neuroprotective potential showing a significant antioxidant effect and augmentation ($p < 0.05$) in nociceptive threshold with the diminution in damage to nerves.

Conclusion: F2 is an optimistic formulation for enhancing the HCE delivery for the effective treatment of neuropathic pain.

ARTICLE HISTORY

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KEYWORDS

Hedyotis corymbosa;
Hedycoyroside; phytosomes;
neuroprotective; neuropathy

1. Introduction

Neuropathic Pain (NPP), which is caused by damage to or injury to the nerves and produces shooting, tingling, shocking, burning or stabbing pain, is the most horrific out of all pain and suffering. To treat NPP many different medications like antidepressants, NSAIDs, anticonvulsant, and opioids can be used. The majority of these include serious side effects while wishing for safe treatment. Herbs as a nature's gift consist of specific glycosides, polyphenols and many more bioactive compounds that mediate the proposed health benefits by functioning as effective antioxidants *in vivo* (Higdon and Frei 2003). Therefore, herbal treatment may provide a high-level alternative in terms of the likelihood of a positive therapeutic result.

Hedyotis species (Rubiaceae) are traditionally used in India and other Asian countries for the treatment of appendicitis, tonsillitis, hepatitis, dysentery and many more diseases and disorders of human beings. The active constituents of *Hedyotis* genus include iridoid

glycosides, triterpenoids, anthraquinones, coumarins, lignans, alkaloids, flavonoids and some compounds exerting anti-inflammatory, neuroprotective, and cytotoxic effects (Gupta 2012, Zahir *et al.* 2013). The whole plant is applied in the clinic against malaria, intestinal abscess, boils, scald, and some tumours. A small amount of some specific iridoid glycosides have been isolated from *H. corymbosa* but the therapeutic activities of these compounds were not investigated. The whole plant of *H. corymbosa* was reported to have three new iridoid glycosides, hedycoyrosides A, B and C. The major compound in *H. corymbosa* leaves is iridoid glycoside, hedycoyroside A (HCA) (Jiang *et al.* 2007). It is about 1/4 of the whole bioactive compound. It was previously reported that HCA exhibited a good antioxidant effect. But it has a significantly lower absorption after oral administration due to its weak permeability and low solubility in the intestinal lumen (Sasikumar *et al.* 2010). The poor oral bioavailability of herbal extracts can be improved by using





Virosome: An engineered virus for vaccine delivery

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ABSTRACT

The purpose of immunization is the effective cellular and humoral immune response against antigens. Several studies on novel vaccine delivery approaches such as micro-particles, liposomes & nanoparticles, etc. against infectious diseases have been investigated so far. In contrast to the conventional approaches in vaccine development, a virosomes-based vaccine represents the next generation in the field of immunization because of its balance between efficacy and tolerability by virtue of its mechanism of immune instigation. The versatility of virosomes as a vaccine adjuvant, and delivery vehicle of molecules of different nature, such as peptides, nucleic acids, and proteins, as well as provide an insight into the prospect of drug targeting using virosomes. This article focuses on the basics of virosomes, structure, composition formulation and development, advantages, interplay with the immune system, current clinical status, different patents highlighting the applications of virosomes and their status, recent advances, and research associated with virosomes, the efficacy, safety, and tolerability of virosomes based vaccines and the future prospective.

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Nanoencapsulation and characterisation of *Hypericum perforatum* for the treatment of neuropathic pain

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RESEARCH ARTICLE



Nanoencapsulation and characterisation of *Hypericum perforatum* for the treatment of neuropathic pain

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ABSTRACT

Aim: This work aimed to encapsulate *Hypericum perforatum* extract (HPE) into nanophytosomes (NPs) and assess the therapeutic efficacy of this nanocarrier in neuropathic pain induced by partial sciatic nerve ligation (PSNL).

Methods: Hydroalcoholic extract of *Hypericum perforatum* was prepared and encapsulated into NPs by thin layer hydration method. Particle size, zeta potential, TEM, differential scanning calorimetry (DSC), entrapment efficiency (%EE), and loading capacity (LC) of NPs were reported. The biochemical and histopathological examinations were measured in the sciatic nerve.

Results: Particle size, zeta potential, %EE, and LC were 104.7 ± 1.529 nm, -8.93 ± 1.71 mV, $87.23 \pm 1.3\%$, and $53.12 \pm 1.7\%$, respectively. TEM revealed well-formed and distinct vesicles. NPHPE (NPs of HPE) was significantly more effective than HPE in reducing PSNL-inducing pain. Antioxidant levels and sciatic nerve histology were reversed to normal with NPHPE.

Conclusions: This study demonstrates that encapsulating HPE with phytosomes is an effective therapeutic approach for neuropathic pain.

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Nanophytosomes;
Hypericum perforatum;
neuropathic pain; sciatic
nerve; antioxidants

1. Introduction

Neuropathic pain is one of the many factors that contribute to the global burden of disease (Sukmawan *et al.* 2021). It is caused by damage to somatosensory nervous system and affects around 7% of the population (Szok *et al.* 2019). Several traditional drugs, including opioids, anticonvulsants, NSAIDs, and antidepressants, are available for neuropathic treatment, but they do not provide acceptable pain relief in many individuals. Most of these people want safe treatment, as they are concerned about serious side effects such as dizziness, nausea, vomiting, stomach ache, heartburn, etc. with the traditional therapy for neuropathic pain. Hence, in this prospect, herbal therapy may thus give a high-level alternative with superior therapeutic results and fewer adverse effects in the future (Kumar *et al.* 2021).

Hypericum perforatum L. (Hypericaceae), mainly known as St. John's wort, is a perennial herb that has recently gained popularity as one of the world's most commonly used medicinal plants (Birt *et al.* 2009, Huang *et al.* 2013). The extracts of *Hypericum perforatum* are employed as medical natural agents because

they have a wide range of therapeutic actions, including antidepressant, antiinflammatory, antibacterial, antioxidant, wound healing, and pain relief (Güneş and Tihmınlıoğlu 2017). It is regarded a safe herbal medicinal agent because it is well tolerated and has few negative effects (Woelk 2000). Recently, *Hypericum perforatum* has also been found to be useful in treatment of oxaliplatin-induced neuropathic pain (Cinci *et al.* 2017). One of the studies found that taking *Hypericum perforatum* extract (HPE) orally can improve morphine antinociception in conditions of neuropathic pain (Zeliou *et al.* 2017). Hypericin is the major bioactive component for the most of HPE functional characteristics. As hypericin is sensitive to high temperatures and pH, it can be encapsulated to improve its stability (Greeson *et al.* 2001).

Traditional herbal preparations have low solubility, stability, and bioavailability, all of which limit their efficiency in therapeutic products (Mathur 2013). Nano-phytosomes are a new lipid-based drug delivery system that overcomes the drawbacks of traditional drug delivery system. Nanophytosomes (NPs) are advanced form of herbal products that are more easily



absorbed, utilised, non-toxic, and biocompatible, and therefore give greater outcomes than traditional herbal extracts (Abd El-Fattah *et al.* 2017). In comparison to conventional herbal extracts, phytosomes are prepared by binding individual ingredients of herbal extracts to phosphatidyl choline, developing in a formulation with better solubility, stability, and absorption, which leads to enhanced pharmacokinetic and pharmacodynamic characteristics (Freag *et al.* 2013).

Phytosomes containing curcumin have been used to treat neuropathic pain in recent years (Pierro and Settembre 2013). One of the study showed that encapsulation of Ginko biloba with phytosomes improve the antioxidant activity (Naik *et al.* 2006). To the best of our knowledge, phytosomes loaded with *Hypericum perforatum* extract have not been studied in the context of neuropathic pain.

From the aforementioned facts, the aim of this study was to develop and characterise NPs containing PSLN-inducing HPE and evaluate its effect for neuropathic pain induced by PSLN in Wistar rats. As this study is preliminary, further research needs to be carried out to develop a high-efficiency nanoformulation for daily use by neuropathy patients, which would be a significant step towards improving their quality of life.

2. Materials and methods

2.1. Materials

Hypericum perforatum herb (whole plant) gifted by Dr. Willmer Schwabe (Noida, India) was authenticated and specimen was kept in the Raw Materials Herbarium and Museum, Delhi (New Delhi, India), with the authentication number NISCAIR/RHMD/consult/2021/3800-1 for future reference. Hypericin standard was purchased from Biogenuix (New Delhi, India), 30% w/w phosphatidylcholine (Sigma Biotech Ltd, New Delhi, India), solvents and other chemical reagents of analytical grade were used during whole study.

2.2. Preparation of plant extract

Hypericum perforatum was coarsely powdered. The extract was prepared by the Soxhlet apparatus using hydroalcoholic (40:60, v/v, ethanol:water) solvent at 75–79 °C for 24 h. The extract obtained was evaporated, dried, and stored in airtight container.

2.3. Preparation of nanophytosomes

The formulation was optimised by Design Expert 13 software and further characterised.

Hypericum perforatum extract was encapsulated into NPs utilising thin layer hydration method with natural soya phosphatidylcholine (SPC) at a molar ratio of 60:80 (mg).

HPE and SPC were weighed out, dissolved in ethanol, and placed in vacuum rotary evaporator in a round bottom flask for 60 min at 60 °C to completely evaporate organic solvent and create a thin dry layer at the bottom of flask. Deionised water (10 ml) was used to hydrate the equivalent thin layer. The phytosomal dispersion was then homogenised at $20\,000 \times g$ (55 °C) for 10 min. The obtained sample was then subjected to four minutes of 70% sonication strength by a probe ultrasound sonicator (Babazadeh *et al.* 2017). At last, NP sample was extruded through 100 nm filters (MF-Millipore® membrane filter, Billerica, MA, 0.1 µm pore size) using an extruder to remove particulates.

2.4. Characterisation of nanophytosome

2.4.1. Surface morphology

A transmission electron microscope was used to analyse the morphology of phytosomes (TEM, Philips CM 10, Amsterdam, Netherlands). Prior to analysis, phytosomes were broken up into smaller pieces by being briefly sonicated in pure alcohol. On a piece of aluminium, apply one drop of this suspension. The phytosomes were ultimately studied with TEM after the entire alcohol had evaporated.

2.4.2. Particle size distribution, PDI, and zeta potential study

A computerised dynamic light scattering (DLS) particle size analyser was used to assess the phytosomes' polydispersity index (PDI), zeta potential, and particle size distribution (Malvern, Zetasizer, Malvern, UK).

2.4.3. Differential scanning calorimetry analysis

HPE, NPHPE, hypericin, and phospholipids thermal curves were produced using differential scanning calorimetry (DSC) (Mettler Toledo TGA/DSC STAR SW9.20, Columbus, OH). The temperature of each sample was scanned over the range of 25–200 °C at a rate of 10 °C/min. A constant 5 ml/min flow of nitrogen was used.



2.4.4. Determination of entrapment efficiency (EE) and loading capacity (LC)

The NPs were transferred to an Amicon® filter (molecular weight cut-off 100 kDa, Millipore, Feltham, UK) and centrifuged at $448 \times g$ for 10 min to calculate the percentage of EE and LC of NPs. The filtrate was then separated and put into HPE. Using a UV-Vis spectrometer, the obtained solution's absorbance was measured at 589 nm (Ultrospec 2000, Biotech, Towcester, UK). The concentration of the unloaded HPE ($\mu\text{g/ml}$) was then calculated by plugging the absorbance value into the regression equation of hypericin standard curve. Finally, using the following equations (Babazadeh *et al.* 2017, Souri *et al.* 2021), EE and LC values for HPE were determined.

2.4.5. In vitro drug release from nanophytosomes

The dialysis bag diffusion technique was used to conduct *in-vitro* medication release. For 24 h before to usage, dialysis bags (cut-off = 12 kDa) were submerged in distilled water. One hundred millilitres of phosphate-buffered saline (PBS, pH 6.8) at 37 °C were added to 20 ml of phytosomes dispersion, which is equal to 1 mg of hypericin, and sealed by double-folding on both sides. The system was put on constant magnetic stirring at a speed of 50 rpm. At predefined intervals, 1 ml of samples from the receiver phase was obtained, and the amount of hypericin was measured using UV-visible spectroscopy, perhaps diluted with methanol. The concentration of hypericin in phytosome was calculated from pure hypericin standard curve, $y = 0.123x + 0.056$, $R^2 = 0.970$ (Patel *et al.* 2019, Singh *et al.* 2021).

In addition to the release profiles, the release kinetics was studied using mathematical modelling. Release kinetic study was performed to describe the release mechanism of hypericin from phytosomes. The release data obtained from *in vitro* release were subjected to zero order, first order, and Higuchi kinetics for model fitting (Weng *et al.* 2020).

2.5. Pharmacological screening

2.5.1. Animals

The pharmacological screening assays were conducted using adult male albino rats, weighing between 150 and 180 g. The Institutional Animal Ethics Committee (IAEC) of I.T.S. College of Pharmacy, Ghaziabad, India, gave their approval to the study's protocol (registration number: 1044/PO/Re/S/17/CPCSEA). Animals were kept in cages and given a conventional feed. The

whole study was carried out in accordance with the IAEC-approved protocols.

2.5.2. Experimental protocol for peripheral neuropathy

The partial sciatic nerve ligation (PSNL/Seltzer model) strategy, which was previously published, convinced peripheral neuropathy (Seltzer *et al.* 1990). Through the intraperitoneal dose of xylazine (5 mg/kg) and ketamine (50 mg/kg), rats were given anaesthesia. Iodine was used to cleanly shave and sanitise the right thigh. The right upper thigh was dissected to reveal the sciatic nerve. The sciatic nerve's dorsal half was then securely sutured with an 8-0 silk suture. The sutures were used to close the incisions after the surgery. After surgery, each rat was kept separated for recovery. The animals showed signs of discomfort and hyperalgesia in their behaviour. Animals were randomly divided into six groups with eight animals each, as stated below:

- Group 1 (control group) received 0.9% saline orally.
- Group 2 (sham) received 0.9% saline orally.
- Group 3 (PSNL) received 0.9% saline orally.
- Group 4 (standard group) was given with gabapentin orally (100 mg/kg).
- Group 5 (test group) received HPE orally (200 mg/kg).
- Group 6 (test group) received NPHPE orally (20 mg/kg).

2.5.3. Behavioural method

Behavioural tests were performed to evaluate allodynia and hot plate method which are the parameters to evaluate neuropathy. The evaluation of sensory loss by quantitative assessment of neuropathy was done by these tests. The tail-flick latency was measured when the tail of the individual animal was dipped into a cup of cold water within 10 °C temperature. The time of tail-flick latency was recorded within the cut off time of 15 s. It was performed on 7th and 14th day, after induction of neuropathy (Na *et al.* 1994). It was evaluated by placing an individual animal on hot plate at 55 °C temperature. The time of hind paw licking latency by the animal was observed and recorded. The cut off timing of 20 s was noted. It was performed on 7th and 14th day of the study (Eddy and Leimbach 1953).



2.5.4. Tissue preparation for biochemical parameters

The entire sciatic nerve was cut out of sacrificed animals. Biochemical estimation was performed after tissue homogenates (10% w/v) were prepared in 0.1 M phosphate buffer (pH 7.4) at 15 000 rpm for 20 min.

2.5.5. Oxidative stress study

The levels of malondialdehyde (MDA), superoxide dismutase (SOD) activity, reduced glutathione, and catalase were measured to determine the oxidative stress. According to the methodology published by Ohkawa *et al.* (1979), MDA levels in the plasma samples were measured using the thiobarbituric acid reactive substances (TBARS) technique. By using the Sedlak and Lindsay (1968) method, the reduced glutathione was calculated and expressed as micromoles (μmol) per gram of tissue weight. SOD was calculated using pyrogallol autoxidation's hindrance (Marklund and Marklund 1974). The absorbance was measured at 420 nm following addition of 25 l of pyrogallol, 100 μl of cytosolic supernatant, to Tris-HCl buffer.

The Clairborne method, developed in 1985, was used to measure the amount of H_2O_2 utilised per minute per milligram of protein in the tissue supernatant. First of all, when the UV range gets smaller, H_2O_2 absorption grows. When H_2O_2 breakdown is performed at 240 nm, there is a decrease in absorption. As a difference in absorbance per unit of time, catalase activity was calculated. Three minutes of one-minute intervals were used to record the change in absorbance at 240 nm.

2.6. Histological examination

Sciatic nerve samples are sliced to a thickness of 4 mm and stained with eosin and haematoxylin after being preserved in formalin fixative solution (10% w/w). Axonal degeneration and histopathological alterations were examined in sections using a light microscope ($\times 400$). The histopathological evaluations performed at the I.T.S. Dental College in Ghaziabad are regarded.

2.7. Statistical analysis

The mean and standard deviation (SD) are used to express all data. To evaluate group significance,

one-way analysis of variance (ANOVA) was utilised. $p < 0.05$ was used to determine statistical significance.

3. Results and discussion

3.1. Characterisation of HPE-loaded phytosomes

The mean particle size and PDI of NPHPE were 104.7 ± 1.529 nm and 0.462 ± 0.02 , respectively, as can be shown in Table 1. The PDI, which gauges the particle size distribution, is a crucial nanoparticle characteristic. The measured particle size is smaller than the previously published data for polyherbal phytosomes (Rathee and Kamboj 2018) and chrysin-loaded phytosomes (Kim and Imm 2020). The biological effectiveness of nanocarriers, including blood circulation time, endocytosis, fusion, adsorption on target cell membranes, and release of loaded substance, is greatly influenced by particle size (Amjadi *et al.* 2019).

The therapeutic effectiveness of nanoparticles depends on their size. Wong *et al.* previously found that insulin-loaded nanoliposomes with smaller particle sizes had stronger anti-diabetic action (Wong *et al.* 2018). By measuring the surface charge of particles, the zeta potential is another significant measure that indicates the physical stability of colloidal systems. HPE-loaded NPs' zeta potential was -8.93 ± 1.71 mV (Table 1), which is comparable to Rathee and Kamboj's finding (2018). The spherical structure and restricted size distribution of phytosomal particles were clearly visible in the TEM picture (Figure 1(A)) of the HPE-loaded NPs. Additionally, the majority of the particles were less than 100 nm, which is consistent with PCS result.

The studies showed that curcumin- and quercetin-loaded NPs both had comparable morphologies (Abd El-Fattah *et al.* 2017, Baradaran *et al.* 2020). The EE and LC values of the HPE-loaded NPs were furthermore $87.23 \pm 1.3\%$ and $53.12 \pm 1.7\%$, respectively (Table 1).

3.2. Differential scanning calorimetry

DSC analysis of HPE, phospholipid, hypericin, and phytosomal complex was reported. DSC analysis was done to confirm the physical interaction existence between

Table 1. Mean diameter, polydispersity index (PDI), zeta potential, % entrapment efficiency (%EE), and loading capacity of *Hypericum perforatum* extract-loaded nanophytosomes.

Mean diameter (nm)	PDI	Zeta potential (mV)	% Entrapment efficiency (EE)	Loading capacity (%)
104.7 ± 1.529	0.462 ± 0.02	-8.93 ± 1.71	87.23 ± 1.3	53.12 ± 1.7

All values were estimated in mean \pm standard deviation ($n = 3$).



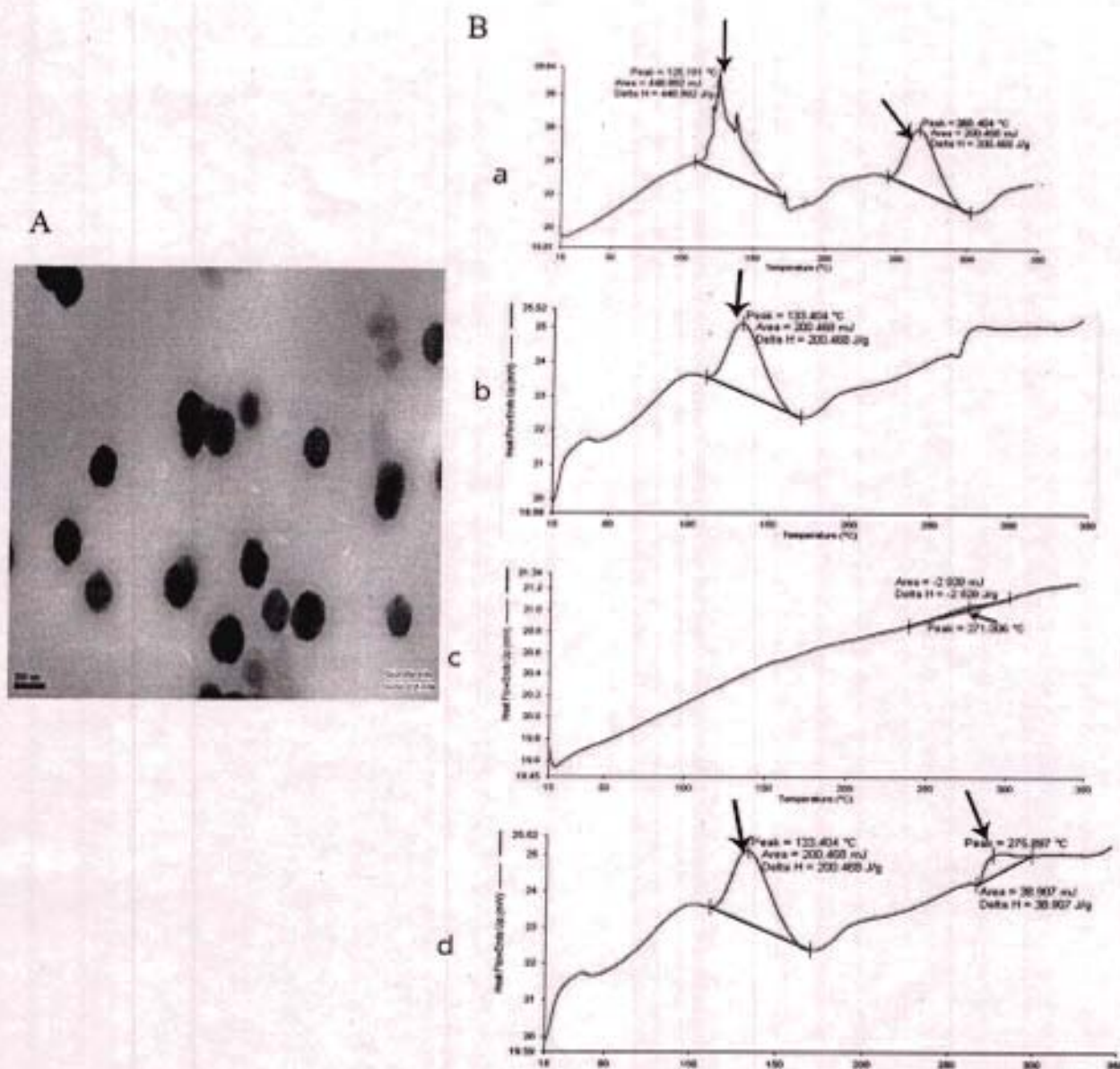


Figure 1. (A) Transmission electron microscopy image showing morphology (particle size and shape) of phytosomes vesicles in the HPE-loaded nanophytosomes. (B) Differential scanning calorimetry thermograms showing the melting point peaks of *Hypericum perforatum* extract (a), pure phospholipid (b), hypericin (c), and *Hypericum perforatum* extract-loaded nanophytosomes (d).

HPE and phospholipids. HPE showed two characteristic peaks at 125.19 °C and 268.404 °C.

The peak of phospholipid was obtained at 133.404 °C. According to the theory, phospholipids melted at higher temperatures, allowing drugs to dissolve in them and partially forming phospholipid complexes. The pure substance hypericin DSC was found at 271.006 °C while the DSC of phytosomes was shifted at 133.404 °C and 275.897 °C (Figure 1(B)). Both peaks in the complex's DSC spectrum can be seen, which indicates that both the drug and the phospholipid are present. Considered to be negligible are hydrogen bonds, which are one type of interaction

between HP and phospholipid (Yanyu *et al.* 2006) or van der Waals force (Lasonder and Weringa 1990).

3.3. In vitro release profile of nanophytosome

In vitro release study was performed to project the performance *in vivo* of phytosome. As shown in Figure 2, *in vitro* release was slow and sustained up to 24 h. The prepared phytosome was known to release $98.01 \pm 12.45\%$ of hypericin in 24 h.

Drug release kinetics data were found to be $R^2 = 0.9757$ for Higuchi model, $R^2 = 0.9174$ for zero order, and $R^2 = 0.1301$ for first order. The value of R^2



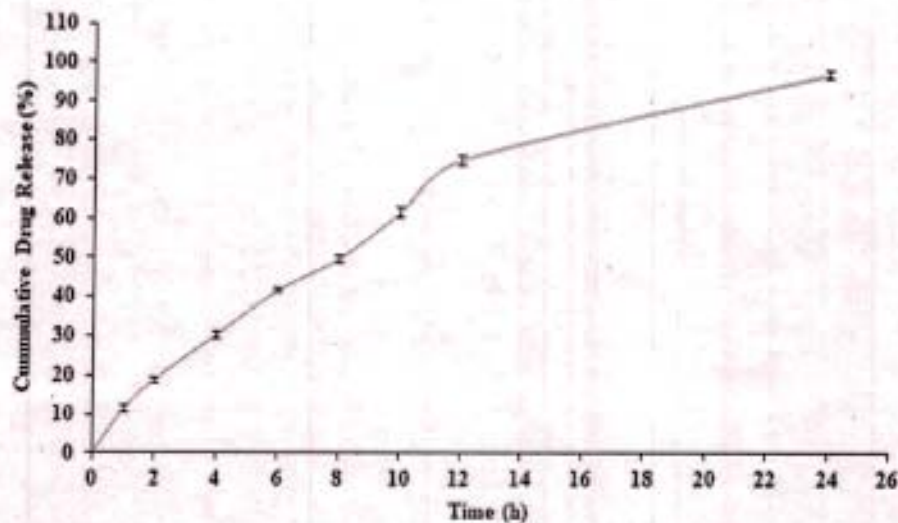


Figure 2. Error bar graph showing % cumulative release patterns of hypericin from phytosome in phosphate-buffered saline pH 6.8 by performing *in vitro* dissolution test of formulations. Values were estimated in mean \pm SD ($n = 3$).

was found maximum for Higuchi kinetics although the release kinetics was supposed to be mixed order for NPs. Further, the mechanism was explored by using Korsmeyer–Peppas model and the value of release exponent n was observed 1.08, which supports non-Fickian diffusion, class II transport of hypericin from phytosomes.

3.4. Behavioural study

3.4.1. Paw withdrawal latency by hot plate method

PWL was evaluated on days 7 and 14. In the sham and PSNL groups, PWL significantly decreased on days 7 and 14 compared to the control group ($F_{5,35} = 108.54$, $p < 0.01$). In comparison to PSNL and sham group, HPE and NPHPE significantly increased paw withdrawal delay on day 7 ($F_{5,35} = 276.76$, $p < 0.01$). When compared to the sham and PSNL groups, withdrawal latency showed a significant improvement on day 14 ($F_{5,35} = 447.29$, $p < 0.01$). Paw withdrawal latency was reported to be considerably greater in NPHPE after day 14 compared to HPE and gabapentin (Table 2). As a result, the NPs-encapsulated HPE enhanced the paw latency in neuropathic pain brought on by PSNL.

3.4.2. Paw withdrawal latency by cold allodynia

Paw withdrawal latency was observed to be significantly higher in the gabapentin, HPE, and NPHPE groups compared to the sham and PSNL animals ($F_{5,35} = 35.26$, $p < 0.01$). It was discovered that increase in the paw withdrawal latency was more effective on day 14 ($F_{5,35} = 665.23$, $p < 0.01$) than day 7 ($F_{5,35} = 345.54$, $p < 0.01$). This demonstrated that

Table 2. Effect of *Hypericum perforatum* extract (HPE)-loaded nanophytosomes on thermal latency by hot plate method in PSNL Wistar rats.

S. no.	Group	7th day	14th day
1	Group I (normal)	12.50 \pm 0.764 ^a	10.60 \pm 0.847 ^a
2	Group II (sham)	10.47 \pm 0.601	8.63 \pm 0.601
3	Group III (PSNL)	4.33 \pm 0.615	3.83 \pm 0.601
4	Group IV (gabapentin)	11.33 \pm 0.78 ^a	9.17 \pm 0.792 ^a
5	Group V (HPE)	11.68 \pm 0.792 ^a	9.33 \pm 0.749 ^a
6	Group VI (NPHPE)	12.27 \pm 0.760 ^{ab}	10.32 \pm 0.864 ^{ab}

The thermal latency was enhanced by nanophytosome of *Hypericum* extract (NPHPE) (group VI) after sciatic nerve injury brought on by PSNL. Groups I, II, and III received 0.9% saline (p.o.), group IV received 100 mg/kg gabapentin (p.o.), group V received 200 mg/kg *Hypericum* extract (HPE) (p.o.) and group VI received NPHPE 20 mg/kg (p.o.). All data were presented as the mean \pm SD. Each group has eight animals; ^a $p < 0.01$ found significant compared with groups II and III; ^b $p < 0.01$ found significant compared with group V. The values were statistically analysed by one-way ANOVA followed by Dunnett's test.

the treatment's daily rise in paw withdrawal delay after the onset of neuropathy (Table 3).

After 14 days of treatment, the allodynia caused by PSNL was reversed. While saline, which was given to the normal group, had no effect on any behavioural reaction, NPHPE's pain-relieving effects were greater than those of HPE.

3.5. Antioxidant effect

When compared to the control and PSNL group, TBARS, GSH SOD, and CAT significantly improved in sciatic nerve after treatment with HPE and NPHPE.

In comparison to the PSNL group, NPHPE and HPE treatment considerably reduced MDA levels. Despite the fact that NPHPE was more efficient than HPE at lowering MDA levels ($F_{5,35} = 98.45$, $p < 0.01$)

NPHPE, HPE, and gabapentin administration significantly increased GSH level compared to the PSNL



group ($F_{5,35} = 44.21, p < 0.01$). This demonstrated a superior level of protection for the management of neuropathic pain brought on by PSLN. NPHPE increased GSH level almost to the same extent as control group.

In comparison to control group, SOD activity in the PSLN group reduced significantly ($p < 0.01$) by using gabapentin (100 mg/kg/day), this lowered level of SOD was significantly elevated ($p < 0.01$). NPHPE and HPE administration significantly reversed the SOD activity induced by PSLN ($F_{5,35} = 61.53, p < 0.01$). Despite the fact that SOD activity was increased by gabapentin and HPE approximately equally, while NPHPE had a greater impact.

A highly improvement in catalase activity was obtained by NPHPE when compared with PSLN group ($F_{5,35} = 7.43, p < 0.01$) (Table 4). It was found that NPHPE was effective equally as control. In comparison to the PSLN group, HPE also showed a substantial protective effect, although it was smaller than NPHPE, demonstrating that NPHPE is more protective to oxidative stress than gabapentin and HPE. The study also revealed the NPs of rutin also showed greater antioxidative property than free form (Amjadi et al. 2021). It was observed that oxidative stress is increased due to

release of free radicals because of induction of neuropathic pain by PSLN. The previous finding in our lab also showed that the oxidative stress was increased due to induction of neuropathic pain. It has been reported that the antioxidative properties were enhanced by encapsulation of curcumin by phyto-somes in inflammation induced mice (Baradaran et al. 2020). Our current study also revealed that NPHPE showed best antioxidative properties by elevating GSH, SOD and catalase and by decreasing the MDA level in sciatic nerve tissue.

3.6. Histopathological evaluation

In comparison to control group, which represents the usual architecture of fibres, PSLN Wistar rats exhibited swelling of myelinated and unmyelinated fibres and alterations in the sciatic nerve's architecture. In rats, treatment with HPE and NPHPE reduced fibre oedema and promoted fibre regeneration in the sciatic nerve. The number of myelinated nerve fibres and the thickness of the myelin sheath were both improved more in the NPHPE-treated groups than in the HPE group (Figure 3).

4. Conclusions

Hypericum perforatum-loaded NPs were developed and characterised with acceptable particle size, PDI, zeta potential, etc. successfully. The administration of HPE-loaded NPs in Wistar rats showed more effectiveness to treat neuropathic pain than HPE. The oxidative stress induced by PSLN in rodents was also improved by HPE loaded NPs. Moreover, the histopathological studies also revealed that HPE-loaded NPs were more effective in repairing the sciatic nerve fibres than HPE. Hence, the current study verified that encapsulation of HPE with NPs is an effective approach to treat the neuropathic pain with good bioavailability and pharmacological activity. The present study introduced a new nanoformulation for neuropathic pain in daily

Table 3. Effect of *Hypericum perforatum* extract (HPE)-loaded nanophytosomes on paw withdrawal latency by cold allodynia in PSLN Wistar rats.

S. no.	Group	7th day	14th day
1	Group I (normal)	8.17 ± 0.13 ^a	10.34 ± 0.15 ^a
2	Group II (sham)	6.98 ± 0.11	8.34 ± 0.13
3	Group III (PSLN)	4.12 ± 0.14	3.62 ± 0.13
4	Group IV (gabapentin)	8.06 ± 0.13 ^a	9.56 ± 0.11 ^a
5	Group V (HPE)	7.44 ± 0.11 ^a	9.57 ± 0.16 ^a
6	Group VI (NPHPE)	8.15 ± 0.21 ^{ab}	10.31 ± 0.16 ^{ab}

The paw withdrawal latency was increased by nanophytosome of *Hypericum* extract (NPHPE) (group VI) after sciatic nerve injury brought on by PSLN. Groups I, II, and III received 0.9% saline (p.o.), group IV received 100 mg/kg gabapentin (p.o.), group V received 200 mg/kg *Hypericum* extract (HPE) (p.o.), and group VI received 20 mg/kg NPHPE (p.o.). All data were presented as the mean ± SD. Each group has eight animals; ^a $p < 0.01$ found significant compared with groups II and III; ^b $p < 0.01$ found significant compared with group V. The values were statistically analysed by one-way ANOVA followed by Dunnett's test.

Table 4. Effect of *Hypericum perforatum* extract (HPE)-loaded nanophytosomes on lipid peroxidation and antioxidant enzymes on neuropathic pain induced by PSLN in rat sciatic nerve tissue.

S. no.	Group	TBARS (nmol/mg protein)	GSH (μ mol/g of tissue weight)	SOD (μ mol/g of tissue weight)	CAT (nmol/min/mg)
1	Group I (normal)	1.180 ± 0.09 ^a	0.35 ± 0.04 ^a	86.89 ± 0.051 ^a	12.98 ± 0.12 ^a
2	Group II (sham)	1.127 ± 0.10	0.33 ± 0.04	48.78 ± 0.053	6.33 ± 0.08
3	Group III (PSLN)	3.91 ± 0.39	0.125 ± 0.03	25.19 ± 0.024	4.26 ± 0.43
4	Group IV (gabapentin)	0.91 ± 0.033 ^a	0.643 ± 0.05 ^a	60.8 ± 0.043 ^a	11.39 ± 0.21 ^a
5	Group V (HPE)	1.03 ± 0.05 ^a	0.224 ± 0.02 ^a	68.56 ± 0.056 ^a	10.07 ± 0.13 ^a
6	Group VI (NPHPE)	0.63 ± 0.041 ^{ab}	0.897 ± 0.06 ^{ab}	84.56 ± 0.031 ^{ab}	12.65 ± 0.23 ^{ab}

The table showed TBARS, GSH, SOD, and CAT levels. All values were expressed as mean ± SD. Each group consists of eight animals; groups IV, V, and VI found significant (^a $p < 0.01$) compared with PSLN (group III); NPHPE (group VI) found significant (^a $p < 0.01$) compared to HPE (group V). The values were statistically analysed by one-way ANOVA followed by Dunnett's test. Groups I, II, and III received 0.9% saline (p.o.), group IV received 100 mg/kg gabapentin (p.o.), group V received 200 mg/kg *Hypericum* extract (p.o.), and group VI received 20 mg/kg nanophytosome of *Hypericum* extract (p.o.).



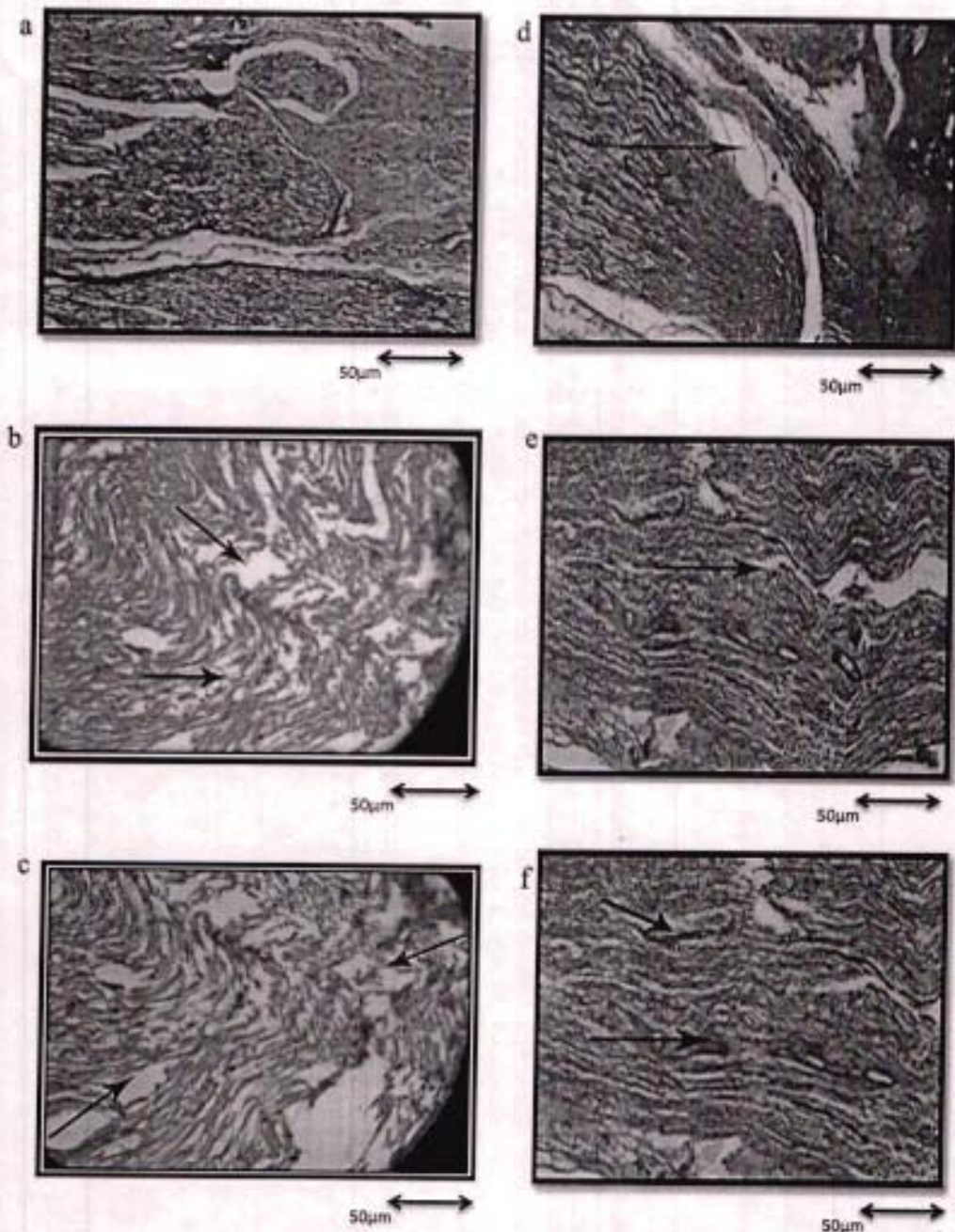


Figure 3. Histopathological study of sciatic nerve on PSNL induced neuropathic pain in different rat groups. (a) Control group (group I received 0.9% saline orally) showed normal architecture. (b) Sham group (group II received 0.9% saline orally) showed slightly swelling in fibres. (c) PSNL group (group III received 0.9% saline orally) showed swelling as well as thickness of the myelin sheath. (d) Treatment with gabapentin 100 mg/kg orally (positive control) (group IV) showed improvement in the swelling as well as the architecture of the myelinated fibre as compared to sham and PSNL group. (e) Treatment with HPE 200 mg/kg orally (group V) showed less oedema, more fibre regeneration as well as improvement in the thickness of myelin sheath along with the architecture of the nerve compared to sham and PSNL group. (f) Treatment with NPHPE 20 mg/kg orally (group VI) statistically reduced swelling and improved the fibre regeneration as well as myelin sheath thickness compared to sham, PSNL, and HPE (groups II, III, and V).

usage to attenuate its complication and improve the life quality of the patients suffering from this devastating disease.





Disclosure statement

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Hepatoprotective Activity of *Lagenaria siceraria* Leaf Extract against Carbon Tetrachloride-Induced Damage in Rats

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Abstract

Traditionally, the juice and decoction of aerial part and leaves of *Lagenaria siceraria* was used for cure and management of hepatic disorders in South Asia. There is a scarcity of scientific details to justify the traditional claim for hepatoprotective potential of leaves of *L. Siceraria*. In the present research work, the hepatoprotective potential was evaluated for methanolic extract of *L. siceraria* leaves (LSME) against carbon tetrachloride induced hepatotoxicity in albino rats. The levels of hepatic biochemical markers were estimated in treated groups. The treatment with LSME (50 mg/kg) altered back the normal levels of biochemical markers as well as done the significant improvement in the damaged hepatocytes. The levels of endogenous liver antioxidant enzymes, catalase, superoxide dismutase and glutathione contents were increased significantly. There was also recorded the significant ($P < 0.001$) depletion in serum glutamic-pyruvic transaminase, serum glutamic-oxaloacetic transaminase, Alkaline- phosphatase and total bilirubin in LSME treated group. From these results, it is suggested that methanolic extract of *L. siceraria* leaves possesses hepatoprotective properties.

Key Words *Lagenaria siceraria*, carbon tetrachloride, Hepatoprotective effect, biochemical enzymes.

Introduction

Liver is the largest vital organ to facilitate intense metabolism and excretion. It plays the vital role in the maintenance, performance and regulating homeostasis of the body. Various biochemical pathways of growth, immunity, energy and reproduction are passed through the liver¹. The major functions of the liver are metabolism of carbohydrate, protein and fat, detoxification, bile secretions and storage of some vitamins. So, keeping the liver healthy is an



important factor for overall health and well being. The environmental toxins, abused by drug habits & alcohol and over the-counter drugs may lead to continuously and variedly exposure to liver unfortunately². This can eventually lead to various liver ailments like fatty liver, hepatitis, cirrhosis and alcoholic liver disease. Now a day's the plant based preparations are employed for the treatment of liver disorders and alleviation of hepatic diseases³. Therefore, a lot of traditional remedies from plant origin are evaluated for its potential for antioxidant and hepatoprotective effect by using experimental rodent model⁴. Carbon tetrachloride (CCl₄)-induced hepatotoxicity is widely used model to evaluate hepatoprotective effects of herbs extracts⁵.

Lagenaria siceraria (Bottle gourd) is an official drug in Ayurvedic Pharmacopoeia⁶. It is one of the most valuable fruit for human being made and gifted by the nature having composition of all the essential constituents that are required for normal and good human health⁷. Juice or decoction of aerial part and leaves of *L. siceraria* is used for cure of jaundice in India traditionally⁸. Leaves paste is used for treatment of alopecia and applied on the head for the headache by ancient people⁹. Leaves are also used as cathartic in constipation¹⁰. The edible part of fruit of *L. siceraria* is rich source of ascorbic acid, β - carotene, vitamin B complex and pectin¹¹. It is also good source of minerals¹². Two sterols namely fucosterol and campesterol were identified and isolated from ethanol extract of dried fruit pulp¹³. HPLC analysis of extract of flowering plant of *L. siceraria* shows presence of flavone-C glycosides¹⁴. Fecal steroid excretion was reported on administration of semi purified dietary fibres isolated from the fruit of *L. Siceraria*¹⁵. In the present work hepatoprotective activity was investigated for methanolic extract of *L. Siceraria* leaves (LSME) in experimental rodents.

Materials and methods

Reagents and chemicals

Carbon tetrachloride was procured from Krishna Chemical Industry Vadodara Gujarat, India; Silymarin was received as a gift sample from Micro labs ltd. India; Kits of standards i.e. SGPT SGOT, ALP and bilirubin was obtained from Erba Diagnostics, India. All the reagents used in experimental work were of analytical grade.

Collection and authentication of plant material

The leaves of *L. siceraria* plant were collected in the month of July from village area of district Bareilly, U.P, India. Leaves were authenticated by Department of Taxonomy, Hindu college (PG) Moradabad, Uttar Pradesh, where a voucher specimen (Taxonomy: HC.MBD/HAP-BK-2010-07-167) has been submitted.

Preparation of extract

Leaves of *L. siceraria* were washed in tap water and dried in shade to avoid degradation of active constituents. Dried leaves were powdered to form coarse particles and stored in an airtight container. The powdered material was extracted by soxhlet apparatus in several batches using different solvents in increasing order of polarity. Petroleum ether, chloroform, acetone, methanol and distilled water were used as solvent. The extracts were concentrated by evaporating the solvent by rotary evaporator (Rotavapor® R-100). Preliminary phytochemical

screening was performed on all extracts of *L. siceraria* to confirm the presence of various phytochemical groups¹⁶.

Estimation of total phenolic content

In the phytochemically active extract, total phenolic content was analyzed by the Folin-Ciocalteu reagent method using gallic acid as a standard. In about 250 ml of methanol:water mixture (60:40 V/V, 0.3% HCl), 100 mg of extract was added and mixed well. Then the mixture was filtered through 0.45 μ m milipore filter. Equimolar quantities (100 ml) of the filtrate and Folin-Ciocalteu reagent (50 %, V/V) were mixed followed by the addition of 2.0 ml of 2% sodium carbonate solution in water. The absorbance of the solution was recorded after 2 hours at 750 nm wavelength (UV spectrophotometer, Shimadzu, UV-2600i). Quantification was based on the standard curve of gallic acid (0–1.0 mg/ml) dissolved in methanol/water (60:40, V/V, 0.3 % HCl). Phenolic content was expressed as milligrams per gram of gallic acid equivalent¹⁷.

Experimental animals

Wistar albino rats of either sex, weighing 150 to 200 gm, were housed in groups of four per cage under controlled light (12:12 light: dark cycle) and temperature ($25\pm 2^\circ\text{C}$). Environmental and behavioral assessment was conducted during the light cycle. Food (Golden feed, New Delhi, India) and water *ad libitum* was provided. The animals were acclimatized to laboratory conditions for seven days before commencement of experiments¹⁸.

All the procedure described in manuscript were reviewed and approved by Institutional Animal Ethical Committee (IAEC) of Teerthanker Mahaveer College of Pharmacy, Moradabad, India (Approval no- 1205/C/08/CPCSEA/2021/IAEC/12).

Acute oral toxicity study

Acute oral toxicity study was performed according to OECD guidelines 423 for LSME (2000 mg/kg body weight) in Wistar albino rats. Hematological parameters, laboratory parameters like body weight, consumption of food, urine examinations, ophthalmologic examinations and behavior were also evaluated during the experiment¹⁹.

Hepatoprotective Screening

The study was conducted by using CCl_4 as toxicant to produce hepatotoxicity and Silymarin as a standard/reference drug²⁰. LSME at a dose of 50 mg/kg was used as test and compared with the results obtained from standard and control. Animals were divided into 4 groups (n=6/group).

- Group 1- Positive control: The animals received distilled water for 7 days and given CCl_4 in olive oil (1:9 ratio) single dose (SD), 10ml/kg orally on 8th day.
- Group 2- Negative control: The animals received saline for 7 days.
- Group 3- Standard: Pre-treated with standard drug Silymarin (100mg/kg body weight /day p.o.) for 7 days followed by SD of CCl_4 on 8th day.
- Group 4- Test: Pre-treated with LSME (50 mg/kg/day p.o.) for 7 days followed by a SD of CCl_4 on 8th day.



After 16 hrs from the administration of last dose of treated groups, the blood samples were collected through retro orbital artery²¹. Serums were separated from the blood samples by centrifugation for 10 minutes at 2000 rpm²². The estimation of biochemical parameters was done for hepatoprotective effect of *L. Siceraria*. Alkaline-phosphatase (ALP), Serum glutamic-oxalo-acetic-transaminase (SGOT), serum glutamic-pyruvic-transaminase (SGPT), and tissue bilirubin level (TBL) were estimated by using standard kits²³. Then animals were sacrificed and their livers were excised & washed in normal saline followed by 0.15 M Tris-HCL buffer. The estimation of tissue lipid peroxidase (LPO) was carried out²⁴. A portion of homogenate after precipitating proteins with trichloroacetic acid (TCA) was used for the estimation of glutathione (GSH)²⁵. The rest of the homogenate was centrifuged at 15000 rpm for 15 min at 4°C. The supernatant was separated for the estimation of super-oxide- dismutase (SOD) and catalase (CAT)²⁶.

Histopathological study

Tissue specimens of liver lobules were used for histopathological examination. Sections of 50 micron thickness were cut by embedding tissue in wax. Haematoxylin and eosin staining reagents were used to stain the sections. All stained sections were examined at 40x and 100x magnification power. Constitution of normal internal structure, swelling/inflammation and necrosis were considered at the time of observation.

Statistical analysis

The analysis of statistical significance was determined by one way analysis of variance (ANOVA) followed by Dunnett's Test. The values were expressed in terms of mean \pm SEM and $P < 0.05$ was considered significant.

Results

The presence of carbohydrates, saponins, steroids, tannins, flavonoids and terpenoids in LSME was revealed through performing the preliminary phytochemical screening. Hence, LSME was found to be phytochemically active. Total phenolic content of LSME was estimated through standard curve equation ($y = 0.0032x + 0.0009$) for gallic acid at 750 nm wavelength. The total phenolic content of *L. siceraria* leaves methanolic extract was found to contain 98.98 $\mu\text{g}/\text{mg}$ of gallic acid equivalent.

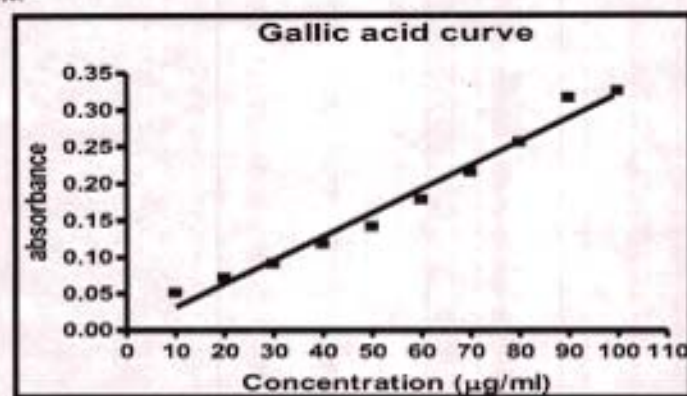


Fig. 1 Standard curve of gallic acid for estimation of total phenolic content of LSME

Acute toxicity of LSME

Hematological parameters were reported normal at the end of the toxicity study. LSME did not produce any mortality and toxic effect up to the dose level at 2000 mg/kg body weight in rats, hence the extract was considered to be safe and non-toxic for further pharmacological screening.

Estimation of biochemical parameters for hepatoprotective effect of LSME

Estimation of the serum alkaline phosphatase activity and total bilirubin is the most widely used parameters for measuring the hepatic damage. There is a significant ($P<0.001$) increase in the levels of bilirubin, SGOT, SGPT and ALP was observed in CCl_4 intoxicated group of this study. LSME possesses significant ($P<0.001$) effect on CCl_4 induced hepatotoxicity (Table 1). The significant depletion of bilirubin, SGOT, SGPT and ALP in LSME pre-treated group revealed the hepatoprotective effect of LSME (50 mg/kg) against CCl_4 induced hepatotoxicity.

Treated group	Dose (mg/kg)	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)	Total bilirubin mg/dl
Positive control	Only vehicle	123.8 ± 1.26	171.2 ± 1.61	122.4 ± 3.96	4.86 ± 0.21
Normal control	Only vehicle	50.32 ± 1.12***	58.8 ± 1.37***	49.04 ± 3.36 ***	1.1 ± 0.17 ***
Standard	100	55.2 ± 1.36***	64.47 ± 1.59***	54.32 ± 3.91 ***	1.94 ± 0.24 ***
LSME	50	58.83 ± 1.11***	79.26 ± 1.66***	61.49 ± 4.08 ***	2.52 ± 0.21 ***

Table 1 Effect of LSME on biochemical parameters in CCl_4 -induced hepatotoxicity

All values expressed as Mean ± SEM (n= 6), *** $P<0.001$ as compared with positive control group (One way ANOVA followed by Dunnett's test)

Effect of LSME on lipid per-oxidation in carbon tetrachloride treated groups

Lipid peroxidase (LPO) levels by thiobarbituric acid reaction showed a significant ($P<0.001$) increase in carbon tetrachloride treated rats. Treatment with LSME (50 mg/kg) significantly ($P<0.001$) inhibited the LPO level which was altered back to normal. The antioxidant effect of LSME was comparable to standard drug Silymarin.

CCl_4 treatment was resulted as a significant ($P<0.001$) decrease in the level of SOD, Catalase and GSH in hepatic tissue when compared with control group. The treatment with LSME at the doses of 50 mg/kg produced a significant increase ($P<0.001$) of SOD, Catalase and GSH levels in comparison of positive control group. The liver of animals treated with Silymarin also showed a significant increase in the levels of antioxidant enzymes when compared to positive control group (Table 2).



Table 2 Antioxidant effect of LSME on CCl₄-induced hepatotoxicity in rats

Treated group	Dose (mg/kg)	CATALASE (U/mg protein)	SOD (%Inhibition of NBT)	LPO (nM/mg protein)	GSH (mM/gm tissue wt.)
Positive control	Only vehicle	13.83 ± 0.10	24.38 ± 0.22	7.19 ± 0.09	7.92 ± 0.18
Negative control	Only vehicle	34.5 ± 0.12***	66.64 ± 0.49 ***	2.61 ± 0.05***	28.14 ± 0.29 ***
Standard (Silymarin)	100	32.68 ± 0.17***	61.33 ± 0.36***	2.89 ± 0.06***	24.57 ± 0.26* **
LSME	50	27.92 ± 1.01***	52.20 ± 0.25 ***	3.23 ± 0.12***	16.32 ± 0.14 ***

All values expressed as Mean ± SEM (n=6) ***P<0.001 as compared to positive control group (One way ANOVA followed by Dunnett's test)

Histopathology of liver

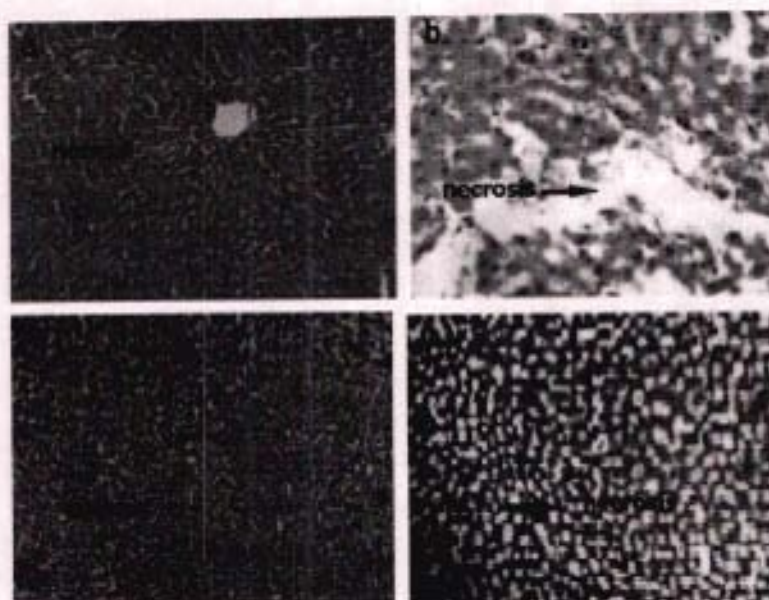


Fig. 2 (a-d) Histopathological images rat liver: a) Negative control group, showing normal architecture; b) Positive control group; showing necrosis of central vein; c): Standard group; showing normal appearance with no evidence of necrosis; d) LSME treated groups; showing less evidence of necrosis compared to positive control group

Histological profile of the animals treated with LSME (50 mg/kg) supported the results obtained by the biochemical investigations. The improvements in tissue regeneration and recovery against CCl₄ induced necrosis of hepatocellular tissues were observed microscopically. The tissue section of liver of positive control group showed that extent of liver damage was more in magnitude as compared to test and standard groups of animals; Whereas test (LSME 50 mg/kg)

and standard (Silymarin 100 mg/kg) groups showed the comparable significant healing effect (Fig. 2) but there was so much difference in the effective dose of test and standard.

Discussion

The organ liver plays a vital role in metabolism of all things including drug and nutrients. Liver has a central role in drug metabolism and it is a most vulnerable tissue for drug toxicity. According to the reports published by USFDA, herbs have been reported to cause liver injury, and 20-40% of all instances of hepatic failure. The asymptomatic elevation of liver enzymes to fulminant hepatic failure is a manifestation of hepatotoxicity due to various environmental toxicants, herbal remedies and clinically useful drugs, like paracetamol, NSAIDs, and gentamycin through the activation to highly reactive free radicals including the oxygen reactive species and super-oxides²⁷. According to literature survey, various enzymatic and non-enzymatic regulations have been developed by the cell to protect from the oxidative stress and other free radicals generated in day to day lifestyle changes. Histopathological investigation along with estimation of SGOT SGPT, ALP, TBL, LPO GSH, Catalase and SOD are the most widely used methods in the diagnosis of status of healthy hepatocytes to confirm their normal functioning and regeneration of new hepatocytes²⁸. The methanol extract of *L. siceraria* leaf reduced the elevated levels of all the biochemical parameters along with significantly inhibition of liver necrosis caused by CCl₄ induced hepatotoxicity.

Conclusion

It can be concluded from this investigation that LSME (50 mg/kg) possess hepatoprotective potential. This effect may be due to the presence of polyphenols and other antioxidants in the extract. The present study submit the evidence for hepatoprotective potential of the leaves of *L. siceraria* and may give a lead to further investigation of active molecule responsible for promising effectiveness in the treatment of hepatic disorders in future.

Declarations

Conflict of Interest

The authors declare that they have no conflict of interest to disclose.

Acknowledgement

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Heavy Metal-Induced Pollution in the Environment through Waste Disposal

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ABSTRACT

Environmental contamination from heavy metals in waste is a growing concern with severe consequences for ecosystems and human health. Industrial, electronic, and household waste commonly contain heavy metals like lead, mercury, cadmium, arsenic, and chromium. Inadequate waste disposal leads to the release of these metals into the environment, contaminating air, water, soil, and the food chain. Rapid industrialization and urbanization contribute to global waste generation, especially in developing countries where improper waste management leads to heavy metal pollution. Dumping waste in landfills and incinerating it without proper controls can result in heavy metal leaching into surrounding soil and groundwater, while incineration of electronic waste emits toxic fumes. Once released, heavy metals persist and accumulate in ecosystems. This paper analyzes the major sources of heavy metals in waste, focusing on various human activities. It identifies significant contributors such as metallurgy, the chemical industry, electrical appliance production, batteries, sewage sludge, coal-fired power stations, waste incineration plants, and household waste. The study also highlights the sources of heavy metals in agricultural regions, specifically pesticides and fertilizer waste. By examining specific types of waste, the paper provides insights into the industries and activities responsible for heavy metal pollution. This analysis serves as a foundation for policymakers, waste management professionals, and stakeholders to develop effective strategies for mitigating and managing heavy metal contamination.

Keywords: heavy metal pollution, waste management, metallurgy, chemical industry, waste incineration plants, household waste, agricultural waste.

1. INTRODUCTION

Environmental contamination caused by heavy metals contained in waste has become a pressing concern with profound implications for both ecosystems and human health. Heavy metals, such as lead, mercury, cadmium, arsenic, and chromium, are commonly found in industrial, electronic, and household waste. When these waste streams are not properly disposed and managed, heavy metals can leak into the environment, contaminating air, water, soil, and ultimately, food. Increasing industrialization, urbanization, and consumption of goods have created a global challenge for waste generation. The inadequate treatment and disposal of waste, particularly in developing countries, often results in heavy metal pollution. Dumping waste in landfills or incinerating it without proper controls can cause the leaching of heavy metals into the surrounding soil and groundwater [1]. Additionally, the incineration of electronic waste, which contains significant amounts of heavy metals, can release toxic fumes into the atmosphere. This can lead to serious health problems for local populations who are exposed to these toxins. Furthermore, heavy metals can accumulate in the food chain, causing contamination of food sources and, ultimately, endangering human health. Once heavy metals are released into the environment, they persist for long periods and accumulate in various ecological compartments. In aquatic ecosystems, heavy metals can contaminate rivers, lakes, and oceans, leading to the disruption of aquatic life. The accumulation of heavy metals in fish and seafood poses a significant threat to human health, as consumption of contaminated food can result in serious illnesses, including organ damage and neurological disorders [2]. Heavy metal contamination in the soil can have detrimental effects on agriculture. Plants absorb these metals from the soil, which can then enter the food chain through crops. This not only affects food safety but also reduces crop yields and overall agricultural productivity. Moreover, when heavy metals contaminate soil, they can migrate into groundwater, polluting drinking water supplies and posing health risks to the human population[3].

Addressing environmental contamination caused by heavy metals in waste requires a multi-faceted approach. Governments and regulatory bodies play a critical role in establishing and enforcing stringent waste management regulations. Proper waste segregation, recycling, and the adoption of environmentally friendly disposal methods, such as controlled landfilling and waste-to-energy technologies, are essential to minimizing heavy metal pollution[4]. Furthermore, the development and implementation of effective treatment technologies are vital to remediation efforts. These may include processes such as physicochemical treatments, microbial remediation, and phytoremediation, which utilize plants to extract and immobilize heavy metals from contaminated soil or water. Public awareness and education are also crucial, as individuals need to understand the importance of responsible waste management practices and the potential health risks associated with heavy metal contamination. Environmental contamination resulting from heavy

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A Case Study on Mini Gas Cylinder Cluster : Evidence from India

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Abstract

Access to cleaner cooking fuel is an essential factor that significantly impacts the living standards of human beings. Liquefied Petroleum Gas (LPG) is one of the clean sources of energy. The requirement of necessary documents and the completion of formal procedures for LPG and PNG connections deprive poor people, migrating workforce, and students who need readily available documents for LPG connections. Sensing this as an opportunity, some manufacturers started producing mini gas cylinders (MGC). The MGC cluster of Meerut, Uttar Pradesh, is a prominent manufacturing hub of such cylinders in India. The cluster had been involved in cylinder production for many years until 2013, when the government granted permission to sell 5kg LPG cylinders through retail outlets of oil distributors, with or without domestic pressure regulators. The research used the case study methodology to identify the problems and issues concerning the promising cluster. This case further corroborated the notion that government support and recognition are essential for the growth and sustenance of any business.

Keywords

Case Study, Mini Gas Cylinders, Mini Gas Cylinder Cluster, LPG.

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A STUDY OF THE IMPACT OF RISK AVOIDANCE AND FINANCIAL WELFARE ON THE INTENT TO INVEST IN THE EQUITY MARKET

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Abstract

This study aims to determine how risk aversion and financial well-being influence the desire to invest in equity products. A seven-item scale was used to evaluate risk avoidance, while an eight-item scale was used to evaluate financial well-being. By distributing a questionnaire to investors in India who participate in the stock market, data for research was gathered. The model was tested and validated using a Structural Equation Modeling (SEM) approach and Warp PLS software. To determine the relationship between the variables, Cronbach's alpha reliability, discriminant validity, hypothesis testing, path coefficients, and model fit were utilized. The analysis revealed that financial security and risk aversion have a substantial effect on the intention to invest in equity products. A sense of financial security does not encourage an investor to participate in the stock market. Instead, it appears to be a barrier to participation in the capital market. This suggests that financial institutions may need to raise awareness of household financial insecurity or employ other creative communication strategies to increase stock market participation.

Keywords: Risk Attitudes, Risk Avoidance, Financial Well-Being, Stock Market Participation, Structural Equation Modeling, Equity Products, Financial Insecurity

1. INTRODUCTION

Behavioral finance is a combination of two disciplines, namely psychology and economics that focuses on the psychology of investors in relation to financial decisions. This combination explains why and how individuals make irrational financial decisions when saving, investing, spending, and borrowing (Belsky, G and Giolovich, T., 1999). It combines personal and social psychology principles with conventional finance theory to examine and emphasize stock market performance. Behavioral finance theory is based on how the thinking process and cognitive errors influence investor decisions and stock market prices (Mate, Rashmi M.

Effect of exogenous application of Sodium Nitroprusside (SNP) and Gibberellic Acid (GA₃) on growth and flowering of Dahlia (*Dahlia variabilis* L.) CV. Kenya

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ABSTRACT: Dahlias (*Dahlia variabilis*) are popular ornamental plants cultivated in many countries which characterized by the rich variety, different forms and attractive flower colors. The present investigation was conducted to study the effect of Sodium Nitroprusside and Gibberellic acid on the growth and flowering of Dahlia cv. Kenya. During two successive season 2021-22 and 2022-23. Different concentration viz. 100 ppm, 150 ppm and 200 ppm of SNP and GA₃ as a foliar spray were used. Result indicated that tallest plant (121.54 cm) was those sprayed with GA₃ at 200mgL⁻¹. Additionally, it showed that foliar application of GA₃ at 200mgL⁻¹ significantly increased the number of branches per plant (12.50), Plant Spread (98.72 cm), number of flowers per plant (15.00), stalk length (19.90 cm) and number of tubers (11.00) while maximum stem diameter (15.67 mm) and highest tuber yield per plant was recorded in GA₃ 100 ppm and GA₃ 150 ppm respectively. The most rapid flower bud initiation (50.21 days after planting) and days to 50% flowering (75.21 days) occurred when plants were sprayed with GA₃ at 200mgL⁻¹. SNP gives better result compared than control but GA₃ more improved the all attributes of dahlia plant. In conclusion, the vegetative flowering and yield characters of dahlia could be improved by application of either GA₃.

Keywords: *Dahlia variabilis*, GA₃, SNP, Morphological parameters, Foliar spray.

INTRODUCTION

Dahlia is a genus of flowering plants in the Asteraceae family, which includes other well-known plants such as sunflowers and daisies. They are native to the mountainous regions of Mexico, Central America, and Colombia (Pandey et al., 2017). Dahlias are renowned for their stunning and diverse range of flower shapes, sizes, and colors, making them popular choices among gardeners and florists worldwide. The flowers can be as small as a few centimeters or as large as dinner plates, and their colors span the entire spectrum from vibrant reds, oranges, and yellows to softer pastels, pinks, and whites (Khurfiwal et al., 2018). GA₃ (Gibberellic Acid) and SNP (Sodium Nitroprusside) play significant roles in influencing flower growth and yield in plants. GA₃, a natural plant hormone, promotes cell division, elongation, and reproductive development. Its application can lead to increased plant height, extended flowering periods, and enhanced flower production, thereby potentially boosting overall yield. However, it is crucial to apply GA₃ judiciously as excessive use may

result in adverse effects on flower quality and growth patterns (Elsadek, 2018). On the other hand, SNP acts as a nitric oxide releaser, regulating various physiological processes in plants. Nitric oxide plays a vital role in flower formation and development. When applied appropriately, SNP can positively impact flower growth and yield. It influences the expression of genes involved in flower development and reproductive processes, leading to improved flower quality, increased flower quantity per plant.

However, the research on dahlia is limited. Plant growth regulators (PGRs) refer to artificially synthesised organic chemicals employed for the purpose of altering the growth of entire plants or specific plant parts. The components of a plant. Photosynthesis is responsible for providing carbon and respiration energy to facilitate plant growth. However, plants also create a class of compounds called plant growth regulators, which play a crucial role in regulating the growth and development of plants. In minute concentrations, these chemicals exert an influence on several physiological processes in

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A Fake News Classification and Identification Model Based on Machine Learning Approach

Authors : Ashish Kumar, M. Izharul Hasan Ansari, Kshatrapal Singh

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Abstract

In the recent past the popularity of the social media platform has increased exponentially and at the same time various challenges have also been increased. One of the major challenges is related to fake news on social media platforms. It is really nontrivial task to filter and distinguish between fake and the real news. In this paper, various machine learning models have been applied to identify and examine the fake news on social media platforms. The Naive Bayes, Support Vector Machines, Passive Aggressive Classifier, Random Forest, BERT, LSTM, and Logistic

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Identify fixed American Sign Language by awareness of Convolutional Neural Network

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Abstract—American Sign-language is a non-artificial language which consists of the verbal characteristics as articulate languages, through grammar that vary from normal English. Sign Language is a pattern of interactive languages that connects with deaf and dumb people to the society. It is represented activity by hands and face. Common citizens are not well aware of the Sign Language. Due to this fact, there is a requirement of an interpreter to ease the conversation. This document will showcase the Convolution Neural network (CNN) replica for forecasting American Sign Language. More than 1000 pictures were recorded to train & instruct the replica. 95% accuracy of identification was achieved in experiment, which displays strong presentation in identification of 24 unchanged American Sign Language samples. Positive growth of this replica could be entertained and this is base to grow the high complex Sign-Language Interpreter.

Keywords : CNN, ASL, ML, NLP etc

I. INTRODUCTION

American Sign-Language (ASL) is an optical verbalization. With signing, the mind procedure semantic data taken by eyes. Sign languages are a worldwide language & every has their own way of understanding and teaching sign language. Similar to other verbal languages, this language has its own rules and grammar. Similar to other languages, ASL is the base language of the all-sign language which is growing and modifying from time to time [1]. According to the research data from the World Health Organization (WHO), In this survey millions of people of the globe are suffering from hearing disabilities. These disabilities can be incomplete or entire incapability to hear and respond back. Hearing

impairment may happen in one or both ears. If this occurs at an early age; it can affect their ability to learn and in verbalization disabilities or known as hearing muteness. An individual with hearing muteness is unable to express his views [2]. These differences separate these peoples from society.

Deaf and dumb individuals face a lot of legal challenges due to interacting barriers that are normally unrecognized by lawyers, society members, judges, policemen. The key question to ask is: What must be done to make an effective conversation in the legal setting? The NAD has won larger control in the legal system for deaf and dumb individuals. Police officers now get more training about the rights of deaf and dumb individuals [3].

It is necessary to continuously think for these people who require our extra concern so that they can have a normal life like you and me. A number of researches have been made to search out an inexpensive & suitable substitute [4]. Therefore, hand sign motions are recognized by vision systems, computing devices can be a theme of deep interest. With broad availability of smart devices such as cell phones including cameras, its approach is to design a computing-based sign language identification model. Backdrop disturbance, colors, and light differ inside the real time world. This results in a least observation rate. Moreover, the current revolution in augmented reality systems and artificial intelligence has represented a large advancement in image data identification [5], [6]. Traditional artificial intelligence depends on physical characteristics (like feature extraction). In place of erecting complicated handmade features, CNNs are capable of mechanizing the processing of feature identification. That



will greatly decrease the man made error and enhance the perception of accuracy rate.

This document will enlighten CNN in identifying the signs of American Sign Language. The CNN is constructed for identifying the data of images that were clicked on smart cameras. The scope is very large to unchanged character identification at this level. Excluding 2 alphabets that involve motion, a total of 24 movements were captured indicating different symbols. 95% rate was recorded for image detection.

Further the document is followed as: Section II contains the parallel task to this language. Section III contains the approach of CNN. Section IV covers the result of experiments that were being organized. At last, Section V contains the detection of this document and recommendations for the work to be done in future.

II. LITERATURE REVIEW

Pramada et.al. (2013) had been looking at image refining & framework of equivalent skill to notice the symbols of Binary Sign Interaction. This type of language symbolizes characters in the form of Binary coded language. All codes of binary are symbolized with fingers of one hand. The picture with normal surroundings is captive and changed into a monochrome image. Further comparison is done with the recorded order of model and presents its equivalent patterns in Text and sound format [7]. Vivek Bedha & N.Radpour proposed a Indepth Convolutional Neural Networks grouping algorithm of ASL for characters and numbers. General CNN model was taken up that consists of a maxpool layer and dropout layer. This system is trained from zero & gives 83% exact rate on confirmation of sign language identification model [8]. Garcia and Viesca put the concept of converting the ASL into characters. They take colored pictures of America Sign Language as guiding parameters for CNN. An upskill Google Net model was taken in this model. Because of alignment in major signs of language, Accuracy rate of 70% was obtained for all letters except J & Z. Further, the number of learning classes was reduced and a confirmation reliability of nearly 97.8% and 73.5% accuracy rate with 5 and 10 letters respectively was achieved [9]. For a hand-based sign language identification model, the provocation relies in continual tracing of hand area, segmenting fist pictures from the surrounding and operating gesture identification. Inequality and obstruction of the joints of the body will enhance the build complication to attain a satisfactory performance. Huang et.al. put a 3- the input captive method that be made up of colored images & body skeletal images captured by Microsoft Kinect. Instead of the conventional handmade feature extraction, he developed a 3D convolutional network for a mechanized part extraction. Maximum recorded rate for his work is up to

94.3% [10]. Moreover, hands based sign language is out the range of argument of this document.

III. PROPOSED METHODOLOGY

a) Statistical record

Making a handful of statistical records is a very well-known section in artificial intelligence. The task is only concerned about the American Sign Language action that includes all characters as shown in the Fig. 1. Characters J and Z were not counted as these letters include motion of hands. 24 gesture pictures were captured to build a dataset. More than 100 images were captured for each symbol or character. There were thousands of pictures, to decrease the training time - pictures were rescaled to 32x32.

American Sign Language Alphabet



Fig. 1 American Sign Language Characters

A total number of 180 recorded pictures were taken for the purpose of training and the rest 20 were taken for testing. Or we can say, 80% of the data collection was required for training purposes & 20% of the data was required to update the rate of perfection of this Sign Language identification model.

b) CNN Model

CNN is an artificial neural network; it is a deep and complete network. It is used for facial recognition, Image segmentation. Further it contains four types of components that are required to shape a CNN model eliminating input/output layers. These are coined as pooling, flattening, complexity layers and Fully Connected layers



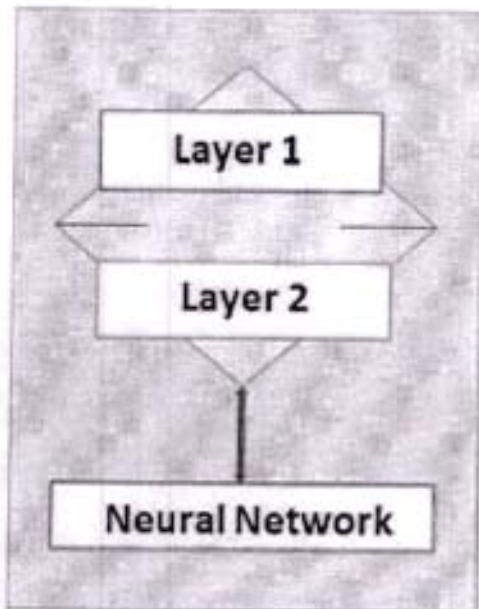


Fig.2- Architecture of CNN model

The Convolutional layer works on the factor extraction of the Images that are inserted. Otherwise, it is known as the filter for detecting image features like edges, axis, curves, pixels, and other image elements. Convolutional Neurons are used to perform Complex operations by examining each pixel of the image that was inserted and transfer the processed data to second layer. The Complex layer holds various filters to determine distinct factors.

Inside convolutional network second layer are representing as a max of pooling layer. Further when this layer is added to the model, it decreases the dimensions of pictures by deducting the pixel quantity in the result from the previous Convolutional layer. Example- Cutting the largest value from the image, and putting it into a duplicate, so that it destroys the remaining data that holds in the drive map.

Third layer is behave like a Flattening layer. Its efficacy is to changes all the collective images into a continuing vector with flattening. This layer is used for the conversion of the data into a 1D array.

Last layer, the combined Layer is the Neural Network to carry out the identification of the images based on the take out inputs from the convolutional layer.

c) Model Architecture

As one input layer, dropout and two cascaded convolutional layers with max pooling are contains in CNN model and also added flatterring layer in this model, Moreover in this model one fully connected layer associated with dropout and 1 output layer associated to SoftMax function.

Inside layer one have two cascaded convilutonal layers accordingly max polling layer. after that we have connected with flatterring layer for converting 3D to 1D arrays

The model of ASL system is broken into following parts such as; Image occupy, Preprocessing, Feature extraction, Feature matching and Pattern recognition.

d) Conception and Training

Demonstration was all organize on a machine with Intel Core i5-7300HQ, 8GB SDRAM and a NVIDIA GeForce GTX 1050. Python programming was used for implementing this model. Image Data and Dropout layer produces for data expansion were make use of keep way from overflowing.

The dataset expansion was applied in a real scenario on the heart of the system that is central processing unit during the time of training of dataset while the developed model was being tested and trained. These Image Data Generator for data enrichment contain of unknown flash into images, anyhow rotated images between 0° - 180° , shifting of the images was done horizontally on the basis of fraction of complete width and vertical shifting on the basis of fraction of complete height.

IV. EXPERIMENTAL OUTCOMES

The model training was carried out by Adam developer with SoftMax loss function. Epoch was set to the maximum range of 100 & training ratio was put at 0.003. During of training loss and accuracy plot are represented in Fig. 3. The chart represents the little loss through limited overflowing after that 100 epochs.

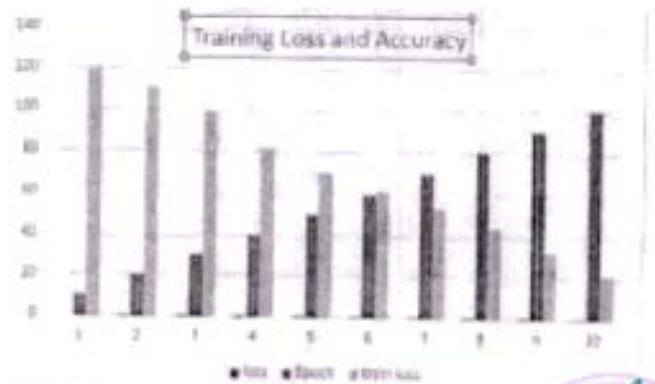


Fig. 3 Training Loss and Accuracy graph for ASL

Scale - * 1-10 = Loss/Accuracy
* 1-120 = Epoch

Handwritten signature and a circular stamp of Anna University, Chennai.

Table 1 represents the relation with older tasks. It is the process which records the maximum expectation rate on American Sign Language

Writers	Illustration	Reliability (exactness)
Sawant Pramada et.al. 2013	Picture Handling and instruction machine methodology on binary sign-language	90.98%
Vivek Bheda & N. Dianna Radpour 2017	Deep CNN on characters & numbers American Sign Language	82.5%
Garcia and Viesca 2016	ASL by CNN with Google Net transfer learning	70%
Lionel Pigou et.al. 2014	2 CNN's on Italian Sign Language To extract different postures/gestures using Kinect of Microsoft	91.7%
This work	CNN on all characters (except J & Z) ASL	95%

V. CONCLUSION

In which document, we experimented with a CNN model for identification of all characters in American Sign Language except J and Z. The experiment output displays, the suggested system is effective in forecasting unchanged characteristic gestures. After making a comparison with previous work, we get a good output. The task will be with real scenario video based sign language. So, what can you do with a sign language degree or training in sign language? There are numerous job opportunities where you can put your knowledge to good use.

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4



Sentiment analysis of Twitter data regarding the agnipath scheme of the defense forces

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ABSTRACT

Due to the popularity of social media today, people frequently share such criticism on Facebook, Twitter, Instagram, and other platforms. Therefore needs to know how your input from users of social media is generated in order to ascertain the public reaction to the policy that has been enacted. However, because of the comments, it is challenging to tell how many people have responded positive or negative. The objective of sentiment analysis of tweets is to provide insight into people's attitudes and perceptions regarding an event. This study illustrates the role of Twitter in the announcement of a new army vacancy through the "agnipath scheme" dubbed "agniveer". The result of this study can be used by the defense forces and government for decision making or policies related to the agnipath scheme. The study studied 4,000 English-language Twitter posts from July 3, 2022 to July 9, 2022. Manual text analysis revealed seven basic groups of tweet sentiments. The tweets' positive, negative, and neutral emotions were shown using orange data mining software, a powerful machine learning, data mining, and data visualization toolset. Result shows that agnipath scheme is mostly accepted by the people.

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1. INTRODUCTION

The fast growth of social media in recent years has made the internet a viable platform for carrying knowledge at little cost. Opinions on a product, company, or circumstance are being mined from comments left on blogs, reviews, postings, and tweets, among other social media outlets. Considerable weight is given to an individual's disposition and emotional state when drawing conclusions about that person's behaviour. Measuring how individuals feel about a topic is called sentiment analysis, also known as opinion mining. Organizations may learn about their customers' feelings and respond appropriately by employing sentiment analysis, a technique that allows us to read and understand people's emotions and place them into meaningful buckets. Detection of the appropriate language is necessary for some analyses (such as those that seek to determine the polarity or emotion of the text, receive feedback on a specific feature, or to conduct a cross-lingual analysis), while others (such as those that seek to determine the text's authenticity) are determined by the text itself. It needs a lot of data, some of which may not be in the right format [1]. As a result, the extracted data is put through a few preparation procedures before being utilised to build the final data set. Tweets put in by people all across the world in response to global events are a gold mine of authentic and



original data. Information posted on this forum is not filtered or gate kept in any way. This has made Twitter a go-to resource for social scientists and academics interested in studying the nuances of human emotion from a variety of angles [2]. Twitter is well-known as a backchannel tool, and in the wake of natural disasters, more people have used it to show their sadness.

Twitter analysis for #Make in India, #Digital India, and #Budget 2019, to learn more about public views on government program. The tweet categorization is implemented using the deep learning technologies of an LSTM (long short-term memory), artificial recurrent neural network. The primary goal of this kind of study is to examine public sentiment towards a government program to better administer that program in the future [3]. The Indian government launched a *agnipath* scheme for the appointment of troops in Army, Navy and Air-force on 14th June, 2022. We will call him *agniveer*. By undertaking qualitative and quantitative analyses of tweets, this study intends to identify the trends of tweets and the social support supplied on social media during the scheme's introduction by human actions.

The following are some of our contributions to this paper: i) this research proposes an effective method for analyzing the sentiments of Twitter data through the orange tool; ii) the collected corpus goes through pre-processing and analysis, and then sentiment analysis is done; and iii) experiments were done with a set of real tweets to show that our method is valid and can be used.

2. THE VIRTUAL WORLD: SOCIAL MEDIA

People in the world today talk to each other through the internet. It was only a dream 30 years ago to be able to say what you thought and have your voice be heard around the world in an instant. Now that social media has come along, it's a reality. In the world we live in now, it's hard to imagine what life would be like without social media. This is a growing trend caused by improvements in information technology.

People use the virtual world not only as a way to talk to each other, but also as a place to say what they think, feel, and believe about many different things. People here don't just talk to each other because they are related or know each other. They also talk to each other on social media because they have the same interests, hobbies, and specializations. Social media has millions of active users, and that number is expected to grow to 4.5 billion by 2022 [4], [5].

2.1. Twitter mining

Twitter mining is now a popular topic since it gives vital information that is utilized and applied in several sectors. It is one of the principal research fields. Various tweets can be gathered and analyzed for research purposes utilising accessible APIs. Twitter APIs are established using authenticated queries.

Twitter users come from many walks of life. Customers range from celebrities to ordinary citizens, business owners and representatives, legislators, and even national presidents and prime ministers [6]. Text analytics refers to the practice of mining information written or spoken in the English language for insights and patterns. Utilize natural language processing to do this text mining [7], [8]. To do this, text mining/analytics use natural language processing. Natural language processing contributes to text mining by assisting the machine in understanding the text and gleaning relevant data from it. Computer science and AI-based ideas are used by NLP to process human language, analyze information, and derive actionable insights. Text analytics is a process, while NLP is the technique used to carry it out.

2.2. Sentiment analysis of twits

In this work, we make an effort to understand the context of the tweets regarding the *agnipath* Scheme for joining the Indian Army that have been posted on Twitter so far. It is recognized that an amount of information can be gleaned from tweets; if the data is properly processed, it may be utilised for decision making and management. Twitter data may be mined for insights into the public's sentiments through opinion mining. This information is incredibly helpful for a variety of organizations and political parties. It is common knowledge that feelings, emotions, and sentiments play a key role in the human experience. Sentiment analysis is the process of extracting such viewpoints [9].

Sentiment analysis is the process of labeling a text's feelings to determine its polarity. It may be separated into three categories: document, sentence, and aspect. When we talk about categorizing thoughts at the document level, we mean doing so throughout the entirety of the content. All sentences are examined at the sentence level to establish their polarity, which can be either positive or negative; mixed opinions may or may not be acknowledged. Aspect level is also known as entity level, and in this context, all features of the phrases are taken into account. In what context does the sentence exist? The entity-level approach is a comprehensive but difficult to implement strategy [10].



3. REVIEW OF LITERATURE

Most users share their personal thoughts and information all the time these days, which has led to a rise in the amount of information shared through social media. This information is a great source for an analyst or researcher seeking vital information for decision-making [11]. Since the beginning of the 21st century, according to [11], sentiment analysis has been one of the most intriguing and active research subjects in the field of natural language processing. The act of analyzing a person's attitude or feeling based on their written words is known as sentiment analysis [12]. Researchers have recently demonstrated new insights on this type of data and been able to categorize feelings [13]. Several methods have been proposed in the literature for doing sentiment analysis, which entails extracting lexical sentiments along with the documents [14], how bi- and tri-grams may be used to deduce an opinion [15]. Since emotions are now a prevalent way to describe sentiments, emojis may be used to represent both positive and negative emotions. It has been noticed that internet resources can offer emotional and social support [16]. Twitter, a micro blogging service, is one of these online platforms that allows users to submit real-time messages, or "tweets." Twitter has become a vital part of the routine of modern society since it provides a robust public forum where individuals may freely discuss their thoughts, ideas, and opinions on a variety of themes. Numerous studies have examined Twitter as a possible method for sharing information, gauging public opinion, and expressing emotions.

By studying tweets on the death of Michael Jackson, the king of pop, we can analyse the crucial role micro blogs play in offering emotional and social support. The findings suggest that Twitter is utilised as an informative support channel [17]. In addition to grief, other kinds of emotions, such as rumor-spreading, statements of hate, and spam, are seen to account for a significant fraction of tweets. Chen *et al.* [18] evaluated the polarity of tweets using sentiment analysis to predict future crimes. Using a machine learning technique [19], conducted a sentiment analysis on the Twitter data of the 2014 FIFA World Cup Soccer tournament. Aharony [20] evaluates Twitter as a channel for communication to determine how three political leaders-the prime minister of Israel, Benjamin Netanyahu, the prime minister of Britain, David Cameron, and the president of the United States, Barack Obama-communicate via Twitter. The statistics indicate that the president of the United States utilises Twitter more than the other two world leaders. However, all three leaders utilize twitter for outreach and openness. Goel and Mittal [21] used Twitter data, self-organizing self-fuzzy neural networks (SOFNN), and Dow Jones Industrial Average (DJIA) values to forecast financial markets. Twitter has also been examined for the dissemination of information during natural or manmade catastrophes, plights, and exigencies. During times of crisis, it is viewed as legitimate by netizens. It has been widely used to comprehend the emotions of people during and after disasters such as "Typhoon Haiyan in the Philippines" [22]. The goal of this study is to investigate and assess public emotions towards COVID-19 vaccinations across Twitter messages (positive, neutral, and negative) as well as the influence tweets have on digital social circles [23].

4. METHOD

This work has been developed by using five phases: i) tweet extraction; ii) tweet content analysis and classification; iii) preprocessing techniques; iv) emotion collection; and v) polarity identification and classification. Each step are explained in detail:

4.1. Tweets extraction

Tweets are required to complete the sentiment analysis task. Here, we retrieved tweets from the Twitter API. We must first develop a Twitter application to obtain API access. The Twitter application may be built with the Twitter app, the consumer key and access token are made available to the user once the app has been built. Both the access key and the consumer secret must remain hidden. Over 4,000 tweets related to the #agnipath scheme were collected and are now available for research.

4.2. Tweet content analysis and classification

The study utilised a naturalistic methodology to the feelings of the tweets and decide the tweet format (i.e., text, picture, or video). Initially, 150 tweets were picked at random and examined based on their sentiment features. Each tweet was thoroughly read and comprehended so that its underlying tone could be recorded. For example, after careful consideration, the tweet "agniveer scheme is a good initiative step for youth to join our country's defense system" was classified as "evishing emotions," whereas another tweet asking, "If this is true, why agniveer?" was classified as "raising questions". "Why should youth have temporary jobs?" was filed under criticism. Consequently, the tweets were classified based on the subject context they included. The attitudes of the 150 tweets were determined by manually analyzing their content. The tweets were classified into seven sentiment categories see in Table 1. This method assisted in identifying the event's numerous themes. The retrieved tweets' major themes and issues were thoroughly studied. For



the development of a theme/emotion, an inductive method was adopted. When a tweet does not fit into an existing category, a new category was created. Human inspection was used to determine the opinions expressed in all 4,000 tweets. We analyzed the 4,000 tweets and classified them into seven broad categories based on the attitudes expressed in each tweet (both those from prior studies and our own) see in Table 1 [1].

Table 1. Structured coding

Category	Definition
Evincing emotions	Share your happiness, your sadness, your anguish, your compassion, and any other negative or positive emotions you have towards the agnipath scheme applicant.
Raising question	People with inquiries about the agnipath scheme and its environs.
Criticism of the government	Government and its policies and flaws are criticised.
Assisting the government	Post in favour of the BJP government.
Increasing dissent	People are voicing objections to the agnipath project.
Distribution of news and data	Share news articles, information, and other relevant items pertaining to the agnipath scheme.
Irrelevant	Posts that have nothing to do with the event.

4.3. Preprocessing technique

This creates a pipeline for text pre-processing. It enables data transformation, tokenization, filtering, and stemming. Transform, will maintain lower case in all tweets, removing accents, parse HTML, and removing URLs: i) transform will keep all tweets in lower case, get rid of accents, parse HTML, and get rid of URLs; ii) filtering, the removal of mention (@), hashtag (#), and RT (retweet) from tweets; and iii) stemming, is the process of removing affixes from root words and changing them into their simplest form.

To create a word cloud in the orange data mining tool, we must first connect the file widget to the corpus widget, and then the corpus widget to the preprocess text widget, where data transformation, tokenization, filtering, and stemming will take place. The preprocessed text will then be linked to a word cloud widget, which will visualize the word cloud. Figure 1 shows the workflow of a word cloud in the orange data mining tool.

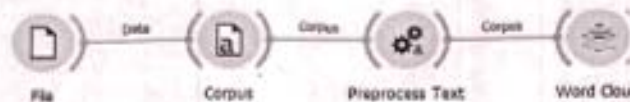


Figure 1. Workflow of word cloud

Jayashankar and Sridaran [24] proposed word clouds or tag clouds as the graphical depiction of words for a certain piece of written content, organized according to their frequency. Figure 2 represents the graphical representations of text data (word cloud) of #agnipath scheme. Figure 2(a) represents the word cloud before the preprocessing, and Figure 2(b) represents the word cloud after the processing.



Figure 2. Word cloud of Twitter data on agnipath scheme (a) word cloud before preprocessing and (b) word cloud after preprocessing

4.4. Collection of emotion

Emotions are complex psychological states that include a subjective experience, a physiological response, and a behavioural or expressive response. On the basis of facial expression, there are six emotion categories commonly used to characterize the fundamental human emotions: anger, contempt, fear, happiness, sorrow, and surprise. "Surprise" is the most ambiguous, as it may be connected with both good and negative emotions [25]. In orange data mining tool we put preprocess data into tweet profiler widget, where we used Ekam (multiclass) algorithm for emotion count. Through select column widget we only select emotion and connect with distributions widget for displaying the emotion through bar chart. Figure 3 shows the workflow of how to calculate emotion in orange data mining tool. Figure 4 depicts emotional value (anger=16, disgust=26, fear=473, joy=2721, sadness=61, and surprise=703) for the #agnipath scheme between July 3rd and July 9th, 2022.

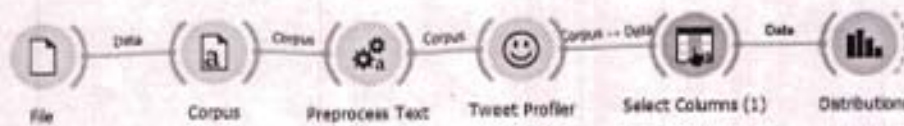


Figure 3. Workflow for calculation of emotion

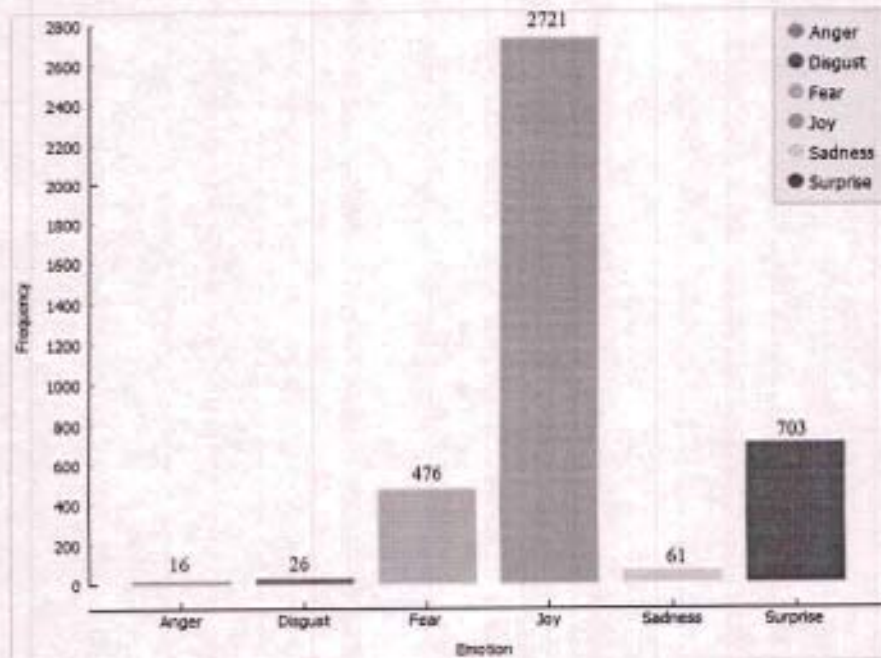


Figure 4. Emotion class bar diagram for #agnipath scheme

4.5. Polarity identification and classification

Polarity is the emotional condition that determines whether a sentence is positive or negative. Orientation, often known as polarity, is the emotion represented in the statement. A text can be categorized as either positive, negative, neutral, and compound. Depending on the comparison with positive and negative terms, it is simple to determine a tweet's score. Figure 5 shows the workflow of polarity class. Orange data mining tool provide six types of tool for sentiment analysis which incorporate within sentiment analysis widget. The tools are Liu-hu, valence aware dictionary and sentiment reasoner (VADER), Multilingual, SentiArt, LilaH, and custom dictionary. We used VADER tool. It is a social media-specific lexicon and rule-based sentiment analysis tool. Sentiment analysis in VADER is grounded in a vocabulary that translates word meanings into numerical representations of how strongly people feel about certain topics. The overall tone of a document may be determined by tallying up the weight of its individual words.





Figure 5. Workflow of polarity class (sentiment analysis)

Figure 6 depicts average sentiment score of 4,000 tweets regarding agnipath scheme. We can observe through graph that neutral sentiment has maximum number (3456.68), compound sentiment (503.19), positive (376.28), and negative (167.01). If we calculate the percentage of the polarity analysis, we get neutral=86.41%, compound=12.57%, positive=9.40%, negative=4.17 % sentiment of agnipath scheme.

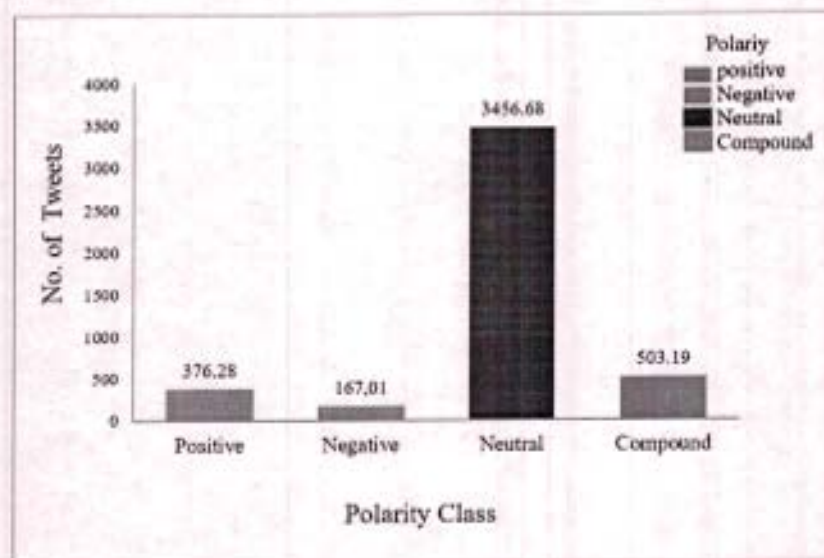


Figure 6. Sentiment score of 4,000 tweets about # agnipath scheme

5. CONCLUSION AND FUTURE SCOPE

The methodology employed in this article was sound and methodical. Before doing sentiment analysis on the tweets, the text was analyzed. The data set utilized in the study is primary and of sufficient quantity. The selection of the tools in this work was based on a comprehensive literature research. We know that a tweet doesn't have a set format; the only rule is that the text can't be too long. Since the tweets came from the Internet, there were no labels showing how people felt about them. The lack of labels for how people felt and the noise in the tweets made the paper harder to understand.

Using the orange data mining tool, a novel method was presented in this article for doing sentiment analysis on a linguistic dataset. The method was described in detail. The result shows that most of the people express joy about the agnipath scheme. and the surprised people comes under the second highest category. Result shows that agnipath scheme is mostly accepted by the people. This means that was not rejected as much as the media was showing the rejection of this scheme.

This study, notwithstanding its merits, has several shortcomings. First, the suggested method can only process tweets in the English language. This is a disadvantage due to the enormous volume of tweets regarding agnipath Scheme available in other languages. We intend to apply this approach to further languages, like Malayalam and Kannad. In addition, our dataset is rather small (7-day period) and restricted to a particular use case. This raises the question of the generalizability of our findings. We intend to collect further information on this occurrence. Furthermore, our present research is restricted to the textual tweets data received from Twitter. In the future, new data such as images, sounds, internet articles, and other forms of multimedia may be contributed to this data collection.

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Social Media as a Marketing Tool: A Case Study of Nestle India Ltd.

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ABSTRACT

The world has become a global village, which is because communication has become a lot stronger than it used to be through different digital platforms. There are so many mediums to communicate with the world now, and many keep evolving; it is hard to put a number to them. Social media is a powerful way for businesses of all sizes to reach prospects and customers. People discover, learn about, follow, and shop from brands on social media. Nestle in India is the prominent Brand to evolve itself as a best 'Social media Marketer' and leveraged the same opportunity. This paper demonstrates the role of social media in marketing of Nestle. Furthermore, this paper highlights the 'Social Media' strategies of Nestle in India as its revolutionary emergence and strong foothold in the Indian market by consistently offering the FMCG products. The study is based on Secondary & descriptive research design which is qualitative in nature.

Key Words: - Social media, Social media marketing, Marketing strategies, Nestle India.

1. INTRODUCTION

Internet, social media, mobile apps, and other digital communications technologies have become part of everyday life for billions of people around the world. Information technology develops rapidly and influences the behavior of many people. Everyone is dependent on the internet to connect with other people and to find the information quickly. This makes smartphones a necessity in their daily activities. Several applications such as online shopping, online transportation, and social media can be downloaded by smartphone users. Among many applications, social media is the most downloaded application. Social media becomes increasingly popular and attractive to be adapted by many business actors due to its low costs and minimum technical requirements. Since the beginning of the 21st century, the use of internet and social media has become a part of business strategy.

For business owners, social is a mean of two-way communication with consumers, to listen to consumer responses and to find out what they want. Companies use social media to gain benefits such as outperforming competitors, lowering costs, managing the operations more effectively, improving relationships with consumers, and improving company image.

In a country like India where there is so much competition in the industry already, Nestle has come a long way and has given its counterparts a run for their money.

This case study will examine how this mega-corporation in the Indian market has maintained a unique brand image for itself through innovative marketing campaigns and strategy that has created a strong brand connection among consumers even in this increasingly digitized world.

2. BRIEF LITERATURE REVIEW

Kaplan and Haenlein [2010], social media is "a group of internet-based applications that is build on the ideological and technological foundations of Web 2.0 and allow the creation and exchange of User-Generated Content".

Strauss and Frost [2012], social media is defined as "online tools and platforms that allow internet users to collaborate on content, share insights and experiences, and connect for business or pleasure".

Larson and Watson [2011], who defined social media as the set of connectivity enabled applications that facilitate interaction and the co creation, exchange, and publication of information among firms and their networked communities of customers.

Wong [2012] in his research found that social media adoption has a positive impact on business performance.

Trainor et al. [2014] stated that the intensity of social-media use in business can have a positive and significant effect on business performance. The company is able to adapt to market needs and interact with customers more efficiently resulting in an increase in sales volume.

Research by Rienda, Fernandez, and Carey [2020] stated that there is a significant influence of social media adoption on business performance.



3. OBJECTIVES

- To understand the 'Social media marketing' strategies of Nestle India.
- To analyze role digital marketing campaigns of Nestle India.

4. RESEARCH METHODOLOGY

This study is based on descriptive research design which is qualitative in nature. The secondary data related to the specific keywords like 'Social media Marketing', 'Nestle India Ltd.', 'Social media as a Marketing', 'marketing strategies' etc. have been explored through the google search. Eventually, the online content and research papers (reputed journals) content is synthesized to arrive at the desired results and conclusion. The analysis has been divided into two parts as the first part is related to the conceptual understanding of 'Social media Marketing' and later one is related to digital marketing campaigns of Nestle India.

5. DATA ANALYSIS & FINDINGS

5.1 Social media marketing

5.1.1 Nestle Social media Marketing :

Get to know the brand identity behind Nestlé

Nestlé is a multinational food and drink processing corporation that is based in Switzerland. It is the biggest food company in the world, measured by revenue. According to its LinkedIn profile, the company has around 273,000 employees and factories or operations in almost every country in the world. Its main products include baby food, bottled water, breakfast cereals, coffee, teas, confectionery, dairy products, ice cream, frozen food, pet foods, and snacks. Its most well-known brands are Nespresso, Nescafé, KitKat, Smurty's, Nesquik, Stouffers, Vittel, and Maggi.

As the world's largest food and Beverage Company, Nestlé's marketing strategy has always been one step ahead. And they understood that the key behind digital success is creating value for customers.

In 2010, Nestlé went through a PR and social media catastrophe that was on everyone's lips at the time. But they didn't give up. They worked so hard on their social media strategy that the brand remained a major player in the food and beverage industry.

They managed to do that through a unified marketing strategy and a strong brand positioning. A significant role in this plays the logo and the slogan, which are easily recognizable.

Nestlé's social media channels

Nestle India has changed its marketing strategies according to the population, expectations, and lookout. Unlike other brands, it didn't wait for any golden opportunity to enter the social media market. Instead, it created its niche and came out from the bottleneck regions to become successful in its digital marketing strategies. From opening business profiles across social media channels to promoting their products and services through influencers, Nestle India has made a firm presence in the digital market.

According to the current statistics, Nestle India has approximately 95 profiles on various social media channels, like 36 Facebook profiles, six Instagram profiles, and 21 Twitter profiles. Due to such a remarkable variation, it has been able to influence the youth and teenagers of the country to try its products, put their faith in the brand, and maintain the online reputation at its optimal level.

- **Nestlé on Facebook**

With 11,624,391 followers, Facebook is by far the biggest social channel of Nestlé. One of the brand's objectives as part of their social media strategy was to improve their social channels, especially Facebook. And they succeeded! Nestlé shares posts related to what the brand is currently up to, its ongoing campaigns, and new product releases on this social platform. The brand also uses this channel to talk about its corporate social responsibility measures. Using an analytics tool like Social insider, you can evaluate a brand's performance on each social media channel.

- **Nestlé on Instagram**

Nestlé has 306,741 followers on Instagram and an engagement of 12K. The brand gained 3,011 new followers in the past six months, indicating that this platform's social media strategy pays off. Like Facebook, Nestlé's Instagram page is designed to announce campaigns, new products, or even competitions. Having separate social media profiles is very smart because it can help a brand organize its marketing campaigns more effectively and target the right audiences. Interestingly, even though Nestlé has many more followers on Facebook, the average engagement rate per post on Instagram was 0.169% in the past six months, compared to Facebook, where the average engagement rate per post was zero. So if you needed another confirmation, there you have it - people tend to engage more with a brand on Instagram than on Facebook.

- **Nestle on Twitter**



Nestle has also penetrated the Twitter market, with approximately 26.7 K followers. This number is something to be addressed and has yet to be generated overnight period with appropriate social media marketing strategies, compelling posts, advertisements about various campaigns, and product launches. So on, the brand has established a strong presence on Twitter. Its average interaction rate per tweet is approximately 0.009%, which is relatively higher than other brands in the FMCG market.

- **Nestle on YouTube**

Whether through in-video or pop-up ads, Nestle has used the YouTube channel, one of the best entertainment platforms in the world, to promote its products and services and maintain a cordial relationship with its existing and new customers. Although the success rate of YouTube channels is less than other social media channels, the growth is consistent and steady.

5.1.2 Nestle Digital marketing campaigns :

Nestle has rolled out several campaigns all these years but here are the few marketing campaigns that have left a lasting impression on the Indian audience. According to Nestle, this campaign is built around the environmental threats of climate change. As a big corporation, the brand is committed to the cause, declaring to take actions in regeneration and thus helping the environment.

A Campaign for the Youth: Karne Se Hee Hona Hai

The Covid-19 pandemic has changed a lot of things for everyone, especially the millennials who were accustomed to doing certain things in a certain way.

This campaign by Nescafe was launched in July 2020 as India was preparing to come out of its series of lockdowns and begin life in the "new normal".

Nescafe, in the above advertisement, encouraged the youth of the country to dream, act, and achieve their life goals.

And in the process, Nestle also highlighted to millennials that "It all starts with a Nescafe". Thus promoting Nescafe and once again establishing that Nescafe plays a vital role in keeping the youth alive and fresh.

A Caring Campaign: Poora Poshan Poori Tasalli

Poora Poshan Poori Tasalli: This campaign was initiated by Nestle Ceregrow in 2019 targeting urban couples who had children between the age of 2-5 years.

In a country like India where parents pay a lot of attention to their child's health and proper nourishment right away from the child's birth.

Nestle very smartly portrayed how Indian mothers are worried about their child's proper nourishment. The brand showcased its product and communicated that Ceregrow not only fulfils the child's hunger but also provides the right kind of nutrients for the child's immunity and overall development and nourishment.

You can watch this short 45-second video by Nestle Ceregrow which delivers the message beautifully and convinces urban parents why Ceregrow is a must-have for their growing toddler.

A Campaign for Maggi lovers: Meri Maggi

Meri Maggi has been one of the most successful mass campaigns led by any brand in India. The Meri Maggi campaign started with the motive to promote Maggi as a snack.

In this campaign, Nestle also encouraged its consumers to personalize Maggi as per their wants and taste and share it on social media pages to get featured on Maggi's official Facebook page.

Nestle started posting pictures of Maggi with different captions to make it relatable for different sets of audiences. Be it a Pyjama Party or a Break after Long Lectures in the college, Maggi made sure it relates to everyone and at the same time creates a strong brand engagement.

6. FINDINGS

- Increase brand awareness
- Build engaged communities
- Sell products and services
- Drive traffic
- Measure brand sentiment
- Provide social customer service
- Advertise products and services to target audiences





- Track performance and adjust larger marketing strategies accordingly
- Direct Contact With Target Audience

7. CONCLUSION

It can be concluded that using social media in marketing does more than improve site traffic and increase your reach. It turns your business into a personality that your audience can communicate and connect with on a deeper level. Regardless of which platforms you use or how you use them, the most important thing to remember is that social media is not a platform to pitch your business.

For India, Nestle chose an emotional and inspiring image to go with, which has played very well for the company. Its consistent efforts in social media marketing and advertising are equally commendable and are one of the biggest hurdles for competitors to overthrow Nestle. Though there is rising competition, Nestle has remained on top for a long.

Over the years, Nestle has proven itself as a leader in the food and beverage industry with product innovation and innovative social media marketing strategies. It creates campaigns that are memorable, relatable, and share-worthy. As it is moving toward developing a solid presence in the future, digital marketing will play an essential role in the future growth of Nestle.

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Applying statistical approach to check the consistency of pairwise comparison matrices during software requirements prioritization process

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Abstract In the field of software engineering, multi-criteria decision making (MCDM) methods have been applied during software requirements prioritization (SRP) process. Among various MCDM methods, analytic hierarchy process is a desired technique to compute the ranking values of the software requirements (SRs). These ranking values are employed to choose the SRs that would be executed during different releases of the software. During SRP, stakeholders specify their preferences on SRs in a matrix, which is known as pairwise comparison matrix (PCM). This matrix is used to compute the ranking values of the SRs. The ranking values of the SRs would be reliable only when the PCMs are consistent. Based on our literature review, we identify that during SRP process less attention is given to statistical based approaches to examine the consistency of PCM. Therefore, to address this issue we proposed a method for SRP which is pliable and genuine because it sets a relevant significance level according to the size of

the PCMs. Finally, the proposed method is discussed by considering the SRs of an institute examination system.

Keywords Analytic hierarchy process · Pairwise comparison matrices · Software requirements selection and prioritization · Statistical approach

1 Introduction

The aim of software requirements prioritization (SRP) process is to compute the ranking values (RVs) of software requirements (SRs) so that it can be selected from the list of the requirements in keeping with consensus of the distinct types of the stakeholders during software development process (Carpertz and Ahmad 2010; Mohammad et al. 2018). Different stakeholders are involved in SRP process; and each stakeholder has different opinion about the SRs. The opinion of stakeholders varies according to their criteria, for example, some stakeholders wants to implement SRs according to their preferences, on the other hand, other stakeholder's focuses on *security*. Here, *preferences* and *security* are two different criteria that must be satisfied during the SRP (Misaghian and Motameni 2018; Sadiq 2017). Prioritization of SRs under many criteria creates a "multi-criteria decision making" (MCDM) problem (Sadiq and Jain 2015). In the literature of MCDM methods, several methods have been proposed for the selection and prioritization of the alternatives; among these methods, analytic hierarchy process (AHP) has been proved to be useful and trustworthy for SRP and "software requirements selection" (SRS) (Karlsson et al.1998). Therefore, it motivates us to choose the AHP as a tool for the solution of the SRP problem. In AHP, consistency ratio is used to

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examine whether the PCM is consistent or not. Consistency of a PCM can be defined as follows (Saaty 1977, 1980):

- A PCM $P = (p_{ij})_{n \times n}$ is said to positive reciprocal if $p_{ij} > 0, p_{ii} = 1, p_{ij} = \frac{1}{p_{ji}}$ for all $i, j \in \{1, 2, \dots, n\}$.
- A positive reciprocal matrix $P = (p_{ij})_{n \times n}$ is said to be perfectly consistent if $p_{ij} = p_{ik}p_{kj}$ for all $i, j, k \in \{1, 2, \dots, n\}$.

The reliable RVs of SRs can be obtained only when the consistent PCM is given. It is troublesome to obtain the high order perfectly consistent PCM due to the nature of human thinking process. A PCM is perfectly consistent, if and only if, $\lambda_{max} \geq n$, where λ_{max} is the principal eigenvalue of a PCM. The consistency index (CI) of a PCM is given by Saaty (1977, 1980):

$$CI = \frac{\lambda_{max} - n}{n - 1} \quad (1)$$

where n is the number of software requirement (SRs) of system. The consistency ratio (CR) is determined by:

$$CR = \frac{CI}{RI} \quad (2)$$

Equation (2), RI is the "average random index". For different values of n , i.e., from 3 to 15, the values of RI is exhibited in Table 1 (Saaty 1977, 1980).

A PCM would be consistent only when $\lambda_{max} = n$. It means that the value of CI will be zero, i.e., $CI = 0$. If $CR < 0.1$, the PCM is said to be acceptable consistent, otherwise, the PCM should be reconstructed until $CR < 0.1$, where 0.1 is the consistency threshold ratio, which is also called Saaty threshold. This threshold value was castigated and refuted in some aspects (Banae and Vansnick 2008; Kwiesielewicz and Van 2004; Karapetrovic and Rosenbloom 1999). For example, it allows "contradictory judgements in PCM" or "it rejects the reasonable PCM". Therefore, to overcome this problem Saaty (1980) revised the value of the CR (0.1); and proposed some new CR threshold values based on the order of the PCM. The revised CR threshold for PCM of order $3 \times 3, 4 \times 4$, and above 4 is 0.05, 0.08, and 0.1, respectively.

Following approaches have been developed to measure the level of the consistency of the PCM. For example, (a) row geometric mean, (b) geometric consistency index, and (c) harmonic consistency index (Crawford and Williams 1985; Aguaron and Morento-Jimenez 2003; Stein and Mizzi 2007). Few methods have been developed to elicit the contradictory judgements and improve them using

transitivity rule (Ishizaka and Lusti 2004; Wang et al. 2009). For Saaty's threshold value (0.1), the statistical interpretation was given by Jong (1984). Statistical approach to Saaty's scaling method for priorities was proposed by Vargas (1982). Statistical approaches were used in Vargas (2008) to compare the CIs. The work of Alonso and Lamata (2004) focused on the estimation of RI. In another study, Alonso and Lamata (2006) suggested the statistical criterion in random matrices. Uncertain judgment estimations of the consistency were studied in Ergu et al. (2011). Based on our literature review, we identify that in SRP process less attention is given to deal with the contradictory judgements in PCM and to avoid the rejection of the reasonable PCM. Therefore, to address this issue, in this paper we proposed a method for the prioritization of SRs in which statistical approach has been used to check the consistency of PCM.

This paper is structured as follows: Sect. 2 presents the related work. Proposed method for the prioritization of SR is explained in Sect. 3. A case study is given in Sect. 4 to explain the steps of the proposed method. Finally, the conclusion and future work are given in Sect. 5.

2 Related work

AHP was proposed by Thomas L. Saaty in 1970 to rank the alternatives or to select the alternatives from the set of alternatives (Saaty 1980). AHP has been applied in the following areas for the selection and prioritization of the alternatives like "software engineering", "management science", "economic analysis", "regional planning and forecasting", etc. In software engineering, alternatives are referred to as SRs (Sadiq and Jain 2015). Several methods have been proposed in the area of SRP to compute the RVs of the SRs using AHP. For example, Achimugu et al. (2014) developed a web based tool to rank the SRs from any geographical location at run time. In their tool, AHP was used to compute the ranking values of the SRs. Fellir et al. (2014) used AHP for the prioritization of the system requirements. Busetta et al. (2017) developed a tool supported gamified collaborative requirements prioritization method in which AHP was employed to select the SRs. Sadiq et al. (2010) considered the AHP for the prioritization of the SRs. In another study, Sadiq et al. (2009) developed a method for the elicitation of SRs and their prioritization using AHP. In addition to this, AHP method has also been applied in the context of agile methods (Sayed et al. 2017).

Table 1 Random indices for different values of n

n	3	4	5	6	7	8	9	10	11	12	13	14	15
RI	0.52	0.89	1.12	1.26	1.36	1.41	1.46	1.49	1.52	1.54	1.56	1.58	1.59



software testing (Sadiq and Sultana 2015; Sadiq and Firoze 2014), software development life cycle (Khan et al. 2014), etc. Garg et al. (2017) developed a "goal oriented approach for SRs elicitation and prioritization using AHP", i.e., *GOASREP*. In another study, Sadiq et al. (2017) applied AHP in "goal oriented requirements elicitation method for the prioritization of SRs"; and they called it *AHP_GOR-E_PSR* method. Sadiq and Afrin (2017) generated the different patterns of the PCM during SRS process to check whether the PCM is consistent or not. Their work was extended by Sadiq et al. (2018) in which they have generated 8 patterns and 512 sub-patterns of PCM and compute the CR of each PCM. In their work, authors maintain a database which contains the information about each PCM and their CR. Based on our review, we found that AHP has been widely used in SRP methods to compute the RVs of the SRs; and in these methods most of the focus is to get the prioritized order of the SRs without discussing the consistency of the PCM of different sizes. In these methods, no systematic approach has been applied to check the consistency of PCM. In practical situations it has been observed that without constructing the consistent PCM, it is not possible to get the reliable ranking values of the SRs (Sadiq and Afrin 2017; Sadiq et al. 2018). Therefore, to address this issue in this paper we proposed a method for the prioritization of the SRs when the PCMs are consistent. To check the consistency of PCM, statistical approach proposed by Lin et al. (2013) has been used in the proposed method. The contributions of the paper are given as below:

- An algorithm has been developed to rank the SRs only when the PCMs are consistent
- Following requirements elicitation techniques have been used, i.e., the survey, interviews, and goal oriented techniques, to elicit the SRs
- Proposed method has been applied by considering the SRs of Institute Examination System (IES)

3 Proposed method

In this paper, we proposed a methodology for the prioritization of SRs when the PCMs are consistent. The steps of the proposed method are given below:

- Step 1: Identification of the stakeholders
- Step 2: Elicitation of SRs
- Step 3: Construction of the PCM during group decision making process
- Step 4: Check the consistency of PCM using statistical method
- Step 5: Computation of the RVs of SRs
- Step 6: Selection of SRs

Step 1: Identification of the stakeholders

Different types of the stakeholders are involved in the development of software projects (Ballejos and Montagna, 2008). Identification and selection of the stakeholders according to the need of the project have great impact on the quality of the SRs; and consequently on the software project (Pacheco and Garcia, 2012). Lack of stakeholders during the software development process may lead to the failure of the software product because stakeholders are the main source of the SRs (Sadiq 2017). Therefore, for the successful development of the software project all the stakeholders must be identified before the starting of the SRs elicitation process. Stakeholder in any project is the "group or individuals who can affect or is affected by the achievement of the organization" (Freeman 2010; Susniene and Purvinis 2015). Two-step process has been used to identify the stakeholders, i.e., (i) specify stakeholder's types and roles (ii) select stakeholders based on types and roles (Sadiq 2017).

Step 2: Elicitation of SRs

Once the stakeholders of a project have been identified then the next step is to elicit the SRs using survey, interview and "goal oriented requirements elicitation technique" (GORET). We have used GORET because it presents the visual representations of the different types of the SRs very clearly in front of the stakeholders; and support for the early requirements analysis. GORET provide an efficient way to communicate requirements to customers (Lapouchnian 2005; Liaskos et al. 2012). In GORET, the high level objective (goal) of an organization or a client is decomposed and refined into sub-goals. The sub-goals are further decomposed and refined into sub-sub-goals. The process of decomposition and refinement of sub-goals is continued until the responsibilities of the last sub-goals are assigned to some agent or system (Lapouchnian 2005; Liaskos et al. 2012).

Step 3: Construction of the PCM during group decision making process

PCM is the key step of AHP which is used to capture the preference of SRs over another requirement. PCMs are constructed after evaluating the SRs on the basis of different criteria during group decision (GDM) process. In GDM, several stakeholders participate to elicit the SRs, and also to specify the preferences of one SR over another SR so that PCM for each and every requirement can be constructed.

Step 4: Check the consistency of PCM using statistical method

A pairwise comparison matrix (PCM) P is perfectly consistent, if there is a principal priority vector $W = (w_1, w_2, \dots, w_n)^T$ with $\sum_{i=1}^n w_i = 1$ and $w_i \geq 0$, such that $p_{ij} =$

$\frac{w_i}{w_j}$ for all $i, j \in \{1, 2, \dots, n\}$, if P is not perfectly consistent then there exist a deviation matrix (B):

$$B = (b_{ij})_{n \times n} = \left(p_{ij} - \frac{w_i}{w_j} \right)_{n \times n} \quad (3)$$

where p_{ij} represents dominance of SR i over j ; w_i and w_j are the priorities of SR i and j , respectively. For perfectly consistent P , the additive error $b_{ij} = 0$ for all $i, j \in \{1, 2, \dots, n\}$.

During the GDM process, decision makers often have uncertain tendency in constructing the PCM (Jong 1984). Here, it is assumed that b_{ij} ($i < j$) are normally distributed with mean zero and mutually independent random variables.

$$\text{Let } \delta_{ij} = \frac{b_{ij}}{\frac{w_i}{w_j}} = \frac{p_{ij} - \frac{w_i}{w_j}}{\frac{w_i}{w_j}} = \frac{p_{ij} w_j}{w_i} - 1 \quad (4)$$

Based on the property of the normal distribution variable, the δ_{ij} ($i < j$) are independent normally distributed with mean zero. Let us assume that all variances of δ_{ij} are σ^2 . So, δ_{ij} follow normal distribution $N(0, \sigma^2)$. Therefore, the value of the λ_{\max} can be computed as Lin et al. (2013):

$$\lambda_{\max} = \frac{1}{n} \sum_{i=1}^n \left(\frac{1}{w_i} \sum_{j=1}^n p_{ij} w_j \right) = \frac{1}{n} \sum_{i=1}^n \sum_{j=1}^n \frac{1}{w_i} p_{ij} w_j \quad (5)$$

We know that $\delta_{ij} = \frac{1}{\delta_j} - 1$; so $\frac{b_{ij} w_j}{w_i} = \delta_{ij} + 1$. Now Eq. (5) can be written as:

$$\lambda_{\max} = \frac{1}{n} \sum_{i=1}^n \sum_{j=1}^n (\delta_{ij} + 1) \quad (6)$$

After simplification of Eq. (6), we have

$$\lambda_{\max} = 1 + \frac{1}{n} \sum_{1 \leq i < j \leq n} \left(\delta_{ij} + \frac{1}{\delta_{ij}} \right) \quad (7)$$

Now the value of CI can be computed as:

$$CI = \frac{\lambda_{\max} - n}{n - 1} = \frac{1}{n(n-1)} \sum_{1 \leq i < j \leq n} \frac{\delta_{ij}^2}{1 + \delta_{ij}} \approx \frac{1}{n(n-1)} \sum_{1 \leq i < j \leq n} \delta_{ij}^2 \quad (8)$$

In the proposed method, the Chi-Squared test statistics has been used to test the consistency of the PCM P . If $\sigma^2 = \sigma_0^2$, then all δ_{ij} follow $N(0, \sigma_0^2)$. Using Eq. (8), the test statistics is denoted by:

$$\varphi^2 = \sum_{1 \leq i < j \leq n} \frac{\delta_{ij}^2}{\sigma_0^2} = \frac{1}{\sigma_0^2} n(n-1) CI \quad (9)$$

To find out the value of CI and CR , the value of σ^2 must be determined. Therefore, to estimate the value of σ^2 the

maximum likelihood estimator (MLE) of variance has been used. As a result, the MLE of the priority vector $W = (w_1, w_2, \dots, w_n)^T$ and the MLE of the variance, i.e., σ^2 are given as below (Devore 2000; Lin et al. 2013):

$$\bar{w}_i = \left(\prod_{j=1}^n p_{ij} \right)^{1/n} \quad (10)$$

$$\hat{\sigma}^2 = \frac{1}{n(n-1)} \sum_{i \neq j} \left[\ln a_{ij} - \ln \left(\frac{w_i}{w_j} \right) \right]^2 \quad (11)$$

where $i, j = 1, 2, \dots, n$

Step 4.1: Construct the test hypothesis: $H_0: \sigma^2 \geq \delta^2$, $H_1: \sigma^2 < \delta^2$, and selecting a suitable significance level of α .

Step 4.2: Compute the correspondence thresholds of CI' or CR' by using the following equation:

$$CI' = \frac{\delta^2}{n(n-1)} \varphi_{\alpha}^2 \left[\frac{n(n-1)}{2} \right] \quad (12)$$

$$CR' = \frac{CI'}{RI} \quad (13)$$

where CI' is the threshold of the "consistency index" and CR' is the threshold of "consistency ratio".

Step 4.4: Calculate CI or CR based on the principal eigenvalue λ_{\max} of the PCM.

Step 4.5: If $CI \leq CI'$ or $CR \leq CR'$, it is concluded that the PCM has an acceptable consistency, otherwise the PCM has an unacceptable consistency.

Step 5: Computation of the RVs of SRs

The RVs of SRs plays a key role to decide which requirement will be implemented in which release of the software (Sadiq 2017; Sadiq and Nazneen 2019). Once we have identified that PCMs for SRs are consistent then these PCMs will be used to compute the RVs of SRs. In this step, AHP has been applied to compute the RVs of the SRs.

Step 6: Selection of SRs

After computing the RVs of SRs, the next step is to select the SRs. Therefore, top n SRs based on their ranking values will be selected for the implementation during the first release of the software. Similarly, the remaining set of SRs will be selected for the other releases of the software depending on their ranking values.

4 Case study

The guidelines proposed by Runeson and Host (2009) have been adopted for conducting the case study research process, which is of exploratory and descriptive type. In this paper, following steps have been included to conduct the case study: (i) case study design and planning, (ii)



preparation and collection of data, and (iii) data analysis. The explanations of these steps are given below:

4.1 Case study design and planning

As suggested by Runeson and Host (2009) following elements have been used for the case study design and planning, i.e., (i) "objective", (ii) "the case", and (iii) "research questions" (RQs).

4.1.1 Objective

The objective of this case study is to prioritize and select the SRs only when the PCMs are consistent.

4.1.2 The case

This is an important element of "case study design and planning" where we discuss about what we are studying. Here, we are studying that how to prioritize and select the SRs of IES with consistent PCM using statistical approach. The IES is useful to perform the following activities related to examination system of an Institution/University: "(i) submission of the online examination form, (ii) generation of the results of the postgraduate courses/undergraduate courses/diploma courses, etc.". All the information related to the examination will also be displayed by IES, for example, last date of the submission of the examination form, date sheet of the examination, etc. (Sadiq 2017; Sadiq and Jain 2014, 2015).

4.1.3 Research questions

Following RQs have been formulated to explain the proposed method:

- RQ-1: How to construct the PCMs for the computation of the RVs of the SRs?
- RQ-2: How to apply the statistical approach to check the consistency of AHP?
- RQ-3: How to select the SRs for the implementation during different releases of the software?

4.2 Preparation and collection of data

To address the above research issues, we prepare the data after eliciting the software requirements of IES. The detailed description about the elicitation of the SRs is discussed in Sect. 4.3. We have collected the data for the prioritization and selection of the SRs after constructing the consistent PCM; and it is also given in Sect. 4.3.

4.3 Data analysis

The objective of data analysis is to transform and model the data with the goal of discovering the useful information for the decision making. Before analyzing the data, we first explain the steps of the proposed method in the following sub-section:

Step 1: Identification of the stakeholders

In this step different stakeholders have been identified based on their types and roles. Based on our analysis, we have identified three primary stakeholders, i.e., (i) Administrators, (ii) Head of the Departments, (iii) Controller of Examinations. Primary stakeholders are those stakeholders who have direct interest in the development of IES. On the other hand, six secondary stakeholders have been identified, i.e., (iv) Requirements analyst-I (for requirements elicitation), (v) Requirements analyst-II (for PCM), (vi) Developer, (vii) Consultants, (viii) Testers, and (ix) Student or End users. These stakeholders have the indirect interest for the development of IES. The list of stakeholders along with their types and roles is exhibited in Table 2. In this study, 54 stakeholders have been identified for the elicitation of the SRs of IES.

Step 2: Elicitation of SRs

Software requirements elicitation is a key activity of software project management in which the needs of the stakeholders are identified so that a successful software project can be developed. Before applying the "goal oriented requirements elicitation technique" (GORET), traditional method of requirements elicitation techniques (RET) have been employed to understand the basic need of the different types of the stakeholders, as listed in Table 2.

Traditional methods of requirements elicitation techniques have been classified into (a) analysis of the existing documents, (b) interview, (c) survey, and (d) questionnaire (Sadiq and Jain 2012). Among these methods, interview is an effective method to understand the need of the stakeholders (Dieste and Juristo, 2011). The responsibilities of stakeholders S4 and S5 were to elicit the FR and NFRs of IES. Therefore, as a first step, they started to work on the traditional methods of requirements elicitation process. The objective of stakeholders S4 and S5 were to forward the form, as shown in Fig. 1, to collect the basic background and their requirements from different students, who are pursuing B. Tech./M.Tech./ Ph.D. in Computer Science and Engineering/Information Technology/Computer Technology/Computer Science and Technology, from Engineering Institution or Universities located in Delhi/NCR region, India. The survey and interviews were carried out at Bakewarr Software Solutions (BSS), New Delhi-110025. The BSS is a new startup in the field of Information Technology and System (ITS) which deals with the training and research in different areas of ITS like Software

Table 2 List of Stakeholders

S. No	Stakeholder's Types	Stakeholder's Role
1	Administrators	S1: To look after all the administrative work during the software development process in an Institution/University
2	Head of the Departments (HODs)	S2: The responsibilities of the HODs are to organize the meeting with their staff and collect the requirements of the IES
3	Controller of Examinations (CoE)	S3: To provide an overview of the all the courses which are running in an Institution/University
4	Requirements analyst-I (for requirements elicitation)	S4: Responsible to elicit the FRs of IES based on Interview from HODs, COE, and Students
5	Requirements analyst-II (to deal with PCMs)	S5: Responsible to elicit the NFRs of IES
6	Developer	S6: To participate during SRP process
7	Consultants	S7: Responsible to implement all the FR/NFRs of IES
8	Testers (Late Phase of RE)	S8: Consultants
9	Students	S9: To generate the test cases so that the SR of IES can be verified and validated
		S10-S54: 45 students are participating to elicit the SR of IES

Software requirements elicitation form

Place of survey/Interview: Bakwan Software Solutions, New Delhi-25, India

Part-A

1. Name of the student:

2. Place of study or work:

3. Name of the Institution/University or company:

Part-B

1. Have you worked earlier on any Information system based projects in your Institution/University or company? If yes, what was your role and responsibilities in that project?

2. What you expect from the Institute Examination System?

3. Is there any specific goal or goals that you want to achieve from Institute Examination System?

4. Can you suggest some stakeholders who can contribute during the requirements elicitation process?

Fig. 1 Form to elicit the software requirements of IES

Engineering, Information Systems, etc. The first author¹ of this paper has also discussed the requirements elicitation process for IES with the students of Ph.D. in Computer Science and Technology and Diploma in Computer Engineering-III Year at Software Engineering Laboratory, Department of University Polytechnic, Faculty of Engineering and Technology, Jamia Millia Islamia (A Central

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University), New Delhi-110025, India. This discussion was carried out during January-June, 2019. Based on the responses received from the students, different goals of IES have been identified. These goals were then refined and decomposed by GORET to elicit the software requirements. Consider the following example for the elicitation of the SRs from one of the goals of the IES, i.e., student's module. The AND/OR graph for the partial view of the student module is exhibited in Fig. 2.

The student's module is decomposed and refined into the following SRs, i.e., login module, fill examination form, check internal assessment marks, check seating plan, and results tracking system. These SRs are connected with *AND connectives*. It means until and unless these five SRs will not be executed, the student module goal can't be achieved. The sub-goal "check seating plan" is decomposed and refined into two sub-requirements, i.e., (i) notice board and (ii) web site. These two sub-requirements are connected with *OR connective*. It means that achievement of any sub-requirement may lead to the achievement of the check seating plan requirement. In the similar way, all the sub-goals of the IES were decomposed and refined, and finally, we have identified the following SRs of IES:

- **SR-1:** Login module for Students, Teachers, and Administration. The user ID and the password of the students will also be forwarded to their parents or guardian
- **SR-2:** To fill the examination form. The information entered into the examination form will be displayed on the hall ticket / admit card of the students
- **SR-3:** Check the internal assessment marks of the students

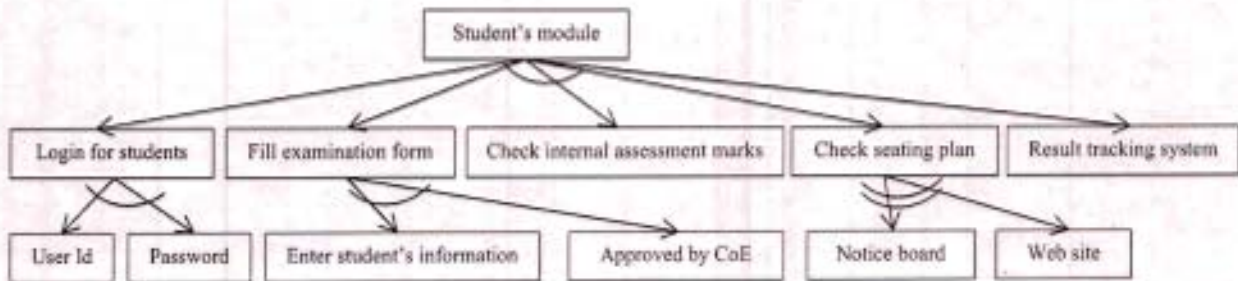


Fig. 2 Partial view of the AND/OR graph of Institute Examination Systems

- SR-4: Check end semester results
- SR-5: Student fee receipt generation after depositing the end semester and backlog paper fee, if any
- SR-6: Generation of seating plan of daily examination before 30 min of the commencement of the examination.
- SR-7: To display the information about the theory and practical examination with codes and date of examinations along with the signature of the controller of examinations (CoE)
- SR-8: To enter the University Examination marks of the Theory and Practical courses (for authorized members only appointed by CoE)
- SR-9: News for filling up the examination form and the generation of the hall ticket
- SR-10: Result tracking system
- SR-11: To generate the mark-sheet of end semester results.
- SR-12: The system should be secure

Step 3: Construction of the PCM during group decision making process

In this step, pairwise comparison matrix was constructed by evaluating the SRs based on the importance of requirements. Saaty rating scale, as shown in Table 3, was used during the evaluation process (Saaty 1965). During the evaluation process, stakeholder S6 found that SR1 is equally important to SR2 so "2" is placed at SR1 row and SR2 column of Table 3. By the rule of Saaty rating scale, SR2 row and SR1 column will hold 1/2 because of the reciprocal property of AHP. SR1 was found to be moderately more important than SR4 so "3" is placed at SR1 row and SR4 column; and 1/3 is placed at SR4 row and SR1 column. In the same way, the entire SRs were evaluated by stakeholder S6; and the results are exhibited in Table 4.

In our work, we have considered importance as criteria for the evaluation of the SRs because it is the most used criteria during the prioritization of the SRs in the literature (Hujainah et al. 2019). The PCM of Table 4 was used to compute the principal eigenvalue; and as result we found that the principal eigenvalue of the PCM of Table 4 is equal to 13.579. Therefore, we get $\lambda_{max} = 13.579$. Based on

the Eq. (1) and Eq. (2) we found that $CI = 0.143586$ and $CR = 0.09323$.

Step 4: Check the consistency of PCM using statistical method

We have developed a program in C, i.e. *PCM_PV*, to compute the values of the "maximum likelihood estimator" (MLE) of the priority vector of PCM based on Eq. (3); and the value of MLE of the variance, i.e., σ^2 based on Eq. (4). Now the consistency of the PCM of Table 4 is checked using *PCM_PV*. As a result, the MLE of the priority vector of P is given by:

$$\bar{W} = (w_1, w_2, \dots, w_{12})^T = (4.028, 3.015, 1.762, 2.183, 1.328, 1.0435, 0.7792, 0.6524, 0.5657, 0.459, 0.3997, 0.2926)$$

The value of MLE of the variance, i.e., σ^2 , is given by:

$$\sigma^2 = \frac{1}{n(n-1)} \sum_{i \neq j} \left[\ln a_{ij} - \ln \left(\frac{w_i}{w_j} \right) \right]^2 = 0.245$$

For $\alpha = 0.10$ and 0.05 , the values of the CV' and CR' are given in Table 5. The Chi-squared test statistic was constructed to test the consistency of PCM by considering the relationship between PCM and deviation matrix (B). The existing methods of SRP based on AHP uses the consistency ratio threshold value (0.1). This threshold value has been criticized and disputed in some aspects as it allows contradictory judgements in PCM or reject the reasonable PCM (Lin et al. 2013). An ideal method of SRP should deal with contradictory judgements and reasonable PCM. The Chi-Squared distribution table is used to find out the critical values of $\chi^2_{\alpha} \left[\frac{n(n-1)}{2} \right]$. For $\alpha = 0.10$ and $\alpha = 0.05$ the Chi-Square distribution are 83.358 and 77.829, respectively (Richard and Johnson 1998).

Here, we found that $CR = 0.09323$ are less than any of the corresponding threshold.

CR' given in Table 5 under condition that the significance level α is equal to 0.10 and 0.05. Therefore, PCM based on the importance of the requirements given in Table 4 has an acceptable consistency. This PCM has also

Table 3 Saaty rating scale

Intensity of importance	Meaning
1	Equal important
3	Somewhat more important
5	Much more important
7	Very much important
9	Absolutely more important
2,4,6,8	Intermediate values (when compromise is needed)

Table 4 PCM based on the importance of the requirements

FRs	SR1	SR2	SR3	SR4	SR5	SR6	SR7	SR8	SR9	SR10	SR11	SR12
SR1	1	2	2	3	4	3	5	5	7	9	9	
SR2	1/2	1	2	2	3	4	3	5	5	7	5	9
SR3	1/2	1/2	1	1/7	2	3	4	3	5	2	7	5
SR4	1/3	1/2	7	1	2	2	3	4	3	5	2	7
SR5	1/4	1/3	1/2	1/2	1	2	2	3	4	3	3	5
SR6	1/2	1/4	1/3	1/2	1/2	1	2	2	2	2	3	5
SR7	1/5	1/3	1/4	1/2	1/2	1/2	1	1/2	2	3	4	3
SR8	1/5	1/5	1/3	1/4	1/3	1/2	2	1	1/3	2	2	4
SR9	1/7	1/5	1/5	1/3	1/4	1/2	1/2	3	1	2	1/2	3
SR10	1/9	1/7	1/2	1/5	1/3	1/2	1/3	1/2	1/2	1	2	2
SR11	1/9	1/5	1/7	1/2	1/2	1/3	1/4	1/2	2	1/2	1	1/2
SR12	1/9	1/9	1/5	1/7	1/5	1/5	1/3	1/4	1/3	1/2	2	1

Table 5 Thresholds of CI and CR

$n = 12$	$\varphi_s^2 \left[\frac{n(n-1)}{2} \right]$	CI'	CR'
$\alpha = 0.10$	82.358	0.153	0.0993
$\alpha = 0.05$	77.929	0.145	0.0949

the acceptable consistency according to Saaty ratio threshold (0.1).

Step 5: Computation of the RVs of SRs

After constructing the consistent PCM using statistical approach, the ranking of the SRs are computed. Based on AHP methods, ranking values (RVs) of SRs are computed; and the results are exhibited in Table 6.

Step 6: Selection of SRs

The objective of this step is to select the SRs based on the ranking values. If we want to implement five SRs in the first release of the software then SRs from SR1 to SR5 will be implemented because of their priority values.

Table 6 Ranking values of the SRs

SRs	SR1	SR2	SR3	SR4	SR5	SR6	SR7	SR8	SR9	SR10	SR11	SR12
RVs	0.232	0.171	0.114	0.142	0.078	0.062	0.049	0.042	0.038	0.027	0.027	0.018
Priority	1	2	4	3	5	6	7	8	9	10	10	11

5 Conclusion and future work

This paper presents a method for the prioritization of SRs using AHP. The PCMs are the important component of the AHP which is employed to capture the preferences of the stakeholders during the decision making process. In the proposed method, statistical approach has been used to check the consistency of the PCM at different significance level, i.e., $\alpha = 0.10$ and $\alpha = 0.05$. We have considered 12 SRs to explain the proposed methodology. These SRs have been elicited from IES using survey, interview, and GORET. Based on our study, we found that SR1 to SR5 are the top five SRs because these requirements have the highest priority. Therefore, these requirements will be implemented in the first release of the software. In real life applications the size of SRs may be large. Therefore, in future, we shall apply the proposed methodology on large scale projects having more than hundreds or thousands of SRs like Replacement Access, Library, and ID card (Lim and Finkelstein 2012). One of the limitations of our work is that we have considered only one criterion during the SRP process, i.e., importance. Therefore, in future we shall try

to apply the proposed method by considering different criteria like, cost, security, efforts, etc., during SRP process.

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Proposed Framework for Dealing COVID-19 Pandemic Using Blockchain Technology

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The production and distribution of COVID-19 testing kits is an urgent and increasingly worldwide requirement, due to the ongoing pandemic. The accuracy of the kit is critically important and to save the world from the faulty kit becomes an issue. The kit before use has to be approved by an authorized medical research agency like US-FDA, ICMR, etc. In this paper, we proposed a framework that ensures that the testing kit is validated by various measures and gives the history of the supply chain of the testing kit. The parties that are used in the supply chain are Notary, Manufacturer, and Validating Party. A Consumer also plays an important role and can punch the batch number to check whether the kit is approved or not. The framework is developed using R3 Corda, a permissioned distributed ledger technology. A permissioned blockchain is used for data privacy and security so that only trusted parties can leave or join the system.

Keywords: Corona virus, Decentralization, HealthCare Industry, R3 Corda, Supply Chain Management,

Introduction

In Nov'2019, Corona virus was originated from Wuhan, China. It is caused by the corona virus called SARS-Cov-2 and was announced as a world epidemic by the World Health Organization (WHO) on 11th March'2020. COVID-19 causes several deaths and many people battled with this deadly virus and recovered. Till 30th September'2020, 33,844,178 cases have been discovered, 25,148,403 are recovered and 1,012,659 are died due to Corona virus. To contend with this pandemic various strategies have been followed by testing, house arrest, or a creating vaccine. COVID-19 testing plays an important role to detect or discover undiscovered cases by various testing methodologies like Antigen, RT-PCR, Serology, etc. The supply chain management (SCM) of these kits is a crucial and necessary step to get an assurance of an accurate kit which can be described in the scope of how to deal with the COVID-19 pandemic using block chain technology.¹ SCM is the process of tracking and managing each process involved from raw to a finished product or from supplier to consumer. For the prevention of faulty kits, there is a requirement for an effective supply

chain management system, and the best accessible answer for developing an ideal SCM system is Blockchain technology. Blockchain² is a decentralized technology which was developed by Satoshi Nakamoto in 2009, in which various nodes or computer share a common ledger that stores immutable records or set of transactions which are secured by a cryptographic function or highly computational mathematical problems. The function used is of the form:-

$$H: K \times M \rightarrow \{0,1\}^n \quad \dots (1)$$

Where H denotes hash and n denotes the number of bits returns by the function. Its first implementation was Bitcoin (crypto currency) in 2014. It overcomes various transaction issues like the double-spending problem, but it is not only limited to the financial sector as blockchain grabs attention in various non-financial sectors like Education, Healthcare, Real Estate, etc., and gets exploring day by day. This paper tells how blockchain technology works, how this can be implemented to deal with the COVID-19 pandemic, how testing kits SCM can be implemented to check whether the kit is validated by an authorized party or not by using a permissioned blockchain platform called R3 Corda. The rest of the paper is

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divided into various sections. Section 2 discusses the existing framework or work of other researchers in the field of healthcare and COVID-19. Section 3 describes the motivation behind the proposed system and the requirement for Blockchain. Section 4 describes the reason for using R3 Corda and compares public and private blockchain. Section 5 describes the implementation and participating parties. Section 6 consists of future scope, a conclusion with the references at the end.

Motivation and Contribution

To give a protected and productive COVID-19 Testing kit gracefully conspire, a comparable sort of system utilized in the Pharmaceutical supply chain or other medical services related territories have been concocted, for example, MedRec, ModelChain, MediLedger, etc. To build such a framework, numerous analysts have utilized various platforms, for example, permissioned Ethereum, Hyperledger Fabric to give transparency. The above platforms utilized are not as much secured and incapable to give protection when contrasted with R3 Corda. Corda is a private blockchain platform that supports Java/Kotlin and for the most part, has an application in the money related area. In the current Covid-19 testing kit supply chain model, the method isn't transparent to the client, and there can be an opportunity that physically kept up records of kits approval can be lost. The producer sends the kit to an approved party for approval; the approving party essentially stores the records as tables. There can be the opportunity that buyers can't get genuine and right information identified with kit approval. This gave the inspiration to build a blockchain platform for kit Supply Chain Management. The commitments in the proposed COVID-19 testing unit SCM structure are as per the following:

- a) Our system gives high security regarding that no unapproved party can be added to the chain and high transparency is additionally given.
- b) The information that store on the chain is permanent and made sure about by profoundly registered cryptographic calculations. Every exchange is approved by a Notary so that any off-base or inaccurate exchange can't be added to the chain.
- c) The mistake can be appeared to the client by punching the Batch Number if the kit isn't approved.

Along these lines, our system is more proficient than the current methodology utilized in testing kit supply chain management. To the best of the creators' information and conviction, this structure can be

executed in true applications and can supplant the current methodology.

Related Work

In the survey, not many endeavors have been made in the supply chain in the healthcare industry or providing a solution to deal with COVID-19 using blockchain. Ijazul Haq⁴ proposes a use case in the field of pharmaceutical industry using blockchain technology to prevent counterfeit drugs and explains how traceability of drug supply can be gained. In the paper, he uses permissioned blockchain and suggests the system with an example that shows how different parties like Nurse, Doctor, and Patient can use the system. MedRec⁵, a white paper published that introduces a system for study purpose in future to researchers and provide storage of secret medical data. It presents a system for saving patient's data and effectively accesses that data by fusing blockchain security. Sandip Jangir⁶ proposes a novel framework using distributed ledger and smart contracts for pharmaceutical SCM. The system is built using the Ethereum platform that achieves privacy, demand-supply management, and real-time tracking of drugs. Proper analysis and experimental results have been taken in terms of User Privacy, Immutability, Data transparency, No single point of failure, High availability, Non-repudiation, and real-time tracking of drugs for proof of concept of the introduced system. Peng Zhang⁷ proposes a framework or decentralized application (Dapp) called FHIRChain for sharing clinical data scalably and securely using blockchain. This work can further be extended to other issues related to healthcare interoperability. William J. Gordon⁸ discusses health data interoperability issues and why there is a shift to patient-driven interoperability from institution-driven interoperability. In the paper, he describes benefits, various limitations, and challenges. Tsung-Ting Kuo⁹ proposes a framework called ModelChain that is used to preserve healthcare data using private blockchain networks by integrating Machine Learning into it. He also developed a new proof-of-information algorithm to find the order of blockchain-based online Machine learning. Si Chen¹⁰ proposes a supply chain quality management framework based on blockchain that provides a theoretical basis to intelligently apply management to the system and can be used in a real-world application. Mohamed Torkey¹¹ provides an innovative framework to deal with COVID-19 by detecting unknown COVID-19 patients using



blockchain. The framework is divided into four main components; they are P2P mobile Application, Infection Verifier System, Blockchain Platform, and Mass- Surveillance System.

Purpose

There can be various issues arise while supplying COVID-19 testing kits such as kits can be faulty, not giving accurate results, the defect can be caused at the manufacturer end or between the supply of the testing kit. The kit when manufactured it has to be validated by authorized medical like the Indian Council of Medical Research (ICMR), US-FDA, etc. There can be a chance when unauthorized or fake marks are printed on the testing kit or counterfeiting of the kit can take place. To deal with various issues discussed, the SCM can be implemented using blockchain and the purpose of the proposed system can be pointed as:-

- I. **To provide Transparency:** - Customers can track the testing kit throughout the supply chain; from manufacturing to the validation done by the validating party this brings transparency. Customers can punch the unique id associated with the kit into the system and can see if the manufacturer or validating paper is legitimate or not.
- II. **To provide Traceability:** - Once the kit is manufactured then it will be registered on the blockchain by the manufacturer in return a unique id or Batch No. will be generated. Using this id the kit will be traced, tracked, and certified at each stage of SCM.
- III. **To provide Security:** - Blockchain is one of the ideal secured technology or ledger systems available right now. Once the information on the blockchain is stored cannot be modified or deleted. In the proposed system, we are using a permissioned blockchain which is more secure than a public blockchain, in which only authorized or legitimated users can participate in pushing the data to the chain. The data privacy and security will be achieved by the fact that data will be stored in the encrypted form using a hash algorithm called SHA-256 and nobody can access or break the security system of blockchain unless 51% attack.

Why R3 Corda?

Corda¹² is a decentralized database platform which mainly focuses on the financial domain and supports

smart contract. In Corda, the smart contract is a pure function that responsible for accepting or rejecting the transaction. R3 Corda is a private blockchain that restricts users to leave or join, when a node transfer a message to another node then the message is only visible between these two nodes unlike in other platforms in which every node can see the message. There are various platforms available in the market to develop blockchain, and they can be categorizing into four board categories called public blockchain, private blockchain, hybrid blockchain, and consortium blockchain. Ethereum¹³ and Bitcoin are some famous public blockchain. Hyperledger Fabric¹⁴ and R3 Corda¹⁵ are some famous private blockchain. Quorum and Hyperledger are some consortium blockchain. Many applications are created using Ethereum & HyperLedger fabric which are widely used but we built our system using R3 Corda because of various reasons such as: Firstly, it is a private blockchain platform and prevents any unnecessary sharing of data. Secondly, in decision making the nodes that are performing transactions can only take part. Lastly, Corda makes its consensus algorithm rather than following developed algorithms. The difference between public blockchain and private blockchain can be shown in the Table 1 given below:-

System Architecture and Parties Involved

Parties Involved

Testing kits play a major role in dealing with the COVID-19 pandemic; as expedite the test sooner we get rid of COVID-19. But for a smooth testing procedure, there should be a proper check on the testing kits to that they are working properly or not giving any false results. Accuracy and efficiency play an important role, and to achieve this testing kit must be validated by an authorized/ trusted party before

Table 1 — Comparison Between Public and Private Blockchain

Parameters	Public Blockchain	Private Blockchain
Access	Open to anyone	Single Organization
Speed	Slower	Faster
Network Architecture	Decentralized	Partially Decentralized
Consensus	Permissionless	Permissioned
Immutability	Complete, Impossible to tampered	Partial, can be tampered
Efficiency	Low	High
Number of Users	Millions	Few Hundreds



use. To ensure this, the supply chain of testing kit should be maintained by using blockchain technology as there can be the chance that the manufacturer can supply the kit directly to the user, or there can be the probability that any fault can occur in between the supply chain. The participating parties in the whole framework are:-

Notary

A notary is a network service by the Corda system that checks whether the transaction or any updation to the chain is valid or not and check for its dependencies. In the dictionary, a notary is a person who is authenticated to do certain legal work, especially to draw up or allot certificates or other legal documents for use in jurisdictions. Notary manages the contract between the manufacturer and validating party, he legitimates the transactions occurring are according to the contract.

Manufacturer

The manufacturer produces a testing kit by considering the terms and conditions prescribed by medical agencies and send the kit to any authorized validating party before supplying it to the consumer. To ensure traceability, all the details of the kit will be maintained on the chain. The details include the Name of Kit, Name of company, Manufacturing Date, Expiry Date, Kit Details. After uploading all the details then a unique UUID i.e. Batch Number will be generated for further reference.

Validating Party

The Validating Party can be any authorized medical research agency like ICMR¹⁶ that has the right to approve the testing kit for further medical use. ICMR follows different rules for each kind of testing kit, but if the kit is US-FDA approved then the kit can be supplied directly. The validating party performs various test reactions, reagents, methodology, and sent the validation report to the manufacturer. If the kit is approved, then only the manufacturer can supply it to the consumer otherwise the manufacturer has to perform corrections that are instructed by the validating party. The validating party has to upload some details for ensuring transparency, which is follows: - Batch Number, approval, comments, Approved By.

After a successful approval kit reaches at consumer end then the consumer will punch the batch number and can see the details which are Batch Number, Manufacturing Date, Expiry Date, kit Details, etc.

Suppose the testing kit is directly supplied by the manufacturer to the consumer without being validated by the validating party then an error will be shown to the consumer by punching the Batch Number to the system.

Software Required

For implementing the above-described framework R3 Corda platform used, and for development of CorDapps, we required some tools that are described below¹⁷:-

a) IntelliJ IDEA 2020.1.2:-

IntelliJ¹⁸ is an Integrated Development Environment (IDE) that supports both Kotlin and Java development. It is available as an Apache 2 Licensed and developed by JetBrains (formerly known as IntelliJ) in January 2001. Corda supports Kotlin plugin version 1.2.71 and IntelliJ Idea versions 2017.x, 2018.x, and 2019.x.

b) JDK1.8:-

Corda requires Java Development Kit (JDK) for development, it requires at least version 8u171. JDK includes its private Java Virtual Machine (JVM) resources like Java Runtime Environment (JRE), Interpreter, Compiler, Archiver, Documentation generator that are required for developing Java-based applications.

c) Postman 7.28.0:-

Postman¹⁹ is a software development tool that is used for testing Application Programming Interface (APIs) which is started as a side project by Abhinav Asthana. The features of postman can be the Compact Layout, API responses for JSON and HTML, HTTP requests with file support, etc.

Implementation

The system for kit validation containing three nodes, they are Validator, Manufacturer, and Notary these nodes/peer Corda sends the requests to the API in the form of an HTTP request and gets the result accordingly. There are three hosts used in the system they are, Corda Vault, Application Tier, and Corda Float. Corda vault stores the transactions that are taking place. In Corda, the transaction types are defined using JVM code that means byte code execution should be fully deterministic. CorDapps (Corda Distributed Applications) are the applications that run or developed on the Corda platform. In CorDapps, classes are defined in JAR files which are

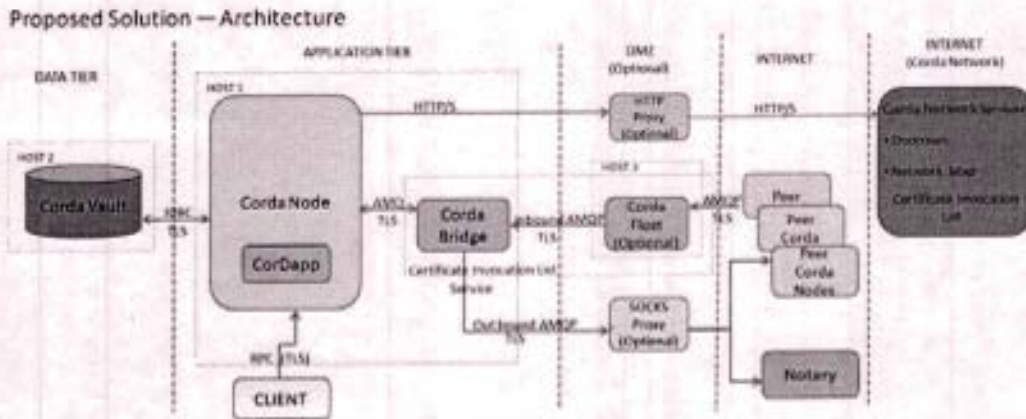


Fig. 1 — System Architecture

programmed in Kotlin and/or Java. The functional aspects of Corda are CorDapps that permit Corda Nodes to achieve agreement on updates to the Vault (DLT ledger/database) for given use cases. Using JDBC, Corda Nodes hold the shared results of CorDapps in a database (vault). The Nodes communicate in a peer-to-peer fashion using AMQP/TLS. The various parties interact with these nodes such as Client applications interact using RPC/TLS and Administrators interact over SSH. Corda Nodes attain an identity certificate via a Doorman service using HTTPS and they learn about other trusted Corda Nodes and their addresses via a Network Map service using HTTPS. Corda Bridge and float uses Corda-firewall.jar. The Bridge catches the inbound messages and sends them to the mutual Artemis line. The Bridge is regularly designed to course through a SOCKS Proxy Server and deliver active messages from the Node to Peers on the Network. The system architecture and workflow of the proposed framework for kit validation can be shown in Fig 1.

Conclusion

In this paper, we proposed a framework for COVID-19 testing kit supply chain management in the field of healthcare using blockchain technology. We pointed out various entities that are involved in SCM and implement this SCM using R3 Corda. We also discussed various tools used and working scenarios. In the future, work can be extended in various ways like by developing solely CorDapp that does not require any third party API to run, replacing unique id to some more secure code or method, adding more intermediate to chain to bring more

transparency, adding more options to the system like uploading files, etc. As we all wish that COVID-19 should end soon, that doesn't mean we cannot use this framework, as this framework can be implemented practically in other areas and can be used further.

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Development Neural Network Models on Prediction of Human Purpose

Mohd Sadim, Ruchi Singhal, Mayank Agrawal, Rajesh Kumar Singh

Keywords: Interaction between humans and robots; prediction of intentions; groundbreaking neural networks;

ABSTRACT

As human-robotic (HRI) interactions are the, more main determinants are predicted. However, the large percentage of robotics follows strict guidelines that seriously limit their mobility and functionality. A key fact which contradicts the understanding of HRI is that robots are unable to understand social desires. The goal of this study is to enhance robotic intelligence by educating people to understand the purposes of humans. Contrary to the previous report, this paper offers a mechanism for predicting human intentions before a single procedure is completed. The test of bouncing a baseball at designated targets is carried out to check the process' performance. The deep learning approach proposed shows that neural networks (CNNs) can be completely used in a new situation. Experimental results suggest that three conventional machine training strategies compete with the proposed CNN-vote system. The CNN Vote Predictor achieves high test accuracy with comparatively less data available in the current context.



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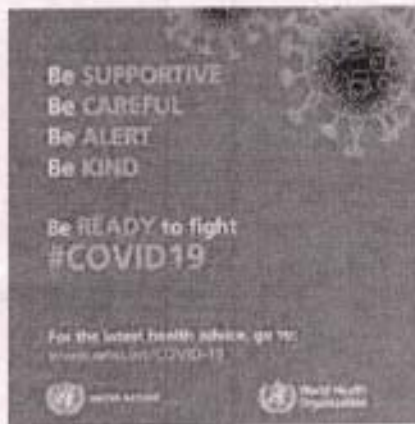
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Enhancing the Performance of Hate Speech Classification Using Dimensionality Reduction Approach

Kaushar Ansari, Anshul Sarawagi

Abstract

In recent times most of the people are using online platforms for sharing their emotions. These emotions can be classified as a positive comment or as a negative comment. But these comments play an important role when comments are made in the form of reviews for any particular purpose. Today all the ecommerce websites, election parties and many other online business forums are predicting these reviews for evaluating the performance of their product or work. Many times people used to post hate speech on social media, so it is very much need that we must predict the hate speech for further improvement. Traditional machine learning algorithms are not able to accurately predict the hate speech. In this work we have applied dimensionality reduction approach for performing the classification of hate speech on the basis of which classifiers has improved the performance. The feature selection approached is done through Information Gain, Term frequency-Inverse Document frequency and Logistic Regression Cross Validation and we have achieved the F1 score of 0.81, 0.90 and 0.87 for the gradient boosting, random forest, and extreme gradient boosting classifiers respectively.

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Imperative Issues and Challenges of Smart Cities in India

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Abstract: Smart city is future which must be taken into account on priority and is a primary need of society. This paper focuses on the role of Information and Communication Technology towards the implementation of smart cities that will help to create a sustainable smart city. It provides a literature review on smart city, methods and applications that leads us to create a city as smart city. It also provides the information regarding functions of a smart city, a comparison between a city and smart city. It also discusses about smart sustainable city and the security issues in a smart city.

Keywords: Internet of Things, Smart city, Sustainable smart city, Information and Communication Technology

1. Introduction

The idea of Smart Cities is advanced in the wake of expanding the urbanization definitely during the most recent 25 years. There is no particular meaning of a Smart City, however, a few researchers have given various definitions. "A Smart City is a resident-driven and chips away at the idea of least government and most extreme administration. It implies effectively available and productive government, brings flourishing through organizations, work creation, and business and adds to keep resident more joyful and give maintainable climate by utilizing Information and Communication Technology (ICT) instruments and best in class advances like Data Analytic, Internet of Things (IoT), and Machine Learning (ML)". At the end of the day, a concise definition may likewise be given as "A Smart City is a resident-driven stage that assists with making life simpler, gives protected, secure, economical and quality foundation, observation, effectively available different method of transportation, well-being, schooling, banking, sanitization services, and public administrations".

Smart urban areas are the advanced metropolitan ideas that are basic necessity for the persons to have a smart standard of living. The only objective is to gather different advancements in order to achieve intelligent and feasible way of adopting it. Here, we have the definition for the smart city which depends upon overall methodology and the focus will be on smart city. This paper presents an in-depth report on the smart city idea in the perspective on India zeroing in on the highlights, choice and assessment models, and approaches. It also examined the current status and difficulties in the perspective of smart city in India. The issues related to smart cities are shown in Figure 1.



Fuzzy Logic Based Environment Control of Operation Theatre

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Abstract: It is very critical and important to maintain the appropriate climatic conditions in the operation theatre. In paper, we present an approach to control total environmental conditions for the operation theatre. Generally for environmental control of operation theatre air conditioning system is installed. In operation theatre environmental parameters like humidity, temperature, oxygen and particles etc, have to be controlled precisely. All the environmental parameters are of nonlinear nature hence, difficult to control or model with the help of conventional control systems. Keeping in mind the complexity and nonlinearity of these parameters a fuzzy logic controller (FLC) for controlling all the environment of operation theatre has been designed. For this fuzzy logic control system temperature, micro particles, humidity & oxygen have been taken as input parameters and based on these parameters speed of AC motor as well as the speed of exhaust motor are controlled. The control system is implemented with the help of different fuzzy rules and their membership functions derived from actual conditions. The performance of the system for control of operation theatre was studied and it has been observed that the result obtained with the fuzzy logic control system provides more effective and economical control. The fuzzy logic control system has been implemented using fuzzy Tech development tool.

Keywords: Operation Theatre Monitoring, Fuzzy Logic, oxygen humidity, Temperature Control.

1. Introduction

A special room designed with completely equipped hospital for performing the surgical operations also known as operation theatre. In 1884 German surgeon Gustav initiate an approach to design an individual operation theatre for the infected and uninfected patient to eliminate the germs with the help of heated and filtered air approach. These Surgical operation theatre is considered as the one of most crucial as well as the complex area in the hospital which is in most careful control of the aseptically conditions of operation theater environment [5]. The main features in this operation that the surgeon mostly observes like the hygiene and the sub elements like heat air and light very carefully. This type of features is not only carries much important conditions of patient health but is necessary to reduce the possibility of complication during surgery and also help for the smooth conduction for the successful operation.

Some uncommon situation containing the temperature in activity theater with specific level, and also decreasing the base level of particles or keeps the idea level for maintaining the dampness level idea for the same and upgrading it into a similar degree of oxygen are vital, henceforth their control is required [6]. To implement the environmental conditions (temperature, humidity, particles and oxygen) in operation theatre, generally air conditioning systems are installed. It is well known that the air conditioning systems consisting of mechanical or electrical components are highly nonlinear. The nonlinearities are difficult to realize with conventional (mathematical) model based controller such as (Proportional-Integral-Derivative) PID. So these controllers may not provide the desired environmental conditions of operation theatre.

Fuzzy logic can provide the best control logic under a highly nonlinear system. The FLC can be designed very easily without the complete mathematical knowledge of nonlinear control. FLC are based on linguistic rules such as "IF-THEN" general structure which is the bases of human logic and It is economical also.

In light of the above FLC for controlling the nonlinear environmental conditions of operation theatre has been designed and studied.

For controlling the environment conditions of an operation theatre temperature, humidity, particles and oxygen have been considered as input parameters. Based on the speed of AC motor and exhaust motor parameters can be controlled and these are called output or controlled parameters. The system is implemented with the help of different linguistic rules and their membership functions derived from actual conditions. After implementation it has been observed that with this system the operation theatre environment, fresh air level, humidity, temperature level (at the desired level) can be managed.

In this research a FLC system has been designed. The benefit of FL is its simplicity. Fuzzy Logic can deal with issues with uncertain and deficient information and it can display nonlinear capacity of self-assertive unpredictability. On the off chance that you don't have a decent plant model or on the off chance that the framework is changing the fuzzy will deliver a superior arrangement, at that point traditional control strategies. For this FLC framework temperature, stickiness, particles and oxygen have been taken as information boundaries; the speed of A C engine and the speed of fumes engine have been controlled as output parameters. The system is implemented with the help of different rules and their membership functions. This work is an attempt to control the optimum conditions required for operation theatre with FLC system.

2. Method- An Approach

First we will discuss about the working and designing for air conditioner followed by the working of Fuzzy logic Controller, then we proposed how the working of air conditioner is manages with the help of Fuzzy logic Controller. In Fig 1 describes the components and the functioning of the air conditioning refrigeration cycle.

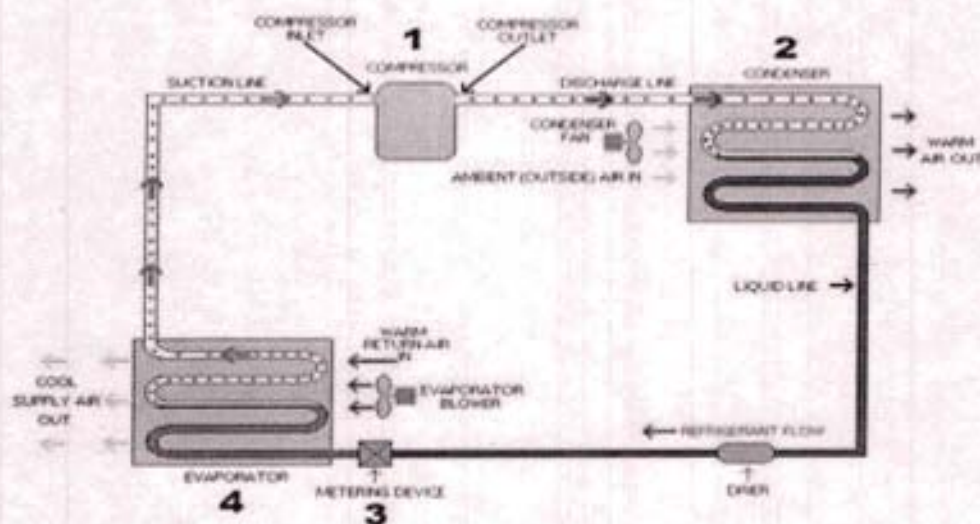


Figure 1. Refrigeration Cycle

The Refrigeration cycle of the airconditioners comprises of the use of chemical that can be easily converted to gas from fluid and back again. The chemical used from the home to the outside air consists of three main parts. These are- Compressor, condenser, evaporator. The condenser and compressor are located on the outside of the air conditioner and the evaporator is located into the home. The compressor is designed to fluid the molecules together and the energy will be high as its temperature. The working liquid leaves the compressor as a hot, high pressure gas and flows into the condenser. In the event that you looked at the air conditioner part outside a house, look for the part that has metal balances all around the blades act simply like a radiator in a car and helps the heat go away, or dissipate, more quickly. At the point when the working liquid leaves the condenser, its temperature is much cooler and it has changed from a gas to a fluid under high pressure. The fluid goes into the evaporator through a exceptionally minuscule, narrowhole. On the other side, the fluid's pressure drops. When it does it starts to evaporate into a gas. As the fluid changes to gas and evaporates, it extracts heat from the air around it. The heat in the air is required to separate the molecules of the liquid from a fluid to a gas. The evaporator also has metal balances to help in exchange the thermal energy with the surrounding air. When the working liquid leaves the evaporator, it is a cool, low pressure gas. It at that point returns to the compressor to start its trip all over again. Connected to the evaporator is a fan that circulates the air inside the house to blow across the evaporator balances. Hot air is lighter than cold air, so the hot air in the room raises to the top of a room. There is a vent there where air is sucked into the air conditioner and goes down ducts. The hot air is utilized to cool the gas in the evaporator. As the heat is removed from the air, the air is cooled. It is then blown into the house through other ducts usually at the floor level. This continues over and over and ever until the room reaches the temperature you want the room cooled to. The thermostat faculties that the temperature has reached the correct setting and turns off the air conditioner. As the room warmsup, the thermostat turns the air conditioner back on until the room reaches the temperature.

2. Fuzzy Logic Controller- FUZZY LOGIC

Fuzzy logic means that there are three steps for the system that process appropriate output as required. They may be classified as-

- a) Fuzzification
- b) Evaluation Rules
- c) Defuzzification

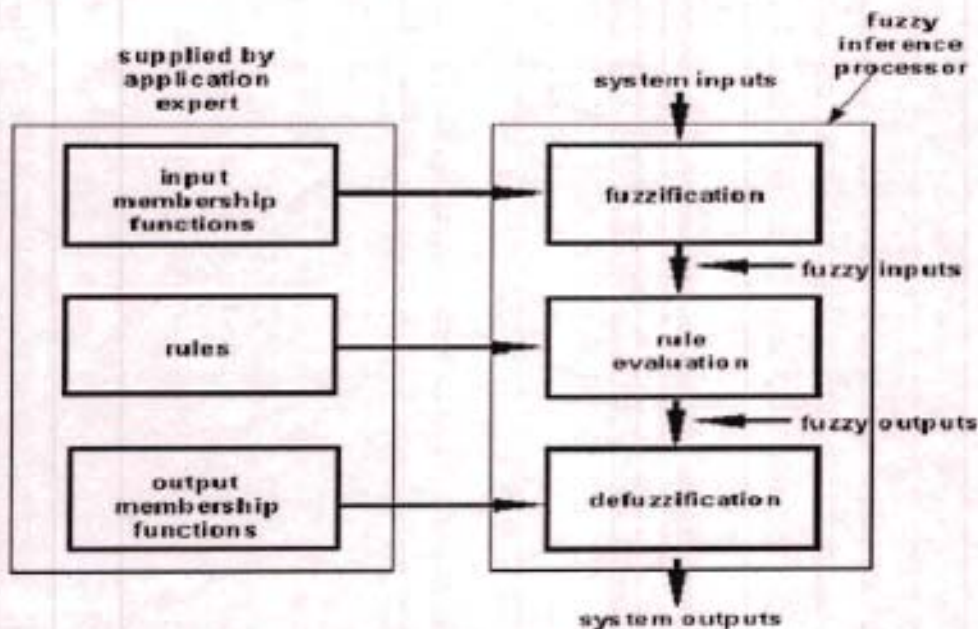


Figure 2. Fuzzy Inference Logic Unit

Fuzzification

The first step in the fuzzy inference process is the fuzzification where a domain conversion is performed in which crisp inputs given to the system are changed into fluffy data sources. Fresh sources of info are fundamentally the specific data sources read by sensors and passed into the control framework to perform preparing, for example, light, speed, temperature, AQI, pressure, rpm's, and so on Each fresh information that is taken by the framework is then handled by the FIU which contains its own gathering of enrollment capacities or it might have the place that can be changed.

Rule Evaluation

It is an arrangement of IF-Zadeh operator and THEN standard for deciding the familiarity guidelines as needed for the ideal operation. The former contained the Input label IS and also equal to either the fuzzy input and the truth table required. The output variable of IS label is totally depend upon the Zadeh function that describes the conclusion utilized.

Defuzzification

In which the output will be in the form of crisp output.

3. Proposed Model

The proposed system will assesses the parameters of the air like temperature, mugginess, oxygen and the quantity of particles. Which then all together and as per the estimations of the information boundaries changes the fan's speed of just as the blower engines and the warming and cooling as per the cooling conditions.

On the basis of their values, Three Expression are defined for the input/ output expression as Low, Medium, High.

Input Parameters: HUMIDITY, OXYGEN, TEMPERATURE, PARTICLES.



Output Parameters : SPEED OF EXHAUST MOTOR, SPEED OF AC MOTOR,

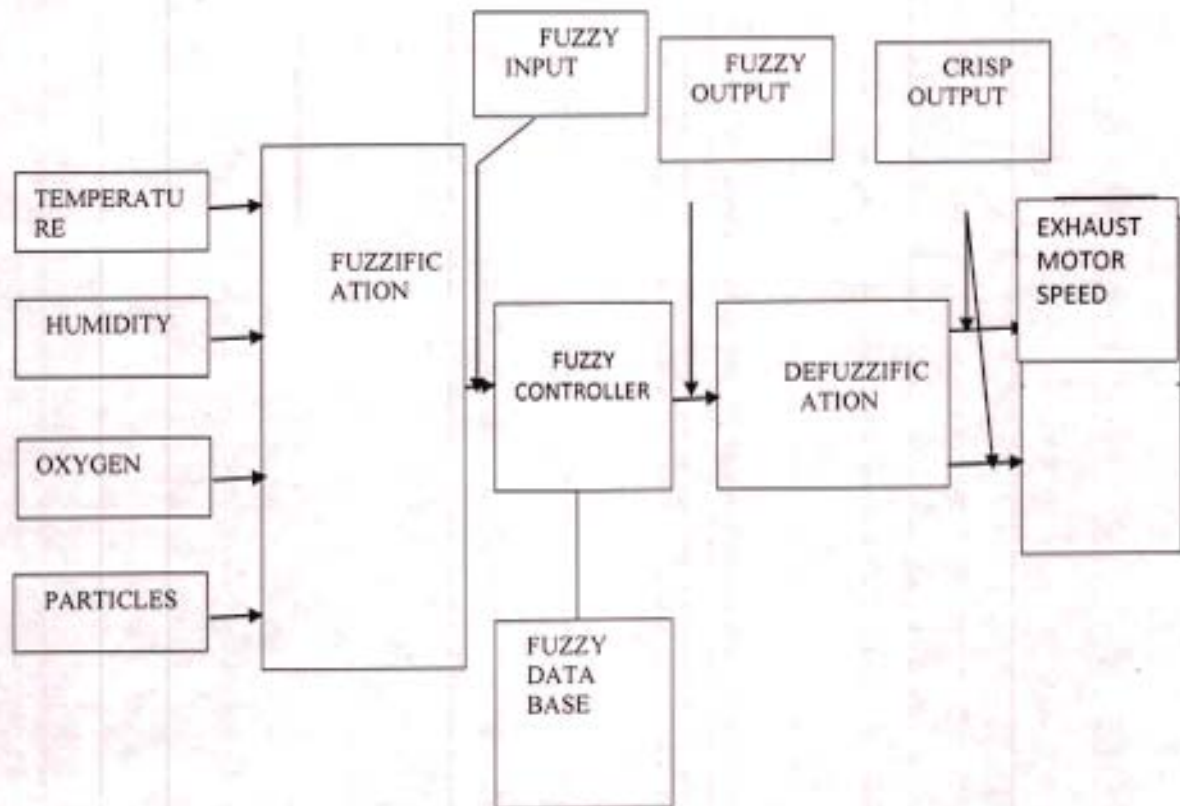


Figure 3. Block Diagram of Proposed Model

Table 1. The input and output Variables / Parameter are shown as-

Variables	Min	Max	Denomination
Temperature of AC in (°C)	16	30	°C
Humidity in (%)	20	70	%
Oxygen in (%)	15	50	%
Particle (Ppm)	1	2000	Ppm
AC Motor Speed (in rpm)	1000	2000	Rpm
Exhaust Motor Speed (in rpm)	1000	2000	Rpm

A. Fuzzification of humidity, temperature and particles of oxygen controlling

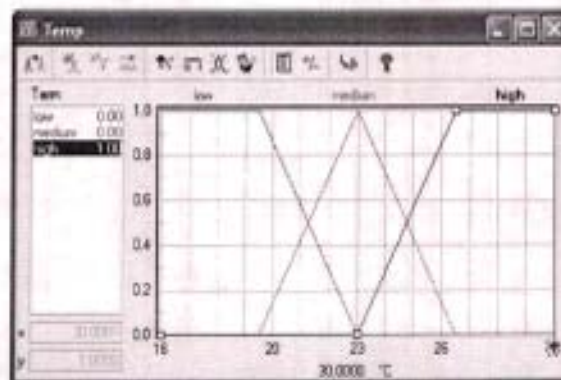


Figure 4.1. Input variable temperature Membership using triangular membership function type

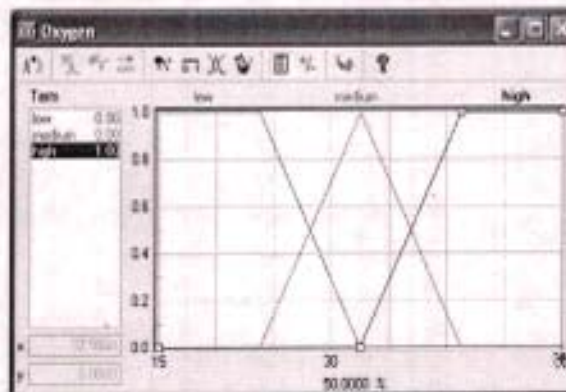


Figure 4.2. Input variable oxygen Membership using triangular membership function type

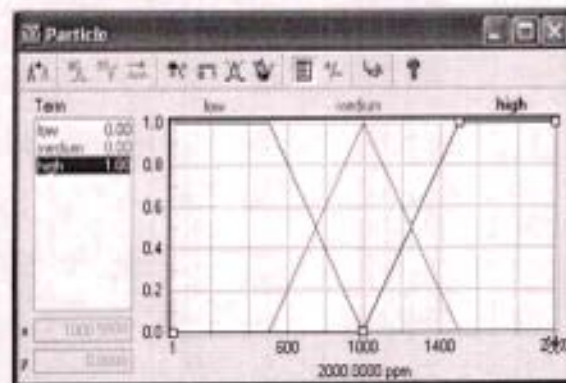


Figure 4.3. Input variable particles Membership using triangular membership function type

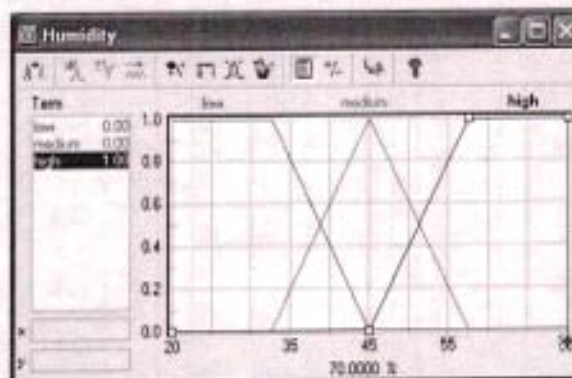


Figure 4.4. Input variable humidity Membership using triangular membership function type



B. Fuzzy Rule base for air conditioning in operation theatre-

The rule base decision system of the fuzzy controller is comprised with the set of rules coded in it. These rules are the intuitions of the human beings based upon their experiences and also they are easy to understand. These rule bases are the qualitative statements written in the form of IF-Then sentences. The rule based defined here for the air conditioner is derived from the common sense and are experimentations in a controlled environment.

A simple form of intuitive rules is like:

"IF 'temperature, oxygen, particles, humidity' are high THEN Speed of A C Motor (S1) and Speed of Exhaust Motor (S2) should be high."

Directives that describe the operation theatre for AC controlling as:

#	IF				THEN		THEN	
	Humidity	Oxygen	Particle	Temperature	DoS	S1	DoS	S2
1	low	low	low	low	1.00	low	1.00	low
2	low	low	low	medium	1.00	low	1.00	low
3	low	low	low	high	1.00	medium	1.00	medium
4	low	low	medium	low	1.00	medium	1.00	medium
5	low	low	medium	medium	1.00	medium	1.00	medium
6	low	low	medium	high	1.00	medium	1.00	medium
7	low	low	high	low	1.00	medium	1.00	medium
8	low	low	high	medium	1.00	high	1.00	high
9	low	low	high	high	1.00	high	1.00	high
10	low	medium	low	low	1.00	low	1.00	low
11	low	medium	low	medium	1.00	medium	1.00	medium
12	low	medium	low	high	1.00	medium	1.00	medium
13	low	medium	medium	low	1.00	medium	1.00	medium
14	low	medium	medium	medium	1.00	medium	1.00	medium
15	low	medium	medium	high	1.00	medium	1.00	medium
16	low	medium	high	low	1.00	high	1.00	high
17	low	medium	high	medium	1.00	high	1.00	high
18	low	medium	high	high	1.00	high	1.00	high
19	low	high	low	low	1.00	low	1.00	low
20	low	high	low	medium	1.00	medium	1.00	medium
21	low	high	low	high	1.00	medium	1.00	medium
22	low	high	medium	low	1.00	medium	1.00	medium
23	low	high	medium	medium	1.00	medium	1.00	medium
24	low	high	medium	high	1.00	medium	1.00	medium
25	low	high	high	low	1.00	medium	1.00	medium
26	low	high	high	medium	1.00	high	1.00	high
27	low	high	high	high	1.00	high	1.00	high
28	medium	low	low	low	1.00	low	1.00	low
29	medium	low	low	medium	1.00	medium	1.00	medium
30	medium	low	low	high	1.00	medium	1.00	medium



#	IF				THEN		THEN	
		Humidity	Oxygen	Particle	Temperature	DoS	S1	DoS
31	medium	low	medium	low	1.00	medium	1.00	medium
32	medium	low	medium	medium	1.00	medium	1.00	medium
33	medium	low	medium	high	1.00	high	1.00	high
34	medium	low	high	low	1.00	high	1.00	high
35	medium	low	high	medium	1.00	high	1.00	high
36	medium	low	high	high	1.00	high	1.00	high
37	medium	medium	low	low	1.00	low	1.00	low
38	medium	medium	low	medium	1.00	medium	1.00	medium
39	medium	medium	low	high	1.00	medium	1.00	medium
40	medium	medium	medium	low	1.00	medium	1.00	medium
41	medium	medium	medium	medium	1.00	medium	1.00	medium
42	medium	medium	medium	high	1.00	medium	1.00	medium
43	medium	medium	high	low	1.00	high	1.00	high
44	medium	medium	high	medium	1.00	high	1.00	high
45	medium	medium	high	high	1.00	high	1.00	high
46	medium	high	low	low	1.00	medium	1.00	medium
47	medium	high	low	medium	1.00	medium	1.00	medium
48	medium	high	low	high	1.00	medium	1.00	medium
49	medium	high	medium	low	1.00	medium	1.00	medium
50	medium	high	medium	medium	1.00	medium	1.00	medium
51	medium	high	medium	high	1.00	high	1.00	high
52	medium	high	high	low	1.00	high	1.00	high
53	medium	high	high	medium	1.00	high	1.00	high
54	medium	high	high	high	1.00	high	1.00	high
55	high	low	low	low	1.00	medium	1.00	medium
56	high	low	low	medium	1.00	medium	1.00	medium
57	high	low	low	high	1.00	high	1.00	high
58	high	low	medium	low	1.00	medium	1.00	medium
59	high	low	medium	medium	1.00	high	1.00	high
60	high	low	medium	high	1.00	high	1.00	high
61	low	low	low	low	1.00	low	1.00	low

#	IF				THEN		THEN	
		Humidity	Oxygen	Particle	Temperature	DoS	S1	DoS
61	high	low	high	low	1.00	high	1.00	high
62	high	low	high	medium	1.00	high	1.00	high
63	high	low	high	high	1.00	high	1.00	high
64	high	medium	low	low	1.00	medium	1.00	medium
65	high	medium	low	medium	1.00	high	1.00	high
66	high	medium	low	high	1.00	high	1.00	high
67	high	medium	medium	low	1.00	medium	1.00	medium
68	high	medium	medium	medium	1.00	high	1.00	high
69	high	medium	medium	high	1.00	high	1.00	high
70	high	medium	high	low	1.00	high	1.00	high
71	high	medium	high	medium	1.00	high	1.00	high
72	high	medium	high	high	1.00	high	1.00	high
73	high	high	low	low	1.00	medium	1.00	medium
74	high	high	low	medium	1.00	medium	1.00	medium
75	high	high	low	high	1.00	high	1.00	high
76	high	high	medium	low	1.00	medium	1.00	medium
77	high	high	medium	medium	1.00	high	1.00	high
78	high	high	medium	high	1.00	high	1.00	high
79	high	high	high	low	1.00	high	1.00	high
80	high	high	high	medium	1.00	high	1.00	high
81	high	high	high	high	1.00	high	1.00	high
82								
83								
84								
85								
86								
87								
88								
89								
90								

During the findings and result derivation these rules are applied for the method desired for the output produced.



4. Result And Discussion

This research shows the simulation consequences of fuzzy rationale control that was mimicked utilizing Fuzzy technology and after effect of fuzzy rationale control that actualized on two yield boundaries portrayed as S1 and S2. The outcome shows the yield of fluffy rationale that executed on two yield boundaries of fluffy logic. Although there is a distinction in a few yield esteem, however the capacity to control the forced air system is effectively planned as per the ideal.

Figure below show the control of air conditioning in Operation Theatre.

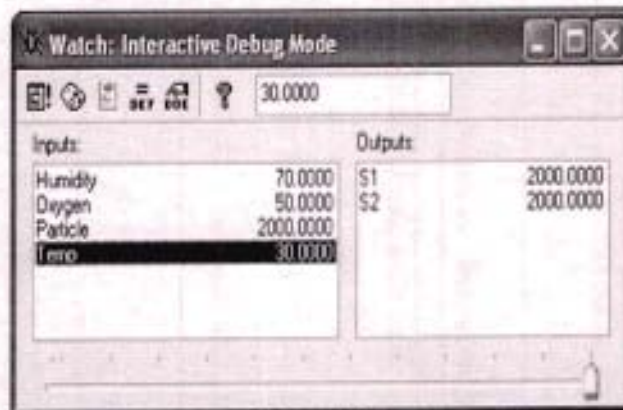


Figure 5.1(a)

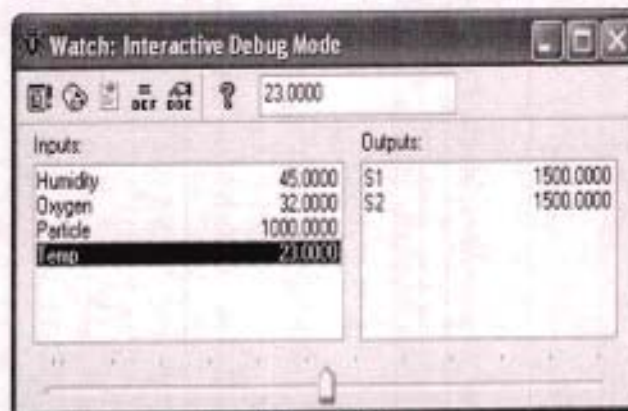


Figure 5.1(b)

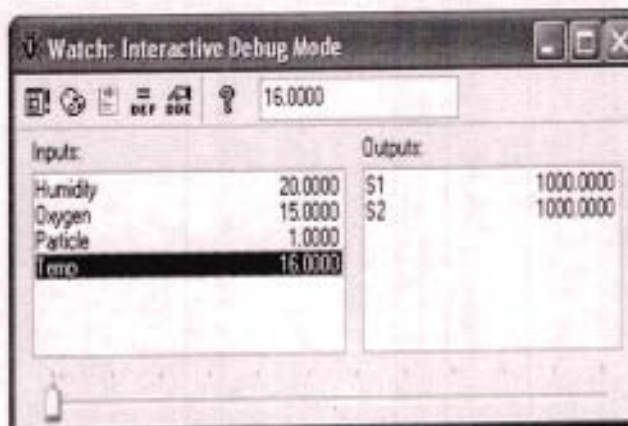


Figure 5.1(c)



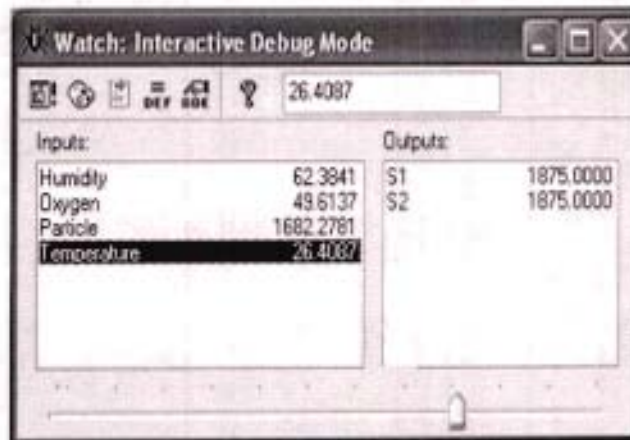


Figure 5.1(d)

These figure shows that (a) when the value of input parameters i.e. is too particle, humidity, temperature, oxygen but the fumes and AC engine speed much maintain at significant level. (b) On lessening the estimation of info boundaries for example moistness, temperature, particle, humidity, temperature, oxygen but the fumes and AC engine speed is likewise diminished to medium. (c) Again on more decrease in the estimation of boundaries esteem for example mugginess, temperature, oxygen but the fumes and AC engine is additionally at low level. (d) When the estimation of temperature is 26.4087 C and at the dampness level 62.3841%, the estimation of particles at 1682.2781 ppm and at the oxygen level 49.6137%; the Fumes and AC engine speed will be encountered as 1875 rpm.

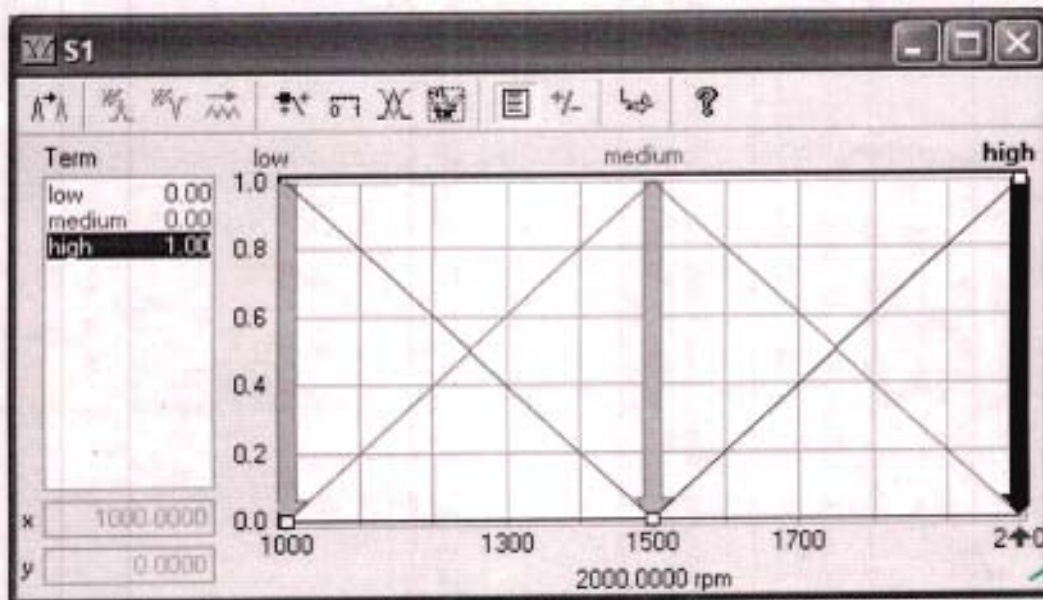


Figure 6. Function of output membership controlling the temperature, humidity, oxygen, particles

Above figure display their yield factors with their related enrollment capacities. Enrollment for the variable speed and plot the function for the AC engine S1/Speed of Exhaust engine S2 utilizing three-sided participation type of work. Finally, in this figure there are three participation of work capabilities are used as Low, Medium, high.

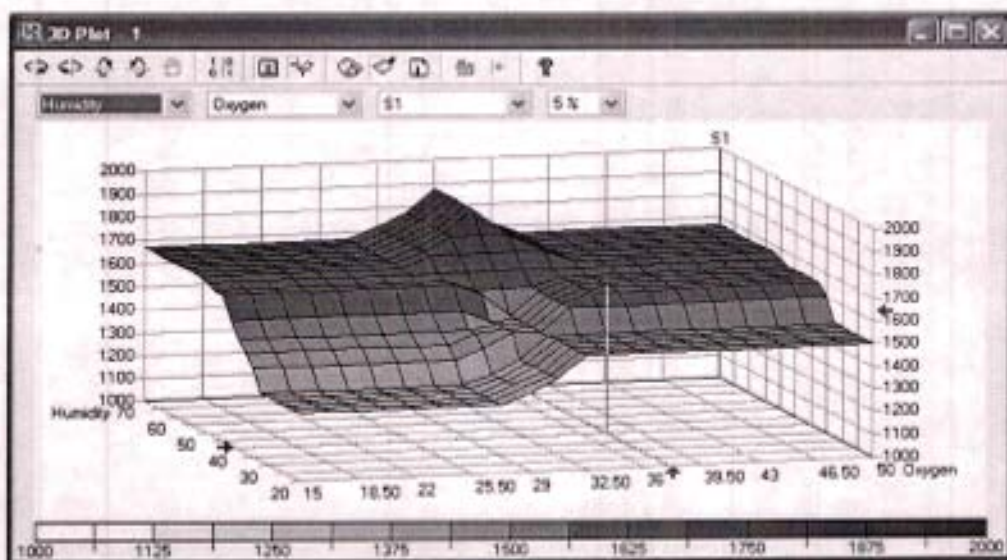


Figure 7. 1 3-D plots for oxygen, humidity & S1/S2

The above figure plot show that when the value of oxygen and humidity increases, the value of output parameter S1/S2 also raised. As per the above figure the humidity will be countered as 40% and the oxygen values will be 37% and their speed is 1632.8300 rpm for S1/S2.

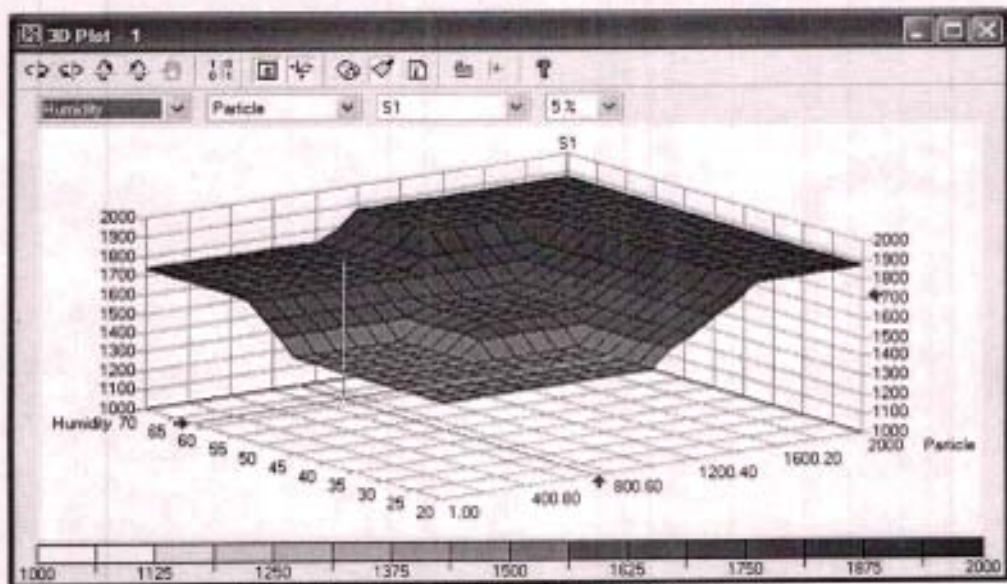


Figure 7.2. 3-D plots for particle, humidity & S1/S2-

The above figure plot shows that when the value of particle and humidity increases, the value of output parameter S1/S2 also raised quickly, according to the above fig. humidity will be countered as 63% and the value of particle is 698 ppm then speed of S1/S2 will be 1735.3400 rpm.



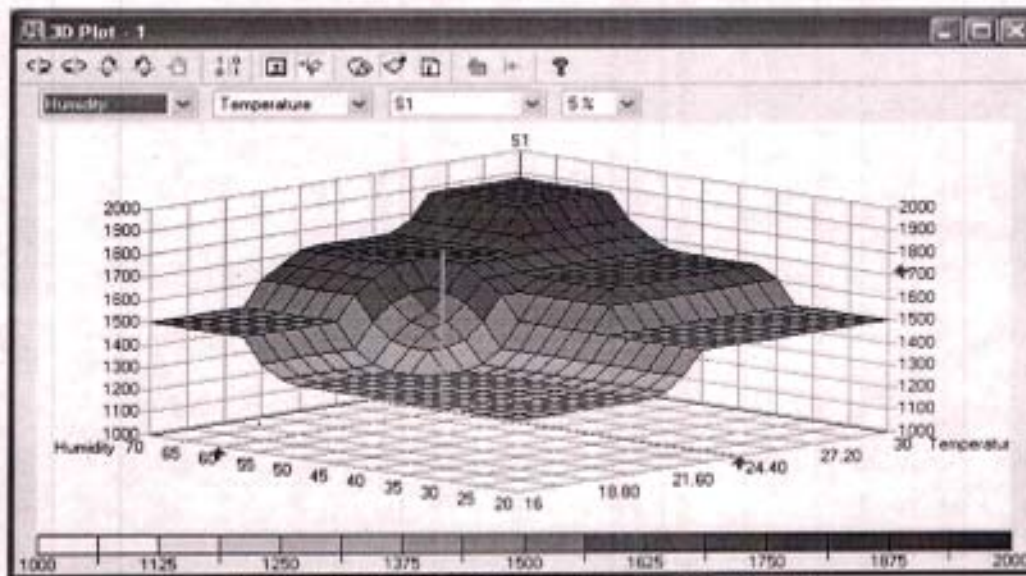


Figure 7.3. 3-D plots for temperature, humidity & S1/S2

The above figure plot show that when the value of input parameter temperature and the humidity increases then the output parameter S1/S2 is also increasing. According to fig. when the value of humidity is at 63% and the value of temperature is 24.40% then speed of S1/S2 will be 1729.0610 rpm.

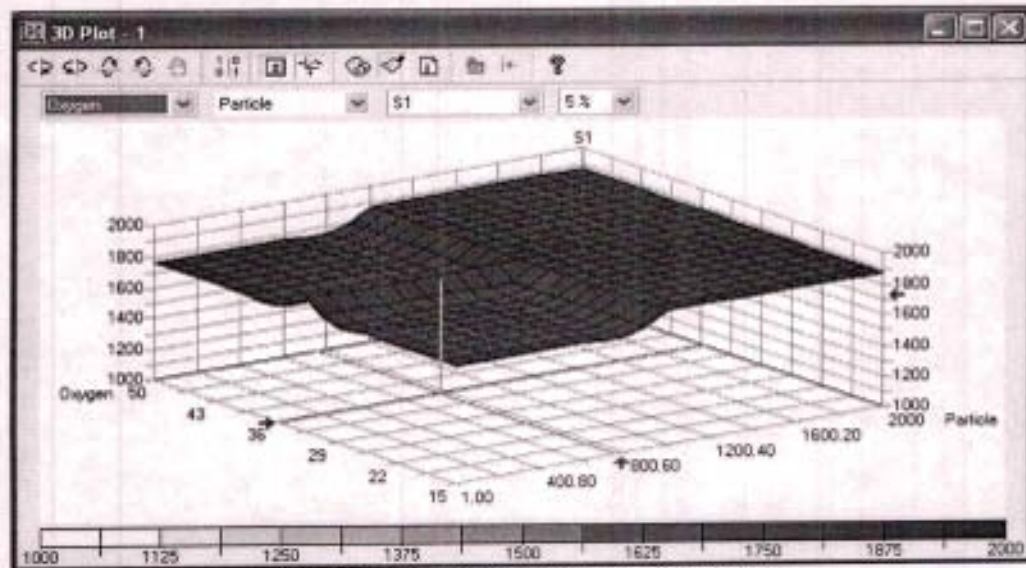


Figure 7.4. 3-D plots for oxygen, particle & S1/S2

The above figure plot shows that when the value of input parameter oxygen and the particle increases then the output parameter S1/S2 is also increasing. According to fig when the value of oxygen is 36% & particle is 800 ppm then speed of S1/S2 will be 1730.3800 rpm.



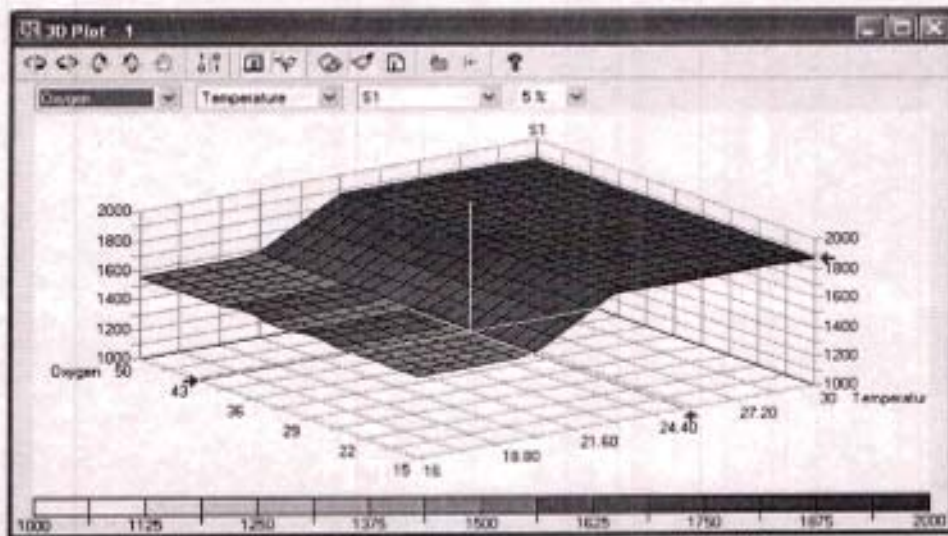


Figure 7.5. 3D plots for oxygen, temperature & S1/S2

The above figure plot show that when the value of input parameter temperature and the oxygen increases then the output parameter S1/S2 is also increasing According to fig when the value of oxygen is 44% and the value of temperature is 25.90 C then speed of S1/S2 will be 1865.6000 rpm.

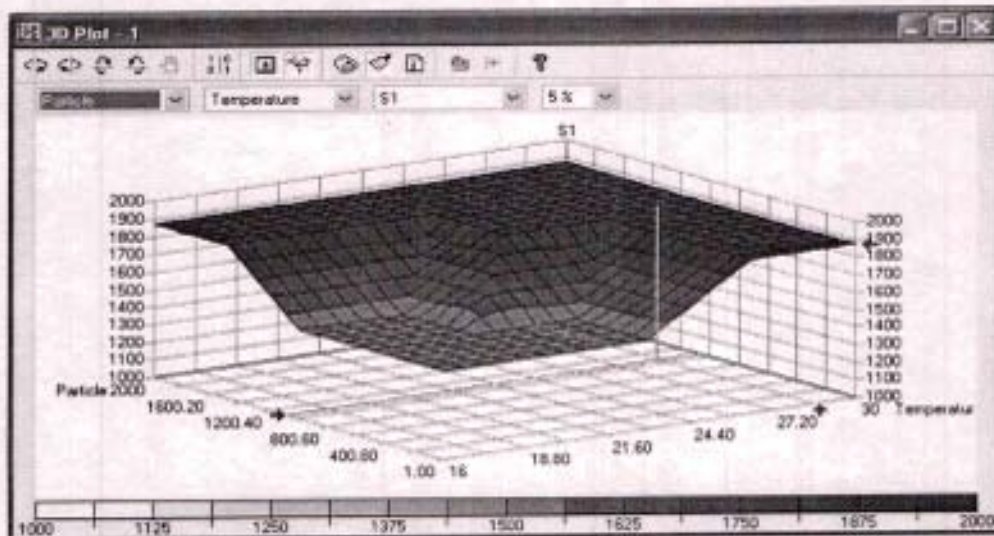
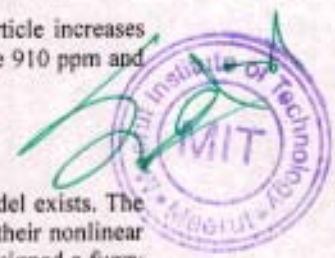


Figure 7.6 . 3-D plots for particle, temperature& S1/S2

The above figure plot show that when the value of input parameter temperature and the particle increases then the output parameter S1/S2 is also increasing. According to fig when the value of particle are 910 ppm and the value of temperature is 28.40 C then speed of S1/S2 will be 1865.0600 rpm.

5. Conclusion & Future Scope

Fuzzy logic techniques are generally used for solving various problems where no definite model exists. The present work also fits into the same category because there are large numbers of parameters and their nonlinear interaction with each other is difficult to model. In the proposed research we have successfully designed a fuzzy logic based controller for environment control of operation theatre. This control approach using fuzzy logic is capable of controlling the nonlinearities of air conditioners and can stabilize the system quickly. All together the system assesses the value of temperature, amount of humidity, percentage of oxygen and amount of particles in air the system then adjust the speed as per the fuzzy rules applies on the speed of AC motors and Exhaust motor. Then, no abrupt difference will be seen on patient and their staff and economical also as it achieve optimum cooling.



The fuzzy logic and artificial intelligence technique may be used to develop hybrid controller for the improvement in performance of the system. In future the fuzzy logic control of environment can be studied by improving the conditions of filtering system with reference to response time etc.

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INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

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Robust and Selective Watermarking using DWT, SVD and Image Depth Map

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Abstract: The spread of digital and media content has increased the risk of copyright violations. Therefore, there is a need for a technique that can either identify or prevent copyright violations. Therefore, there is a need for a technique that can either identify or prevent copyright infringement. For this reason, either cryptographic or non-cryptographic methods can be employed, but the simplest and cheapest option is non-cryptographic, which anyone can use. Therefore, watermarking or steganography may be used. Watermarking is the concealing of digital content into the image to be watermarked. Watermark employs several embedding techniques, including DWT, DCT, SVD, etc. Both DWT and SVD have been utilised in numerous watermarking techniques that have yielded positive results and repelled numerous attacks. In numerous studies, DWT has achieved superior performance. The watermark image utilised DWT and SVD in this research. The watermarked image is processed using DWT and then divided into smaller parts. The blocks are then processed using SVD, and the watermark's binary pixels are inserted on the greatest values of singular values. The alpha values determine the durability of the watermark and the image. The experimental results of the suggested method indicate an improvement in the perceived attributes of the watermarked image, as revealed by their analysis. It was examined against attacks such as AWGN, Median Filters, etc.

Index Term: - Cryptography, Watermarking, Discrete Wavelet Transform (DWT), Singular Value Decomposition (SVD), Discrete Cosine Transform (DCT).

1. INTRODUCTION

A watermark is a distinguishable image on paper that appear as different shades of brightness or darkness when can be viewed by transmitted light and is generated by changes in the density of the material [1]. To prevent duplication, watermarks have been placed to postal stamps, banknotes, and other official papers.

1.1 Introduction to Watermarking

Like traditional watermark, digital watermarking are only discernible under specific condition, that is, when an algorithm is applied, and are otherwise unnoticeable. [2] A digital watermarking that alters the carrier signal to the point where it is discernible is useless. In digital watermarking, the signal can be audio, photographs, videos, texts, or 3D models. A signal that may simultaneously carry many watermarks. Digital watermarks do not alter the size of carrier signals, unlike metadata that is added to the carrier signal.

The required characteristics of a digital watermarking depend on the application. For copyright marking of media file, a digital watermarking must be quite strong against adjustment that can be made to the carrier signals. So that integrity must be maintained, a fragile watermarking should be utilised.

Both steganography and digital watermarking use steganographic techniques to conceal information inside noisy signals. In contrast to steganography, digital watermarking attempts to manage robustness as a primary priority usually. As digital copies of data are identical to the original, digital watermarking is a passive security measure. It only labels data but does not degrade it or restrict access to it. Digital watermarking can be used for source tracking. A watermark is an image that is placed into a digital signal at each distribution point. If a copy of the work is later discovered, from the copy watermark can be extracted and the source of distribution can be determined. Reportedly, this technology has been used to identify the source of illegally pirated movies. Watermarking is the process of embedding the author's identity into digital data so that it can be used to verify the claim when necessary.

Several ways, such as the company's logo, the author's banner, or some sort of signature, can be used to generate a watermark, which can also be created for every image [9]. This is not the primary issue; the embedding and extraction of the watermark is the primary concern. These are the primary means by which the author can assert ownership of an image. The majority of image watermarking research has thus far been conducted in these areas; the watermark remains unaltered even if the watermarked image has experienced significant distortions or alterations.

1.2 Types of Watermarking

Watermarking techniques can be categorised based on a variety of variables. Watermarking can be classified according to the following characteristics:

1. *Domain Based*
2. *Document Based*
3. *Visibility based*

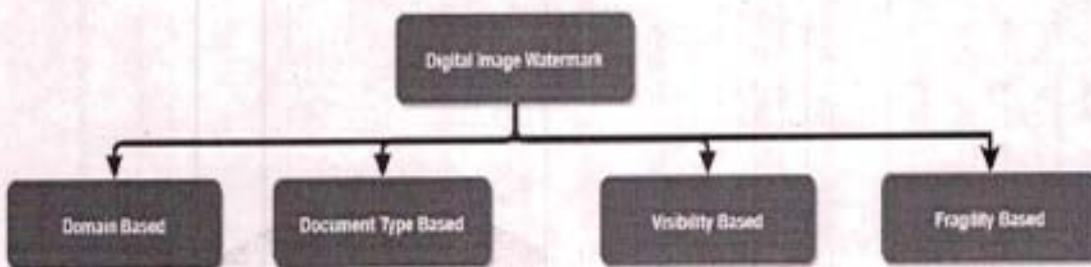


Figure 1 Bases of Watermark Categorized

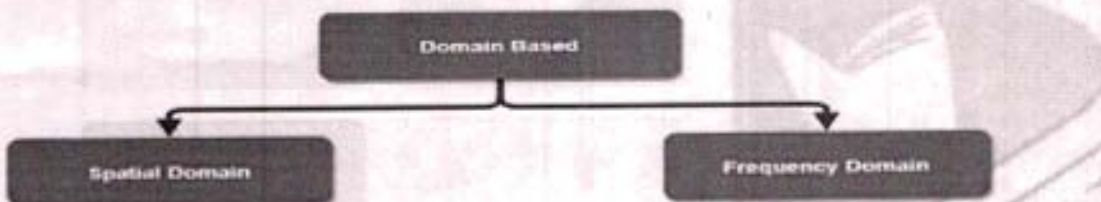


Figure 2 Based on Working Domain

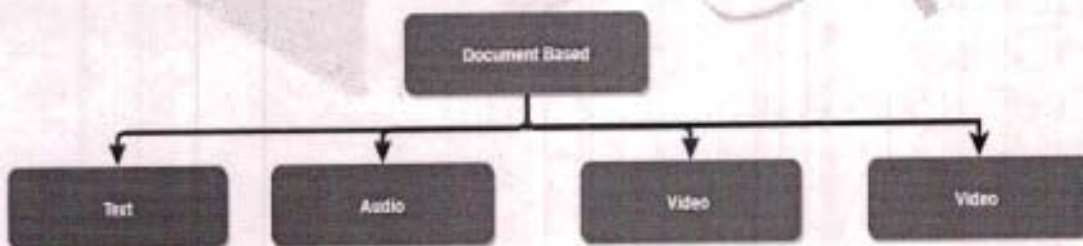


Figure 3 Based on Type of Document

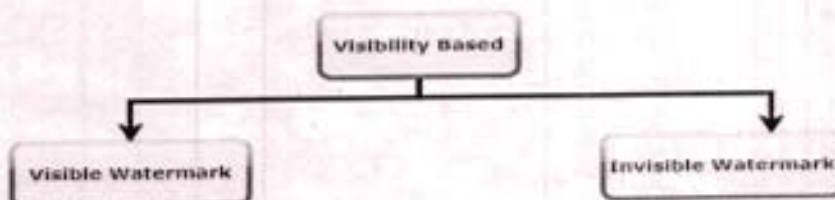


Figure 4 Based on Visibility



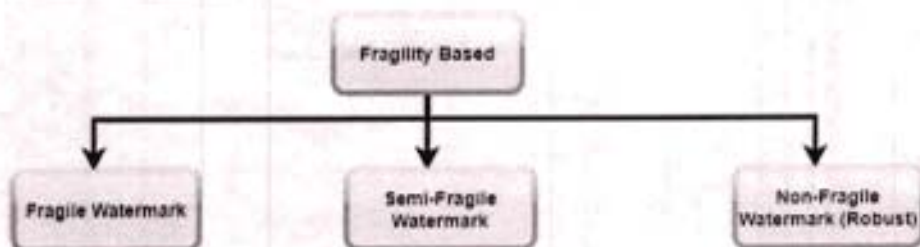


Figure 5 Based on Fragility

1.3 Characteristics of Watermark

The following is a list of characteristics [14] of watermarking:

Imperceptibility: This indicates that the watermark is present in the image, but it cannot be seen by the naked eye due to the way the image was processed.

Robustness: It is utilised in situations in which the watermark has been subjected to various image processing operations such as cropping, resizing, blurring, and so on.

Capacity: The quantity of watermark that an image can retain or that an algorithm can implant without significantly affecting the original image is referred to as the watermark capacity.

Security: A safe and secure watermark is one that cannot be located or removed by an adversary even if they had the key.

False-positive: It is the prerequisite that the detector must indicate the presence of a watermark, even though one does not exist.

1.4 Application of Watermark

Watermarking can be used to authenticate nearly all types of documents. There are numerous applications for watermarking; hence the following are some of the applications [14] for watermarking:

Information Security

Copyright Protection

Content Identification and Management

Content/object authentication

Others application

1.5 Objectives

The Objectives of the proposed work are:

Develop a watermarking algorithm that can embed the watermark adaptively, i.e., with various strengths for the background and foreground, while staying undetectable in the image.

Achieve a high PSNR and SSIM for watermarked images compared to the host image.

To accurately extract the watermark image from the watermarked image.

Apply image processing attacks and determine if the system can withstand these attacks while maintaining the integrity of the watermark, and if so, to what extent.

II. LITERATURE SURVEY

The important qualities of a watermarking algorithm [32] are its fragility, visibility, and capacity. A watermark, which is usually dealt with, should be robust, invisible, and have a large capacity. It should be noted that if there is a need for the validity check of an image, then the watermark should be fragile and invisible. However, for the authenticity of the image, several other methods are used. Our major aim for this survey is to search for robust and invisible watermarks. It should be noted that capacity [34] is usually not important because only a small amount of information, like images, text, etc., is added as a watermark. It is not too important to consider it as a part of the survey. As previously stated, spatial domain watermarks are lower resistant to image processing modifications. The transform domain [36] must be included for the survey. It improves the capacity of watermark placement in images **Error! Reference source not found.**

Burgett et al. introduced the first efficient watermarking technique in the DCT domain [23]. Their approach partitions the image into 8x8 blocks for which the DCT is figure out. In each block, a pair of coefficients is selected. To embed a bit, the coefficients are swapped so that, depending on the bit value, the value of positional difference is either positive or negative.

The method described in **Error! Reference source not found.** models the watermarking channel as a generalised channel with fading and mean additive noise that is not zero. Utilizing an adaptive extractor, they present an optimised watermark extraction approach for their quantization-based watermarking. In [20], four DWT levels are selected as the watermarking domain. This is settled to strengthen the approach while concealing the watermark.

The technique of watermarking in short frequency coefficients of the host image using a sample projection approach is analysed in [21].

The algorithm described in [35] embeds the watermarking in JPEG2000-compressed and encrypted image. This approach utilises the stream cypher technology during the encryption phase. A quantize watermarking technique based on feature modulation was developed in [38]. In this method, the feature signal is the normalised correlation (NC) between the host signal and a certain random signal. In the [31] literature, many embedding, and extraction techniques have been investigated. In [24][25], for instance, GA is used for watermarking purposes. One of the most important applications of watermarking is the detection of forgeries. In [28][37], watermarking is utilised to detect tampering in various multimedia settings. The paper [29] proposes a watermarking approach based on CT. The watermark is embedded in the DCT coefficients of CT's detail blocks. They are inserted in complicated blocks with a greater strength factor. This is done to achieve greater durability. Also, to achieve imperceptibility, smooth blocks are embedded with a weaker force. Using edge concentration and block entropy, the difficulty of each detailed block is determined.

III. PROPOSED APPROACH

Watermarking involves two sub-procedures: embedding the watermark and extracting the embedded watermark. The watermark embedding module includes inserting the watermark into the host image by modifying the intermediate values of the host image part (singular values) with the watermark part (watermark pixels). For controlling the strength of the watermark and the image, another parameter called image depth maps (here it is ground truth images) is used.

In the extraction of watermarking module, the watermark that was inserted in the embedding module is taken out of the watermarked image. For that, the procedure is just the reverse of that embedding.

Some basic terminology used in the watermarking process is as follows:

Host Image: It is the image on which the watermark must be applied. This is a color RGB image.

Watermark Image / Content: This is the data which must be embedded in the original host image to make it a watermarked image.

Watermarked Image: It is an image with watermark content embedded into it. It is obtained after applying a watermarking process.

Ground-Truth Image: It is an image that is obtained after applying Image Depth Map techniques. The Image Depth Map is a separate topic of interest. This would be used to separate the foreground and background images of the host image according to object interest.



3.1. Transformation Techniques

1. *Singular Value Decomposition*: - SVD is a technique for linear transformation in algebra. SVD [4] is the decomposition of a given matrix into two orthogonal matrices and a diagonal matrix. If X is a $m \times n$ matrix that is to be decomposed using SVD, then U and V are orthogonal matrices of dimensions $m \times m$ and $n \times n$, respectively, and Σ is a diagonal matrix with the dimensions $m \times n$, same as X .

$$X = U\Sigma V \dots \dots \dots (1)$$

Σ is known as the singular values (SVs) matrix in equation (1), can have many zeroes at the bottom and rightmost corners of the diagonal matrix. Left singular vectors (LSC) and right singular vectors (RSC) are U and V , respectively. The LSC of X is derived from eigenvectors of XX^T and the RSC of X is derived from eigenvectors of $X^T X$. SVs of the image represent the brightness, whereas singular vectors (SCs) determine the image's geometry. [5]

2. *Discrete Wavelet Transform (DWT)*: - It is extensively utilised in the domains of signal processing and image processing. DWT is a multi-resolution analysis that decomposes an image into fixed-bandwidth frequency channels on a logarithmic scale. One of the benefits of DWT is its resemblance to a structure according to a particular resolution and decomposition at each level

3. *Blocking*: - Blocking is the division of a huge matrix into smaller matrices. Typically, a large matrix is partitioned into 4x4 blocks. Before dividing, the compatibility of the matrix for division is examined. If not, compatibility is achieved by rounding up to the nearest multiple of four. After division, the two dimensions are preserved and can be utilised as necessary.

4. *Alpha Selection*: - The Image Depth map provides ground-level photographs. Consequently, for the alpha selection technique, the ground truth image would be required to depict the foreground and background twenty-six portions of the image. The empirically determined effective foreground and background alpha values are 0.96 and 0.98 for the foreground and background, respectively. The ground truth image is initially binarized using well-known thresholding techniques, such as OSTU global thresholding.

5. *Watermark Intermediate Value Modification*: - To embed the watermark into the host image, certain prerequisite values must be established in the preceding phases. The host image is pre-processed to obtain a group of 4x4 blocks containing the greatest single values from each of the 4x4 input blocks. These singular values are derived from the SVD procedure described in above techniques.

$$A = \alpha B + (1 - \alpha)t \dots \dots \dots (1)$$

B is a component of the host image following pre-processing. α is the image part's strength factor utilised in the watermarked image. t is the portion of the watermark image to be utilised in the watermarked image. This equation is also known as alpha blending. [12][13][15][16].

3.2 Watermark Techniques

The Watermark Embedding Module embeds the watermark into the image. The implementation is described in depth. The procedure entails separating the RGB components and applying the watermark to each of them, i.e., red, green, and blue. The watermark in these components will be utilised to determine whether version of the watermark extracted from the watermarked image being processed here is the most accurate. This describes the input, output, and overall operation of the watermark embedding process.

INPUT:

Host Image: Let us say H1.png

Watermark Image: Let it be W1.png

Ground-truth Image: For Depth Map parameter. Let it be G1.png

OUTPUT:

Watermarked Image: After applying watermark in Host Image.

Measuring Parameter Value: PSNR and SSIM

ALGORITHM:

Initialization Of the Input Variables

Read H1.png using imread () function in MATLAB. Store it in variable, say imgH1.

Read W1.png and store it in a variable, wimg1.



Read GT1.png and store it in a variable, `gting1`

`imgH1`, `wimg1`, and `gting1` are all 2D arrays of the pixel value.

2. Host Image Pre-Processing

Split the RGB components of image `imgH1` and proceed for each component the underneath steps. Let it be `redComp`, `greenComp` and `blueComp`.

Apply Discrete Wavelet Transform (DWT) level-1 on `imgH1`, to get four components `cA`, `cH`, `cV` and `cD`.

$$[cA, cH, cV, cD] = \text{dwt2}(imgH1, 'Haar')$$

"`cA`" is an approximate image, not exactly the copy of original one, it looks but it is not. "`cH`" is the horizontal details of the image; "`cV`" is the vertical details of the image; "`cD`" is the diagonal details. "`dwt2()`" is a built-in MATLAB function for 2-D DWT transform.

Now `cA` is selected for the further processing.

Divide the `cA` into 4×4 . It is stored in `block4x4`. A point to be noted is that `block4x4` is a 3D array, multiple 4×4 blocks are stored in layers one after another.

Apply singular value decomposition (SVD) on the `block4x4`.

$$[u_{comp}, s_{comp}, v_{comp}, singularValue] = \text{svdTransform}(block4x4)$$

`ucomp` is a 4×4 orthogonal matrix (3D array), `scomp` is 4×4 diagonal matrix (3D array), `vcomp` is a four-by-four orthogonal matrix (3D array). These have multiple entries against each four-by-four entry of `block4x4`. `singularValue` is 3D array of dimension $4 \times 4 \times M$, with entries having first elements of four-by-four of `scomp`. `svdTransform()` is a user-defined function which applies the SVD on each of the 4×4 block of `block4x4` using a built-in MATLAB function `svd()` giving the output as mentioned above.

3. Ground Truth Image Pre-Processing

Apply DWT on `gting1`.

$$gtcA, gtcH, gtcV, gtcD = \text{dwt2}(gting1, 'Haar')$$

b) Convert `gtcA` to binary image

$$bwImage = \text{imbinarize}(gtcA);$$

"`bwImage`" is matrix having entries 0 and 1.

c) Each entry of one is replaced by the alpha value of the foreground, and zero is replaced by the alpha value of the background. The α value of the foreground and background must be selected experimentally. It is the strength factor of the host image part used in watermarked images, and $(1 - \alpha)$ is the strength factor of the watermark part. Applying these operations, in the end, we get a 2-D array.

d) Blocking of `alpha2DArray` to 4×4 blocks

$$alpha3DArray = \text{blocking4x4fromnxdn}(alpha2DArray)$$

4. Watermark Image Pre-Processing

Adjust the size of the watermark image to match the size of `singularValue`.

$$sz1 = \text{size}(singularValue)$$

$$dimensionsOfWatermark = \left\lceil \sqrt{\text{ceil}(sz1(1) * sz1(2) * sz1(3))} \right\rceil \left\lceil \sqrt{\text{ceil}(sz1(1) * sz1(2) * sz1(3))} \right\rceil$$

$$watermarkResized = \text{imresize}(wimg1, dimensionsOfWatermark)$$

watermarkResized is resized version of watermark. Its dimension is matching with *dimensionsOfWatermark*

Division of the *watermarkResized* into 4x4 blocks

$$\text{blockedWatermark4x4} = \text{blocking4x4fromn \times n}(\text{watermarkResized})$$

blockedWatermark4x4 is a four-by-four blocked watermark image.

DCT is applied on *blockedWatermark4x4* to get *dctBlockedValue*

$$\text{dctBlockedValue} = \text{dctBox}(\text{blockedWatermark4x4})$$

dctBlockedValue is DCT output of 4x4 blocks in the previous steps.

5. Watermark Intermediate Value Modification

In this section, value modifications are made. *singularValue* from host image pre-processing, *dctBlockedValue* from watermark pre-processing, and *alpha3DArray* from GT Image preparation are the values used. The operation listed below is used to change *singularValue*. It is applied to each (i, j) array element.

$$\text{modSingularValues4x4} = \text{singularValue} * \text{alpha3DArray} + (1 - \text{alpha3DArray}) * \text{dctBlockedValue}$$

This is called alpha-blending.

6. Post- Processing

Retiling *singularValue4x4mod* to s_{comp} to generate modified singular values and take inverse SVD.

$$[s_{compMod}, \text{imgblock4x4}] = \text{insvd}(\text{singularValue4x4mod}, u_{comp}, s_{comp}, v_{comp})$$

Retile *imgblock4x4* blocks to form an approximate image i.e., *cAmod*.

Apply Inverse DWT to get final watermarked image.

$$\text{wdimgFinal} = \text{idwt2}(\text{cAmod}, cH, cV, cD, 'Haar')$$

cAmod is a modified approximate image *cH*, *cV* and *cD* are the values obtained in step 1.

7. Output

wdimgFinal is written to an image file. It is compared with Host Image H1.png, it should be visibly same. So, its PSNR values and SSIM values are recorded to compare how similar they are.

3.3 Watermark Extraction

INPUT:

Host Image: Let us say H1.png

Watermark Image: Let it be W1.png

Ground-truth Image: For Depth Map parameter. Let it be G1.png

OUTPUT:

Watermark Image: The watermark image which was embedded into the host image.

Measuring Parameter Value: PSNR and SSIM, to measure the accuracy of watermark extraction.



FULL ALGORITHM:

1. Initialization Of Input Variables

imgH = read H1.png, Host-image

gimg = read GT1.png, ground-truth image

wmdimg = read wimg1File.tiff, watermarked image.

wimg = read w1.png, watermark.

2. Host Image Pre-Processing

Split the RGB components of image imgH1 and proceed for each component the underneath steps. Let it be redComp, greenComp and blueComp.

Apply Discrete Wavelet Transform (DWT) level-1 on imgH1, to get four components cA, cH, cV and cD.

$$[cA, cH, cV, cD] = \text{dwt2}(imgH1, 'Haar')$$

cA is an approximate image, cV is the vertical details of the image; cD is the diagonal details. `dwt2()` is a built-in MATLAB function for 2D DWT transform.

Now cA is selected for the further processing.

Divide the cA into 4×4 blocks. It is stored in block4x4. A point to be noted is that block4x4 is a 3D array, multiple 4×4 blocks are stored in layers one after another.

Apply singular value decomposition (SVD) on the block4x4

$$[u_{comp}, s_{comp}, v_{comp}, singularValue] = \text{svdTransform}(block4x4)$$

3. Ground Truth Image Pre-Processing

Apply DWT on gimg1.

$$gtcA, gtcH, gtcV, gtcD = \text{dwt2}(gimg1, 'Haar')$$

Convert gtcA to binary image

$$bwImage = \text{imbinarize}(gtcA);$$

bwImage is matrix having entries 0 and 1.

Blocking of alpha2DArray to 4×4 blocks

$$alpha3DArray = \text{blocking4x4fromn}(alpha2DArray)$$

4. Watermarked Image Pre-Processing

Apply DWT on wmdimg

$$[wcA, wcH, wcV, wcD] = \text{dwt2}(wmdimg, 'Haar');$$

Select wcA for further processing

Divide wcA into 4×4 blocks.

$$wimgblock4x4 = \text{blocking4x4fromn}(wcA)$$

Apply SVD on wimgblock4x4



$$[WU_{comp}, WS_{comp}, WD_{comp}, wSingValue] = svdTransform(wimgblock4x4)$$

5. Watermark Value Detection

$$extWimg4x4 = \frac{wSingValue - singValue * alpha3Darray}{1 - alpha3Darray}$$

In this part, the extraction of the watermark from the watermarked image is done. For applying the operation, we would need value *wSingValue* from the Watermarked Image pre-processing step, *alpha3Darray* from the GT Image pre-processing, *singValue* from Host Image pre-processing. These are used to calculate the watermarked content using the formula

6. Post-Processing

extWimg4x4 is a DCT value of watermark extracted, it is need to applied inverse DCT on *extWimg4x4* to get a reconstructed *invDCTBlocked*.

Since *invDCTBlocked* is in blocked form it is needed, to be retiled into an image. So, after retiling the blocks into image we get the watermarked image.

$$extWatermarkImage = retiling4x4(intoImage(invDCTBlocked, dimensionofWimg))$$

7. Output

extWatermarkImage is a watermark extracted image. It is exactly same visually, so for measuring the similarity of the two images, MSE and SSIM is calculated. For binary watermark, the 1s and 0s are replaced by a minimum threshold e.g., 251 and 53, respectively. After that, all the steps are same.

IV. RESULT AND ANALYSIS

In this part, the algorithm for watermarking has been evaluated and the measured values of various parameters have been recorded. The Peak-Signal-to-Noise-Ratio (PSNR) and the Structural Similarity Index (SSI) are the criteria used to analyse the watermarked images (SSIM). SSIM and Mean-Squared Error (MSE) are more often employed to evaluate the retrieved watermark image. However, Bit-Error Rate (BER) can also be employed, but it is more appropriate for binary data. The foreground and background alpha values are set at 0.96 and 0.98, respectively. The watermark picture has a size of 64 by 64 pixels, whereas the host image has proportions of 512 by 512 pixels. A grayscale and binary watermark have been applied. Additionally, the size of GT images is the same as the size of the host image.

$$MSE = 0 / \sum_{i=1}^m \sum_{j=1}^n [x(i, j) - y(i, j)]^2 \quad (1)$$

$$PSNR = 10 \log_{10} \frac{\max(x(i, j))^2}{MSE} \quad (2)$$

$$SSIM(x, y) = \frac{(2\mu_x + c_1)(2\sigma_{xy} + c_2)}{(\mu_x^2 + \mu_y^2 + c_1)(\sigma_x^2 + \sigma_y^2 + c_2)} \quad (3)$$

$$BER = \frac{\sum_{i=1}^m \sum_{j=1}^n x_{ij} - y_{ij}}{\text{total number bits}} \quad (4)$$



TABLE 4.1 ANALYSIS OF WATERMARK EMBEDDING MODULE

S.No	Image File Name	PSNR	SSIM
1	Baby3.png	49.27	0.9998
2	Bowling.png	47.34	0.9997
3	Bowling2.png	44.14	0.9991
4	Cloth1.png	48.86	0.9998
5	Cloth2.png	48.19	0.9999
6	Cloth3.png	45.38	0.9998
7	Cloth4.png	47.67	0.9997

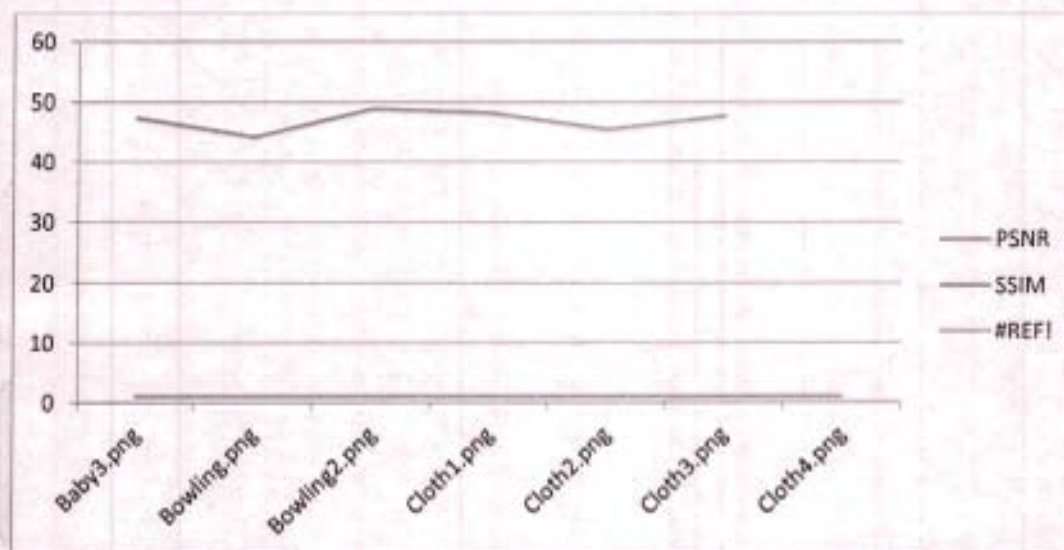


Figure 6 PSNR and SSIM Values

The PSNR values are exceptionally high, indicating that the signal-to-noise ratio is exceedingly high. The SSIM values are close to 1, indicating that the watermarked image is nearly identical to the original image. The average degree of resemblance is 99.48 percent, making it the same image.

TABLE 4.2 ANALYSIS OF WATERMARK EXTRACTION MODULE

S.No	Image File Name	SSIM	MSE
1	Baby3.png	1	0
2	Bowling.png	1	0
3	Bowling2.png	1	0
4	Cloth1.png	1	0
5	Cloth2.png	1	0
6	Cloth3.png	1	0
7	Cloth4.png	1	0

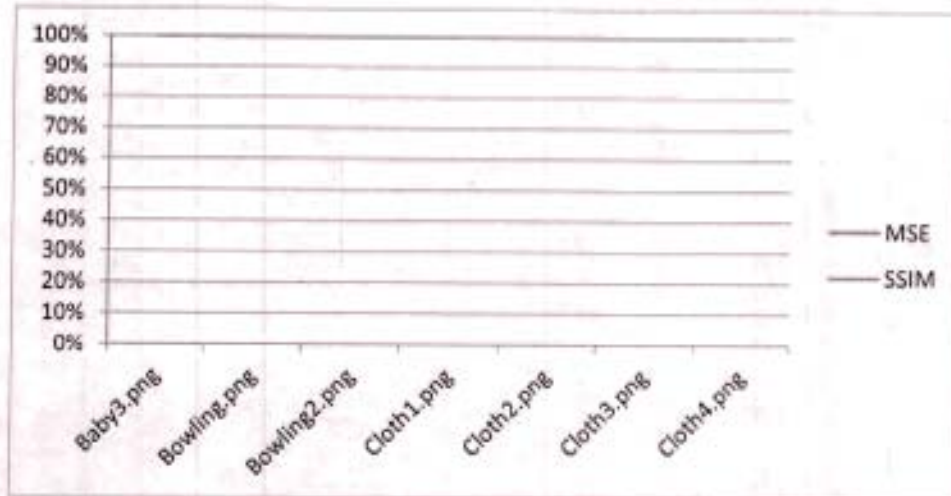


Figure 7 SSIM and MSE Values

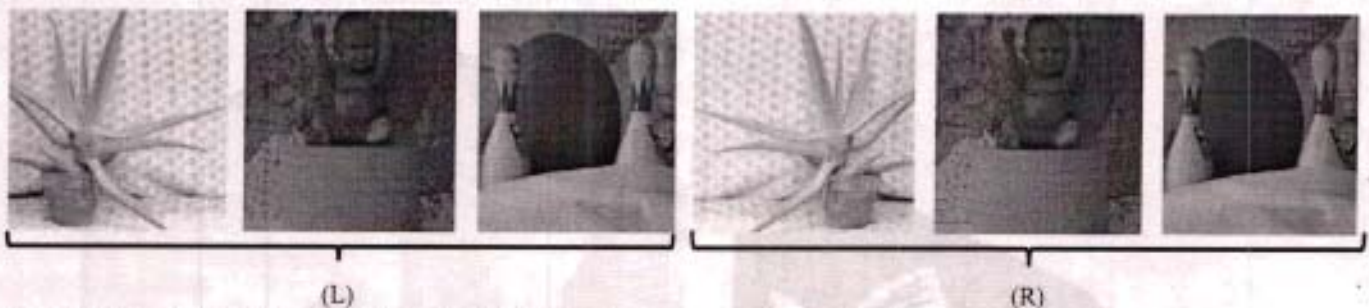


Figure 8 Image (L) Before Watermark and Image (R) After Watermark

V. CONCLUSION AND FUTURE SCOPE

The goal of this project was to design an algorithm that can insert a watermark into an image by using image processing tools like DWT and SVD. The watermark should have been invisible to the human visual system. The binary watermark was taken into account for the watermarking process. In addition to that, RGB images were chosen for this process. In the previous chapter, we have seen that the method is working positively for both processes, i.e., forward, and reversed process. By forward, we mean embedding, and reverse means extracting the watermark image. We applied the image processing attack on the watermarked images and then we tried to extract the watermark out of them. We tried AWGN, Median Filter, Scaling, and JPEG compression on the watermarked image, and then extraction was applied to find out the extent to which the watermark had been extracted from the image. To measure the values, MSE, BER, and SSIM are used. It has been observed that the algorithm performed very well with the bare images and watermarked images. However, it is not exactly similar in the case of applying DIP attacks and extracting watermarks from them. It was expected that at least the algorithm might perform very well with AWGN and the Media filter, but it is not that way. The reason behind the lack of performance as per the expectation is that while generating the image from DWT to SVD and back, the values are modified to insert the watermark, but in doing so the image pixels generated after embedding are all floating-point numbers. Also, when it is rounded-off to the nearest whole number, some of the value gets lost due to floating numbers. This problem needs to be addressed in future research.

Future scope:

Although there can be various improvements to be made to this research, some of the points to highlight for future scope are as follows:

1. The problem with lossy type casting is that it causes the watermark to be distorted initially.
2. The method can be extended to grayscale watermarks and RGB watermarks as well.
3. It has been noted that while applying the JPEG compression, the watermark gets destroyed, at least till quality factor 75%.

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अन्नं बहुकुर्वीत तद्वतम्!

किसान भारती

वर्ष: 53, अंक: 11

अगस्त 2022

संरक्षक

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फसलों के हानिकारक नेमाटोड (सूत्रकृमियों) का प्रबन्धन

ऋषिपाल, सी.एस. प्रसाद एवं वाई.पी. मलिक

नेमाटोड (सूत्रकृमि) सामान्यतया सभी प्रकार के वातावरण उष्ण, उप-उष्ण कटिबंधीय, शीतोष्णक, समुन्द्र व पहाड़ी की चाँटी तक पाये जाते हैं। यह जीव वर्ग का 80 से 90 प्रतिशत बहुकोशिय जीव है। नेमाटोड (सूत्रकृमि) ज्यादातर जलीय जीव है ये घरातलीय अनुकूल भी हो गये हैं। नेमाटोड (सूत्रकृमि) सामान्यतया भूमि में पाये जाते हैं कुछ फसल के वाहय भाग पर अन्यथा ज्यादातर भूमि में पाये जाते हैं। ये भूमि में 1.8 से 120 मिलीयन प्रति वर्गमीटर की संख्या में पाये जाते हैं। संसार में नेमाटोड (सूत्रकृमि पहाड, पर्वत, घाटी, लावस व समुन्द्र में एक परत के रूप में पाये गये हैं। इस समय लगभग 15000 से 500000 या इससे अधिक प्रजातियों का आकलन किया गया है।

विश्व आधार पर 34 प्रतिशत फसलें रोगों, कीटों और खरपतवार के कारण हानि होती है। कुछ जातियों में लगभग 7 प्रतिशत उदारणतया यह आंकलन किया जा चुका है लगभग 35 प्रतिशत फसल वार्षिक हानि व 12 प्रतिशत रोगों नेमाटोड (सूत्रकृमि) द्वारा, 7 प्रतिशत कीटों, 3 प्रतिशत खरपतवार व 11 प्रतिशत नेमाटोड (सूत्रकृमि) द्वारा हानि होती हैं। नेमाटोड (सूत्रकृमि) द्वारा आलु, टमाटर, बैंगन, भिण्डी और पेपर 12.2, 20.6, 16.9, 20.4 और 12.2 प्रतिशत है। इस लेख में महत्वपूर्ण पदप परजीवी नेमाटोड के पहचान, जीवन चक्र व प्रबन्धन के बारे में जानकारी दी गयी है जिसे पाठक लाभ उठा सकें। नेमाटोड (सूत्रकृमि) आकार में लम्बवत गोलाकार, नाशपाती, किडनी व धागे के आकार या मादा आकारनुसार परिवर्तित होता है। शरीर का अग्र भाग हल्का शंकु जबकि पिछला भाग नुकिला शंकु व मुख्य एक्स शरीर लम्बवत और चौड़ाई व्यास में पाया जाता है। शरीर का मध्य भाग क्यूटिकल एपिडमिस कोशिका से बन जाती है मुख आगे की ओर खुलता है। मुख में आगे छिद्र गुफा जो संख्या में 6 स्टाइलेट होते हैं अतः अग्रः आन्त तब आन्त व नर व मादा में अलग-अलग पाये जाते हैं। नेमाटोड (सूत्रकृमि) नर मादा से अपेक्षाकर्त छोटा होता है। नेमाटोड (सूत्रकृमि) में विसर्जन तन्त्र पाया जाता है जबकि श्वसन व सरकुलेटरी तन्त्र नहीं पाया जाता है। नेमाटोड (सूत्रकृमि) में विभिन्न प्रकार का प्रजनन पाया जाता है जैसे स्वतन्त्र रहने वाले

नेमाटोड वो भूमिगत में, सामान्य द्विप्रजनन कुछ जातियों में नर नहीं पाया जाता है। उनमें अनिशोषित प्रजनन पाया जाता है। परजीवी नेमाटोड (सूत्रकृमि) अण्डे जमा करते हैं, पौधों के टिशु या भूमि में एकल या समूह में अण्डे देते हैं अण्डे से लारवा बहार निकल कर पौधे के टिशु की खोज करता है। इसकी चार अवस्थाएँ होती है और फिर वयस्क बन जाता है। पादप परजीवी नेमाटोड (सूत्रकृमि) प्रथम अवस्था अण्डे में और स्टाइलेट द्वितीय अवस्था में शुरू हो जाते हैं। पादप परजीवी पौधे में घुस जाता है और पौधे के टिशु खाने लगता है। जब वाहयः टिशु को खाता है, तो वाह परजीवी कहलाता है।

पादप परजीवी नेमाटोड (सूत्रकृमि) से प्रभाव पड़ता है फसल उत्पादन पर इसके प्रकोप से सभी फसलों में हानि होती है इनके आक्रमण से बाग, रसोई की फसलें भी प्रभावित होती है।

बीज गाल (पिटिका) नेमाटोड (सूत्रकृमि) एंगुनिया ट्रिटीसाई वितरण एवं पोषक

यह नेमाटोड (सूत्रकृमि) विश्व में गेहूँ उत्पाद क्षेत्रों में पाया जाता है। इसका मुख्य श्रोत बीज कोकल रोग से संक्रमित होने के कारण बुवाई के समय बीज के साथ भूमि में प्रवेश कर जाता है। एक बीज गाल (पिटिका) में लगभग 3000 से 12000 द्वितीय अवस्था जूवेनाइल सुसप्तता अवस्था पाये गये

प्रबोधन पर्यवेक्षक, जैविक नियन्त्रण प्रयोगशाला, प्रध्यापक (बीट विज्ञान विभाग), सरदार वल्लभ भाई पटेल कृषि एवं प्रौद्योगिक विश्वविद्यालय-मेरठ उ.प्र.; सह प्रध्यापक (अलसी इकाई) (बीट विज्ञान विभाग), चन्द्रशेखर आजाद कृषि एवं प्रौद्योगिक विश्वविद्यालय-कानपुर, उ.प्र.

हैं। जब ये जूवेनाइल नमी भूमि के सर्म्पक में आते हैं और नमी सोखने के कारण मुलायम हो जाते हैं। ये गाल (पिटिका) को फाड़कर द्वितीय जूवेनाइल कार्यशील हो जाते हैं और भूमि से 10 से 15 दिन में बाहर आ जाते हैं और बीज के जमने के समय जूवेनाइल हमला कर देते हैं और बीज के जमने वाले भाग पर गाल बनाते हैं। उपर तने में वाहयः भाग पौधे के घारो और बढ़ने वाले क्षेत्र में और पत्ती की शीथ में एक पानी की पतली परत के कारण बीज में उपरी परत के सहारे उपर चढ़ जाता है। पत्ती के बढ़ने वाले भाग से पत्ती की छोटी पर चढ़ जाते हैं। ये पौधे की बढ़वार अवस्था में फूल जननाग तन्त्र पर नेमाटोड (सूत्रकृमि) हमला करता है और वाहय परजीवी जूवेनाइल अन्तः परजीवी जे में बदल जाता है। इस अवस्था में नेमाटोड (सूत्रकृमि) गुफा में बन्द हो जाता है और अतिरिक्त बढ़ने के कारण आरम्भिक फूल जो घटनाक्रम में गाल (पिटिका) में बदल जाता है। जूवेनाइल जल्दी से 3 से 5 दिनों में फूल में आफमण कर नर व मादा में परिवर्तित हो जाता है। बहुत से नर व मादा हरे गाल (पिटिका) में उपस्थित रहते हैं। नर व मादा द्वारा प्रजनन करने के बाद मादा अण्डे देना प्रारम्भ कर देती है और अगले 6 से 12 दिनों के अन्दर 1000 अण्डे नये गाल (पिटिका) के अन्दर दे देते हैं जो बाद में विकसित बीज में बनते हैं वयस्क मर जाते हैं और अण्डे से द्वितीय जूवेनाइल निकलते हैं जब फसल पकने वाली होती है गाल (पिटिका) भूरे रंग में परिवर्तित हो जाते हैं द्वितीय अवस्था सुस्पता अवस्था में गाल (पिटिका) में भर जाते हैं बीज द्वितीय अवस्था जे 28 वर्ष पुराने बीज गाल (पिटिका) में जीवीत अवस्था में पाये गये हैं। फसल की कटाई के समय स्वस्थ बीज के साथ गाल (पिटिका) बीज भी एकत्र कर लिये जाते हैं। जब अगले वर्ष की फसल की बुवाई की जाती है अगले वर्ष जीवन चक्र फिर आरम्भ हो जाता है।

क्षति के लक्षण

नेमाटोड (सूत्रकृमि) से संकमित नवजात

पौधा हल्का सा आधारीय भाग फुला हुआ व बीज के जमाव के 20-25 दिन बाद तने पर निकली हुई पत्ती नवजात पौधे की मुड़ी हुई और चोटी पर से मुड़ी हुयी बढ़वार के समय पायी गयी हैं। संकमित नवजात पौधे की बढ़वार रुक जाती है और पौधा मर भी जाता है। संकमित पौधा सामान्या दिखाई देता है। पौधे में बहुत सी फुटाव निकल आता है, और पौधे में बाली 30-40 दिन पहले निकल आती है। बाली छोटी हरी जो लम्बे समय तक सामान्य बाली की अपेक्षा हरी रहती है। बीज पत्र खुले व गाल (पिटिका) में बदल जाते हैं। इस रोग का मुख्य लक्षण कुछ या सब बीज कोकल में बदल जाते हैं। कोकल या गाल (पिटिका) छोटा, गहरा व स्वस्थ बीज की अपेक्षा भूरा और अनियमित आकार में बदल जाता है। गाल (पिटिका) के आकार के अनुसार एक गाल (पिटिका) में 800 से 3500 की संख्या में पाये जाते हैं।

यह नेमाटोड (सूत्रकृमि) बीजाणु सह सहायक के कारण पीली बाल या टुण्डा रोग हो जाता है। नेमाटोड (सूत्रकृमि) बीजाणु को फैलाने का काम करता है। इस रोग के कारण नवजात पौधे की पत्ती व बाल पर हल्का पीला सा पर्दाथ जमा हो जाता है। आरम्भिक लक्षण टुण्डा समान बाल कोकल रोग के कारण नमी के वातावरण में उत्तक पर पीला कीचड़ गिरता हुआ देखा गया है और जो बाद में कड़ा हो जाता है और सूखने पर भूरा हो जाता है। संकमित पौधे से बाल बूट से नहीं निकल पाती और न ही बाली में दाना बनता है। जीवाणु लक्षण दिखाई देते ही बाल को नष्ट कर देना चाहिए। उत्तरी भरत में बीज गाल नेमाटोड (सूत्रकृमि) एंगुनिया टिट्टीसाई जिम्मेदार गेहूँ में बाल कोकल रोग व टुण्डा या पीला मौजक रोग को फैलाता है। इस रोग के बीजाणु क्लेविबेक्टर टिट्टीसाई के कारण इसके द्वारा 1 प्रतिशत से कभी-कभी 80 प्रतिशत तक हानि होती है। इस नेमाटोड (सूत्रकृमि) के कारण उत्तरी भरत में लगभग 10,000 टन गेहूँ की जिसकी कीमत 70 मिलीयन रूपयें हैं।



नियन्त्रण

1. बीज की सफाई

टुण्डा रोग या बाल कोकल रोग रहित या नेमाटोड (सूत्रकृमि) रहित बीज लेना चाहिए। इसे प्राप्त करने के लिए बीज को छन्नी से छान्ने पानी में 20 प्रतिशत का ब्राइन घोल में डालकर तैरते हुये बीज अलग कर लेने चाहिये। प्रमाणित नेमाटोड (सूत्रकृमि) रहित बीज खरीदने चाहिए।

2. गर्म पानी से उपचारित करना

बीज को 4 से 6 घन्टे ठन्डे पानी में भिगोना चाहिए। तब 54° सेन्टीग्रेड गर्म पानी में 10 मिनट तक उपचारित करना चाहिए।

3. फसल हेर फेर कर बोना

नेमाटोड (सूत्रकृमि) खेत से बहार करने के लिए पोषक फसल की 2 या 3 वर्ष तक बुवाई नही करनी चाहिए।

4. रोग रोधी किस्म

नेमाटोड (सूत्रकृमि) अवरोधी प्रजातियों खेत में बोनी चाहिए।

5. अवांछित पौधे निकालना

नेमाटोड (सूत्रकृमि) संक्रमित पौधे पता लगाकर अगेती अवस्था में नष्ट कर देना चाहिए।

6. सूप से फटकाना या हवा में उड़ाना

यह विधि भी सहायक गाल को बहार करने के लिए इस विधि से 100 प्रतिशत गाल (पिटिका) अलग नहीं होते हैं।

मूल गॉठ नेमाटोड (सूत्रकृमि) मेलोइडोगाइन प्रजातियों

पहचान व जीवन चक्र

विश्व के विभिन्न भागों में इस नेमाटोड (सूत्रकृमि) की 80 प्रजातियों पायी गयी है और उनमें

से 13 प्रजातियों भारत में पायी जाती हैं। इस नेमाटोड (सूत्रकृमि) के 2000 से अधिक पोषक पौधे हैं। इसे सब्जी, दलहन अच्छे पोषक पौधे हैं, जबकि अनाज वाली फसल कम पोषक हैं। इस नेमाटोड (सूत्रकृमि) का घातक समस्या सब्जी फसले जैसे बैंगन, भिन्डी, टमाटर, कद्दू, आलू, मिर्च, इसके अतिरिक्त दलहन फसलें अरहर, हरा चना, काला चना, और लोबीया, फल फसलें विशेषकर पपिता और अंगूर रेशे वाली फसलें जूट और कपास तेल वाली फसलें विशेषकर मूंगफली और सूरजमुखी आदि फसलें हैं। मूल गॉठ नेमाटोड (सूत्रकृमि) पोषक पर एक ही स्थान पर रहने वाला अन्तः परजीवी है। मादा नेमाटोड लगातार 10-12 दिन तक अण्डे देती हैं। और एक मादा लगभग 200-400 अण्डे देती हैं। जबकि अच्छे पोषक पौधे पर 2000 तक अण्डे देती हैं। मादा अण्डे की थेली में अविभाजित पूर्ण विकसित व द्वितिया अवस्था जूनेवाइल पाये जाते हैं। पूर्ण अवस्था 10-15 दिन में पूर्ण हो जाती है। अण्डे में प्रथम अवस्था जूवेनाइल विकसित होता व द्वितिया अवस्था अण्डे से बहार निकलकर भूमि की ओर जाता है और पौधे की मूल की छोटी में प्रवेश करता है। नेमाटोड (सूत्रकृमि) की द्वितिया अवस्था ही संक्रमित या परजीवी अवस्था है। नेमाटोड (सूत्रकृमि) द्वितिया अवस्था कई महीनों तक भूमि में जलीय दशा में अवातावरणीय अवस्था में रहते हैं। लारवा मूल को बेधकर मूल के कोर्टक्स अविभाजित वाहक क्षेत्र में लम्बत मूल के सिरे से प्रवेश कर वहाँ लारवा की सभी अवस्थाये पायी जाती हैं। और परिवक्ता अवस्था तक खाता है। जब द्वितिया अवस्था खाना प्रारम्भ करता है जड़ में गोल आकार का फूला हुआ दिखाई देता है। जनन अंग आकार में बढ़ जाते हैं, द्वितिया अवस्था के देर की अवस्था में जन्नांग भिन्न हो जाते हैं। मादा में जननांग भी आकार के नर में आई आकार के विकसित हो जाते हैं। लगभग एक सप्ताह बाद द्वितिया अवस्था से तृतीय अवस्था बहार निकलता है। जो द्वितिया अवस्था जैसा दिखाई देता है लेकिन स्टाईलेट से खाली रहता है। तृतिया अवस्था जल्दी से चतुर्तिया अवस्था में आ

जाती हैं। 3 से 4 दिन में स्टाईलेट दोबारा से आ जाते हैं स्पाइकूल विकसित और अन्तिम अवस्था में पाचन व प्रजनन तन्त्र विकसित हो जाते हैं। जीवन चक्र गर्मि व बर्शात में 21 से 27 दिन जबकि शरद ऋतु में लम्बी अवधि 50 से 80 दिन से उपर होता है, यह वातावरण की दशा विशेषकर तापक्रम पर निर्भर करता है। एक वर्ष में 7 से 8 पिढ़िया पायी जाती है। पिढ़ियों की संख्या तापक्रम व उचित नमी और पोषक पर निर्भर करती हैं।

हानि के लक्षण

संकमित पौधे में प्रायः पत्ती पिली, छोटी, पौधे की बढ़वार रुक जाती है, हल्के व कम फल बनते हैं, और गर्म दिनों में पौधे मुरझा जाते हैं। नेमाटोड (सूत्रकृमि) से भूमि संकमित होने के कारण पौधे कम निकलते हैं। इस नेमाटोड (सूत्रकृमि) का नवजात पौधे पर अधिक प्रभाव पड़ता है और निकलते ही सूख जाते हैं उदारहणः समय से पूर्व फलों का शुरु हो जाना इसके लक्षण के कारण हैं सामान्य लक्षण टुकड़ों में बढ़वार जो कि अनियमित संख्या का खेत में वितरण इस कारण नेमाटोड टुकड़ो में निकलते हैं। भूमि में निचे मूल में गाल जो नेमाटोड (सूत्रकृमि) से संकमित की पहचान हैं। पौधे की मूल पर गोंठ का आकार व आकृति जाति, निमेटोड की संख्या, पोषक का आकार, फसल की आयु पर निर्भर करता हैं। मूल गोंठ के कारण मूल का वजन कम हो जाता है और मूल तंत्र छोटा होने के कारण पौधे खाद्य कम लेने के कारण पौधे की वृद्धि कम हो जाती है। इस नेमाटोड (सूत्रकृमि) से सब्जी वाली फसल अधिक प्रभावित होती है और कद्दू वर्गिय सब्जी में गोंठ भारी पायी जाती है मूल गोंठ नेमाटोड (सूत्रकृमि) द्वारा 11 प्रतिशत हानि सब्जी फसल में और प्रक्षेत्र में अधिकतम 25 से 50 तक होती हैं।

नियन्त्रण

1. कृषि कियाए विधि

- खेत की गर्मी के दिनों में मई-जून में 2-3 गहरी जुताई, फसलों को बदलकर व बुवाई की समय

बदलकर बाने से इसके संकमित को कम किया जा सकता हैं। फसल-चक्र में अनाज वाली फसल बोनी चाहिए।

- फसलों के अवशेषो को जलाकर नष्ट करने से इनकी संख्या को कम किया जा सकता है। अन्तः फसल जैसे-प्याज, लहसुन, को पोषित फसल के बीच में बोने से इसके प्रकोप को कम किया जा सकता हैं।

2. अवरोधि प्रजातियाँ

- मूल गोंठ अवरोधी प्रजातियाँ बोने से आर्थिक हानि से बचा जा सकता हैं।
- टमाटर-पंजाब एन. आर. 07, हिसार ललित, बनारस जैन्ट आदि।
- बैंगन-माइजर हरा, विजय संकरण, ब्लैक ब्यूटी, पुसा परपिल लोंग।
- भिण्डी-लॉग ग्रिन स्मूथ, आई. सी. 9273, आई. सी. 18960 आदि।
- मिर्च-पूसा ज्वाला, कैप 63, ज्वाला, लोबिया-सी. 152 तम्बाकू-एन.सी. 45।

3. रासायनिक विधि

- पौध वाली फसलों की नर्सरी को 3 ग्राम या 0.3 ग्राम प्रति मीटर से उपचारित करना चाहिए।
- सीधे खेत में बाने वाली फसलों जैसे भिण्डी कद्दू, लोबिया, के बीजो को कार्बोफुरान 2-3 ग्राम प्रति कि.ग्रा. से उपचारित करना चाहिए।
- बैंगन व टमाटर की पौध को 0.1 प्रतिशत मोनोकोटोफोस के घोल या 0.05 प्रतिशत ट्राइजोफोस के घोल में जड़ों को खेत में लगाने से पूर्व 6 घंटे पहले भिगों लेना चाहिए।
- खेत में प्रयाप्त मात्रा में लैविक करंज, महुआ, और नीम की खली 2.5 टन प्रति हैक्टेयर के हिसाब से डालना लाभदायक पाया गया हैं।

4. जैविक नियन्त्रण

- फंगस पेसीलोकमाइसस लिलासीनस और जीवाणु



पस्टुरिया पनीटरन्स मूल गॉठ का मुख्य जैविक नियन्त्रण पाया गया है।

धान का मूल नेमाटोड (हिरसचमनियाला प्रजातियों)

पहचान व जीवन चक्र

यह स्थान्तरण अन्तः परजीवी नेमाटोड है। दोनों नर व मादा वयस्क पूरा जीवन वर्मिफोरम हैं। इस निमेटोड की विश्व स्तर 22 प्रजातियों पायी जाती हैं। हिरसचमनियाला प्रजाति विश्व स्तर पर आर्थिक दृष्टि से महत्वपूर्ण प्रजाति है। इसकी जनसंख्या नमी क्षेत्रों के अपेक्षा निकास का उचित प्रबन्धन वाले क्षेत्रों में अधिक होती है। ये सूखे में 5 महिने से उपर और पानी में 7 महिने जीवीत रह सकते हैं। धान इसका मुख्य पोषक है, घास कुल के पौधों को भी खता है इसके अतिरिक्त सब्जी, मक्का, गन्ना आदि। इस नेमाटोड (सूत्रकृमि) की सभी अवस्थाएँ मूल के निचे छोटी वाले भाग में प्रवेश करता है। अण्डे जड़ के अन्दर देता है। अण्डे से जूवेनाइल पौधे के कोर्टेक्स भाग में 5-6 दिन में विकसित होते हैं। अण्डे फुटने के बाद जूवेनाइल लगातार मूल के अन्दर घुमकर खाना प्रारम्भ कर देता है। यह वयस्क बनने तक चार निर्मोचन करता है। प्रथम चिर्मोचन अण्डे के बहार करता है। यह पूरा जीवन चक्र लगभग 30 दिन में पूरा कर लेता है। एक फसल में 3 से 4 पिढ़ि पायी जाती हैं। जूवेनाइल या वयस्क भूमि से नई मूल की खोज में निकलते हैं। अधिकतर नेमाटोड (सूत्रकृमि) की संख्या फूल आने के समय देखी गयी है। व मूल की कमिक क्षति कटाई तक होती है।

क्षति के लक्षण

इस नेमाटोड (सूत्रकृमि) की भूमि के उपर कोई विशेष लक्षण आसानी से पोषक तत्वों की कमी और असन्तुल सूक्ष्म जैव तत्वों के कारण स्पष्ट नहीं दिखायी देती है। तब भी मूल की क्षति होने के कारण पौधे की वृद्धि रुक जाना, क्लोरसिस व पौधे की फुटान कम देखी गयी है। संकमित होने पर फूल 10

से 12 दिन देर से आना इस कारण फसल देर से पकती है। कभी-कभी द्वितीया संक्रमण जीवाणु व फंगस को देखा गया है, इस अवस्था में जड़ भूरी और काली अन्त में सड़ जाती है। जड़ का निरिक्षण करने पर जड़ का उतक क्षय और खोखली और बहुत से खाली स्थान कोर्टेक्स में पाये जाते हैं। इस नेमाटोड (सूत्रकृमि) द्वारा लगभग औसतन 25 प्रतिशत उपज की हानि का आंकलन किया गया है। इसकी हानि ज्यादा पूर्वी और दक्षिण राज्यों में लगातार धान की फसल उगाने व अनुकूल वातावरण के कारण होती है।

नियन्त्रण

1. खेत की गर्मी में मई-जून के दिनों में 2-3 गहरी जुताई करके छोड़ देना चाहिए।
2. फसल चक्र में मूंगफली, जूट, गेहूँ और आलु या अन्त फसल साथ में ट्रेप फसल सेसबानिया रोस्टरटा जो खेत नेमाटोड की संख्या कम करने में सहायक है।
3. खेत में सरसों व नीम की खली डालने से निमेटोड की संख्या बढ़ने से रोकता है।
4. भूमि उपचार कार्बोफुरान ग्रेनुलस 1 कि.ग्रा. प्रति हैक्टेयर बीज की नर्सरी और बीज की दुवाई के 20 दिन पहले या पौध स्थान्तरण के समय डालने पर निमेटोड की संख्या को कम करेगा व खेत में दूसरी मात्रा 15 दिन के अन्तराल पर देनी चाहिए।
5. पौध की जड़ फोस्फामिडान 0.2 प्रतिशत घोल में पौध के स्थान्तरण से पहले डुबा लेनी चाहिए।

अनाज (सिरियल) का गॉठ निमेटोड (इंटरोडेरा एविनी)

पहचान व जीवन चक्र

यह नेमाटोड (सूत्रकृमि) गेहूँ और ज्वार के मोत्या रोग का रोगकारक है। यह नेमाटोड (सूत्रकृमि) विश्व के गेहूँ और ज्वार उत्पादक क्षेत्रों में अधिक पाया जाता है। भारत में राजस्थान, पंजाब, हरियाणा,



दिल्ली, हिमाचल प्रदेश, जम्मू कश्मीर, मध्य प्रदेश और उत्तर प्रदेश में प्रत्येक वर्ष लगभग 50 प्रतिशत फसल इस नेमाटोड (सूत्रकृमि) द्वारा संक्रमित होती है। अधिक हलकी मृदा में लगभग 80 से 100 प्रतिशत हानि होती है। इस नेमाटोड (सूत्रकृमि) के गेहूँ मुख्य पोषक लेकिन राई, जई और कुछ घास परिवार के पौधे भी परजीवीत होते हैं। अनाज सिस्ट गॉठ नेमाटोड (सूत्रकृमि) मूल में एक ही स्थान पर रह कर खाने वाला कीट है। अण्डे गॉठ के अन्दर देता है व कई वर्षों तक जीवित रहते हैं। ये गॉठ से अलग होकर भूमि में निकल जाते हैं और अगले वर्ष में प्राथमिक संक्रमित करते हैं। नीबू के आकार की गॉठ मार्च अप्रैल और अक्टूबर-नवम्बर तक 400 अण्डे गॉठों में रहते हैं, इस समय अण्डे में द्वितीय अवस्था जूवेनाइल शुरुपता अवस्था में रहते हैं। फसल के शुरुवात में फसल की अगली बुवाई के समय नवम्बर से जनवरी संक्रमित जेवेनाइल द्वितीय अवस्था गॉठ से निकलने प्रारम्भ हो जाते हैं। एक सीजन में 50 प्रतिशत अण्डे फुट जाते हैं, और बाकी अगले सीजन तक सुरक्षित रहते हैं। मूल के संकुचन से सिस्ट के निकलने पर सामान्य प्रभाव पड़ता है ज्यादातर लारवा निकलते हैं। जब फसल 4 से 5 सप्ताह पुरानी व तापक्रम 16 से 18° सेन्टी ग्रेड हो जाता है जेवेनाइल की द्वितीया अवस्था सामान्यतया मूल की चोटी से प्रवेश करते हैं मेरीसेमेटिक भाग नेमाटोड (सूत्रकृमि) भेदक का पक्षीय स्थान है। शरीर के विकसित होने में तीन निर्मोचन 4 सप्ताह में लेता है भोजन लेने के बाद द्वितीय अवस्था चौड़ाई में बढ़ती है। नादा नीबू का आकार लेती है, और सफेद रंग की होती है। मूल में प्रवेश के 4-5 सप्ताह बाद मूल से बहार निकलते देखा जा सकता है। मादा तभी मर जाती है और उसकी शरीर की भित्ति कठोर व गॉठ से भरी जो अगले सीजन को संक्रमित में काम आती है। नर वयस्क गोलाकार केंचुए जैसा होता है। नर का विकास 3 निर्मोचन बाद जो लारवे होते हैं, लारवें आखरी निर्मोचन के बाद वयस्क में बदलता है। इसका जीवन चक्र 9 से 14 सप्ताह में पूरा कर लेता

है, और वर्ष में एक पिढ़ी पायी जाती है।

क्षति के लक्षण

इस नेमाटोड के संक्रमित के लक्षण प्रक्षेत्र में टुकड़ों में दिखाई देता है। आरम्भिक अवस्था के समय जो कम में अन्दर का प्रक्षेत्र 3 से 4 वर्ष में फँस जाते हैं। आरम्भिक लक्षण लगभग 3 से 4 सप्ताह बाद फसल की बुवाई के जमाव के समय दिखने लगते हैं इसके अतिरिक्त बढ़वार रुकना और सामान्य उतकक्षय: संक्रमित पौधे पीले और हरे पीले रंग के दिखाई देते हैं पत्ती रंगहीन पीले स्टीफर में बदल धने और पत्ती नुकीली धारीदार अपेक्षा कृत स्वस्थ पौधे के फुटाव बड़ी मात्रा में कम होना तथा तना पतला कमजोर हो जाता है ऐसे रोग ग्रसित पौधे फूल आने से पूर्व पकजाना और बाली में बहुत कम दाने बनते हैं अधिक संक्रमण होने पर दाना नहीं बनता है और बहुत कम दाने बनते हैं। संक्रमित जड़ छोटी और और बहुत सारी शाखायें जो गुच्छों के रूप में दिखाई देती है। और पौधे के आधार पर मुसला जड़ विकसित हो जाती है। राजस्थान दशा में एक सफेद गॉठ सामान्यतया विकसित हो जाती है जड़ पर फरवरी के मध्य जो की मिश्रित नेमाटोड के आक्रमण का दिखाई पड़ता है रेशे वाली जड़ें मुख्यतया भूमि से आंसानी से खिचे जा सकते हैं, हल्की भूमि इसका प्रकोप अधिक और 45 से 48 प्रतिशत हानि का आंकलन किया जा सकता है। इसके 6 अण्डे प्रति ग्राम मिट्टी में पाये जाते हैं।

नियन्त्रण

1. कृषि कियाए विधि

- गॉठ नेमाटोड एक विशेष पोषक और सूखे न सहने वाला है फसल चक्र व गर्मी की जुताई से प्रबन्धन किया जा सकता है।
- पोषक अवरोधी फसलें जैसे-सरसों, चना, कारिन्डर, गाजर, फेन्चबीन, आदि से 2 वर्ष लगते हैं।
- अपोषक फसल उगाने पर संक्रमित फसल में 60



प्रतिशत तक निमेटोड की संख्या कम की जा सकती है।

- जून-मई के महीने में 2-3 गर्मी की जुताई करने पर नेमाटोड की संख्या करने में लाभदायक पाया जाता है। क्योंकि गर्मी के महीने में गॉट पोषक कम हो जाते हैं। अगेती बुवाई गेहूँ फसल हानि को कम करते हैं 1 से 1.5 महीने की फसल नेमाटोड के आक्रमण सहन करने की क्षमता रखती हैं।

2. अवरोधी प्रजातियों

भारत में गेहूँ की निमेटोड अवरोधी प्रजातियों सी.सी.एन.आर.वी.-1, राज एम.आर.-1, उगाई जाती हैं।

- ज्यों की प्रजातियों राज किरन, आर. डी. 2035, आर. डी. 2052, और सी. 164 अवरोधी प्रजातियों व संकमित प्रक्षेत्र में उगाई जाती हैं इन प्रजातियों में मादा अण्डे देने में असफल होने के कारण नेमाटोड की संख्या कम हो जाती है।

3. रासायनिक विधि

- भूमि को 3 ग्राम कार्बोफुरान या फोरेट 10 ग्राम प्रति 2 कि.ग्रा. प्रति हैक्टियर डालने पर नेमाटोड पर नियन्त्रण पाया जा सकता है।

4. एकीकृत नियन्त्रण

- मई-जून के महीने में गर्मी की जुताई और अपोषक फसल के बुवाई जैसे- सरसों, चना या अवरोधी प्रजाति बाने पर नेमाटोड की संख्या कम करने में लाभदायक पाया गया है।

धान का तना नेमाटोड (हिटीलेन्चस एन्गुलस)

पहचान व जीवन चक्र

इस नेमाटोड (सूत्रकृमि) की 50 प्रजातियों पायी जाती हैं, जिनमें एक दर्जन आर्थिक आधार पर महत्वपूर्ण प्रजातियाँ हैं। भारत में डिटिलेन्चस एन्गुलस एक महत्वपूर्ण उफरा रोग फैलाने की जिम्मेदार है। यह नेमाटोड (सूत्रकृमि) धान उत्पादक

क्षेत्रों एशिया अफ्रिका, बंगलादेश, ब्रमा, मलेशिया, थाईलैन्ड, फिलीपिन्स, इजिप्ट, और भारत हैं। भारत में व्यापक प्रचलित आसाम, पश्चिम बंगाल, बिहार और उत्तर प्रदेश हैं। उफरा रोग मुख्यतया गहरे पानी वाले क्षेत्रों में या वे क्षेत्र जहाँ खेत में गतिहीन पानी भरा हो पाया जाता है। यह धान का घातक कीट है और भारत में इसके कारण 5 प्रतिशत से अधिक उपज में कमी आंकी गयी है। यह नेमाटोड (सूत्रकृमि) धान की फसल का एक वाहय परजीवी तथा धान की प्रजातियों का मुख्य पोषक है।

फसल के पकने के समय बयस्क और चतुर्थ अवस्था जूवेनाइल नेमाटोड (सूत्रकृमि) खाता है। धान के सूखे भाग पर चारों ओर जीवित रहता लिपटा रहता है। और है 6 से 15 महीने तक समुचित दशा मिलने पर ये बिना खुले सकिय रहता है पोषक पौधे की खोज में यह नेमाटोड (सूत्रकृमि) पानी में 4 महीने से उपर जीवित रहता है। बीज के जमने के बाद पौधे में नेमाटोड (सूत्रकृमि) प्रवेश कर खाने लगता है। प्रजनन पोषक पौधे के अन्दर मई-जून और नवम्बर सामान्यतया कली फूटन के बाद करता है। प्रत्येक मादा 50 से 100 अण्डे देती है। प्रथम अवस्था का निर्मोचन अण्डे के अन्दर होता है। जीवन चक्र का विकास द्वितीया अवस्था लारवा 15 दिन लेता है, सामान्य तापक्रम अनुकूल आद्रता और पोषक की उपस्थिति जीवन चक्र को कम करती है। क्योंकि धान के तने पर सीधा अण्डे देती है। नेमाटोड (सूत्रकृमि) विपरित परिस्थिति में 15 महीने से अधिक जीवित रहता है बढवार काल के अन्त में नेमाटोड (सूत्रकृमि) जीव अवरोधी पौधे के पकने व सूखने के समय बनता है।

क्षति के लक्षण

उफरा रोग के लक्षण स्पष्ट रूप से पौधे के नवजात अवस्था या फसल की अगेती अवस्था में नहीं दिखाई देते हैं। पत्ती हल्की पतली और कुछ समय उतकक्षय देखे जा सकते हैं। संकमित नवजात पौधे पर उफरा रोग के लक्षण 2 महीने की फसल पर



आसानी से देखे जा सकते हैं। पत्ती का उत्कृष्ट भाग भूरे से गहरा भूरा हो जाता है। इस रोग के दो प्रकार के लक्षण देखे जा सकते हैं। बाल के निकलने व निकलने के आधार पर देखा जाता है उफरा प्रथम व द्वितीया और बाल लीफ शीथ के अन्दर पूर्णतया रह जाती हैं। फुला हुआ उफरा जाना जाता है उफरा प्रथम संक्रमण जल्दी होने पर बाल निकल आती हैं। उफरा द्वितीया पकी हुयी उफरा से जाना जाता है। बाल का टुकड़ों में निकलना और बाद की अवस्था में क्षति का कारण व निचे वाली का भाग खाली रहता है। खेत में उफरा रोग के लक्षण टुकड़ों में दिखाई देते हैं। उफरा रोग से 20 से 90 प्रतिशत का आंकलन व औसत हानि 30 प्रतिशत का आंकलन किया जा चुका है। वर्षा ऋतु में मानसून जल्दी आने पर उफरा रोग बढ़ता है।

नियन्त्रण

1. खेत की गर्मी में मई-जून के दिनों में 2-3 गहरी जुताई करके छोड़ देना चाहिए। गहरी जुताई करने से निमेटोड नष्ट हो जाते हैं।
2. फसल को काटने के बाद अवशेषों को नष्ट कर देना चाहिए। इससे नेमाटोड की संख्या में कमी आती है।

3. खेत में उचित जल निकास और फसल से अवाछिनिय पौधे बहार निकालने से नेमाटोड के बढ़वार में कमी आती है।
4. फसल चक्र अपनाने व मिश्रित फसल सरसों के साथ बाने से फसल में नेमाटोड की संख्या कम आंकी गयी है।
5. उफरा रोग रोकने में कुछ रासायनिक प्रभावित पाये गये हैं लेकिन ये मंहंगे और गहरे पानी वाले धान के लिए हानिकारक हैं।
6. भूमि उपचार डाइजिन, डाइसल्फूटोन और फेनसल्फोसियान लाभदायक पाया गया है। कार्बोफूरो 3 जी 1 कि.ग्रा. खेत में पौध स्थान्तरण से पूर्व बखेर कर नेमाटोड नियन्त्रण में लाभदायक पाया गया है और उपज में 38 से 39 प्रतिशत अधिक उपज प्राप्त होती है।
7. पौधे की जड़ को मिरल 3 प्रतिशत और टेक्टो एफ-1 5.5-10 और 2.5-10 प्रतिशत उफरा के संक्रमण को कम करता है।
8. रायदा 10-06 गहरे पानी वाली प्रजाति और पदमा वाली एक अगेती प्रजाति नेमाटोड के नियन्त्रण में सहायक हैं।

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पाठकों से निवेदन

पाठक बन्धुओं! हमें आपका सतत सहयोग प्राप्त हो रहा है जिसके लिए हम आपके आभारी हैं। जैसा कि आपको विदित ही है कि इस पत्रिका में अधिकतर लेख वैज्ञानिक पृष्ठभूमि पर आधारित होते हैं जिस कारण आपकी सहभागिता नहीं हो पाती। सम्भव है कि आपके पास भी कोई ऐसी जानकारी, अनुभव अथवा तकनीक हो जिसे आप अन्य पाठकों के साथ बांटना चाहते हों। अगर ऐसा हो तो संकोच न करें और हमें अपनी जानकारी/अनुभव/तकनीक टंकित करकर सम्पादक, किसान भारती, संचार केन्द्र, गोविन्द बल्लभ पंत कृषि एवं प्रौद्योगिक विश्वविद्यालय, पंतनगर-२६३१४७, उधमसिंहनगर, उत्तराखण्ड के पते पर भिजवाने का कष्ट करें। यदि आपके द्वारा दी गयी जानकारी अथवा अनुभव किसानोपयोगी पाया गया तो हम उसे पत्रिका के आगामी अंकों में प्रकाशित करने का प्रयास करेंगे।

सम्पादक



Synthesis and Biological Evaluation of Betulonic Acid Derivatives as Antibacterial Agents

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ABSTRACT: Betulonic acid is pentacyclic triterpenes of the lupane type, which were isolated from birch bark. Betulonic acid and its derivatives can serve as interesting structural plan of action for the advancement of new therapeutic agents because of its high accessibility and a wide scope of natural activities. It has significant organic properties, for example, antiviral, antitumor, calming, antimicrobial, hepatoprotective, just as immunostimulant exercises. A series of novel synthetic and semisynthetic derivatives of BetA have been synthesized using different synthetic techniques and subjected to various testes and investigated for their therapeutic activities. Amino acid alkyl esters were prepared using amino acids-glycine. Presence of amino acids enhances the anti-inflammatory activity. Amino acids possess anti-inflammatory activity therefore the derivative of betulonic acid via amino acid esterification enhances the antiinflammatory activity of the synthesized compound. The synthesized betulonic acid derivatives were subjected to biological screening to evaluate their antimicrobial and anti-inflammatory effects.

KEYWORDS: Antineoplastic, Bhojpatra, Betulinic Acid Antibacterial, Anti-inflammatory etc.

I. INTRODUCTION

[1-2]Lupane triterpenoids of plant origination, for example, betulin and betulonic and betulonic acids show an assortment of organic movement and are fascinating as beginning materials for synthetic and biocatalytic changes

Betulin is commonly isolated from the bark of birch tree (*Betula utilis*) plant species. Birch tree is familiar for a long time for its curing properties; the oil obtained from birch bark was used as medicament for the remedy of skin diseases like dermatitis and psoriasis. The bark of the tree

was used to prepare teas for treating infections of digestive tract by Native Americans, so it is regarded that the use of this medicinal plant as a source for the isolation of active constituents could be a vital source for curing many ailments. The major reason behind selecting this research work is that betulonic acid has better solubility than betulin. As well as the presence of amino acids enhances the anti-inflammatory activity. [3]Amino acids possess anti-inflammatory activity therefore the derivative of betulonic acid via amino acid esterification enhances the antiinflammatory activity of the synthesized compound.

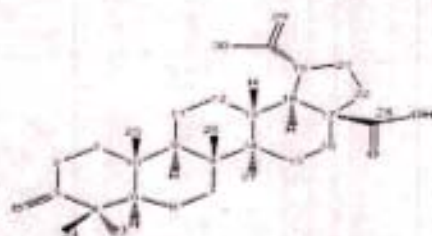
Betulonic acid—a potent leading biological moiety

[4]Betulonic acid [lup-20(29)-en-3-oxo-28-oi] has valuable biological properties such as antiviral Antitumor, anti-inflammatory, antimicrobial, hepatoprotective, as well as immunostimulant activities. Until the 2000s interest to betulonic acid was basically because of its role as the precursor for synthesis of betulinic acid, which is a fruitful drug in case of human melanoma.

[5]The antitumor action of BetA has drawn in the consideration of the pioneers who aim to evolve novel antitumor agents. Betulonic acid is the one of the major successful parts of numerous customary Chinese medications.

[6]It is also established as an intermediate in synthesis of several triterpenoid derivatives with anti-inflammatory, antiviral, and antiproliferative properties. Being an auxiliary metabolite of birches betulonic acid was found in particular as minor component in bud extracts. Today's flow interest for medicinal chemistry is synthesis of betulonic acid peptide derivatives, since they show high antiviral activity and can act as inhibitors of the tumor cell growth.





Structure of Betulonic acid

II. MATERIALS AND METHODS

Extraction and Isolation of Betulin Present research project work is based on the approach of semi-synthetic work on natural products. Lot of research work has been conducted on bhojpatra adopting both natural and synthetic approaches. But here, we have consolidated both natural as well as synthetic work. In this reference few reported extraction and isolation procedures were carried out which are as follows:

- Initially 15 gm of bhojpatra was boiled in methanol for about 10-15 minutes in iodine flask along with few porcelain chips. Then its solid part was separated from solvent part. The solvent front was then concentrated to the maximum and
- kept overnight in fridge. This procedure was found to be the simplest and provides with maximum yield of betulon in impurified form.
- When impurified betulon obtained, its recrystallisation process was carried out with butanol or isopropanol and water.
- TLC of the synthesized compounds was carried out in order to ascertain its purity.

Mobile phase for TLC: -

Ethyl acetate: benzene: formic acid (36:12:5)
Detecting agent: Anisaldehyde (0.5mL) + H₂SO₄(1 mL 97%) + Glacial acetic acid (50 mL)

Result \Rightarrow Less violet spot- Betulin
 More violet spot- Betulonic acid

Preparation of Betulonic acid Preparation of Jone's Reagent

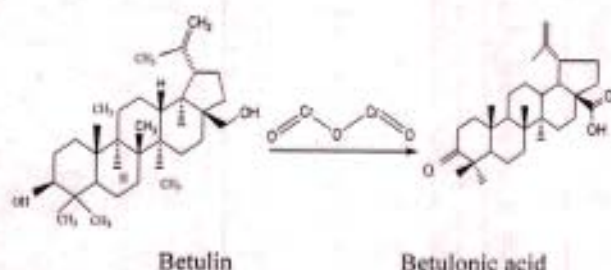
Jone's reagent was produced by dissolving chromium trioxide (70 gm, 0.70 moles) in 100 ml of water placed in a 500 ml beaker. The beaker was then kept in an ice bath. 18 ml of sulphuric acid (61 ml, 1.10 mole) and then 200 ml of water was further added on with constant manual stirring. The above mixture was then cooled to 0°C - 5°C.

Preparation of Betulin to Betulonic Acid:-

Jone's reagent was added drop by drop to a betulon (1 gm, 2.26 mmol) solution mixture in 50 ml acetone cooled to 0°C. Continuous stirring the above resulting mixture was done around 1.5 hrs at a temperature of 0° C, a further addition of 25 ml methanol was done, then for next 5 minutes the solution was stirred. 40 ml of water was further added. Vacuum was applied to remove the acetone and by using 40 ml of ethyl acetate the aqueous residue was extracted. The next step involved the separation of aqueous layers from the ethyl acetate layer. Washing of ethyl acetate layer was done first with 20 ml of water followed by washing with 15 ml of brine. Magnesium sulphate was employed for drying ethyl acetate layer, filtered and further removal of ethyl acetate layer was done under vacuum. Then column chromatography of residue was carried out using 60-200 mesh silica gel employing petroleum ether/ ethyl acetate (4:1) to produce betulonic acid (770 mg), whose melting point was detected to be in the range 247° C- 249°C. The reaction resulted in a 75% yield of betulonic acid.



Chemical reaction:



General Procedure for Preparation of Amino Acid Alkyl Ester Hydrochlorides Derivatives of Betulonic Acid

In a round bottom flask 0.1 mol amino acid was placed. Newly prepared distilled chlorotrimethylsilane (0.2 mol) was added steadily to this under continuous stirring with a magnetic stirrer. 100 ml of methanol was then added to the above mixture. The resulting solution was then mixed at 25°C. The resulting mixture was concentrated with the help of rotary evaporator as the reaction completed, to yield the product amino acid ester hydrochloride. Following are the above mentioned method amino acid alkyl ester derivatives which were synthesized:

- I. Glycine methyl ester
- II. Glycine ethyl ester
- III. Glycine propyl ester

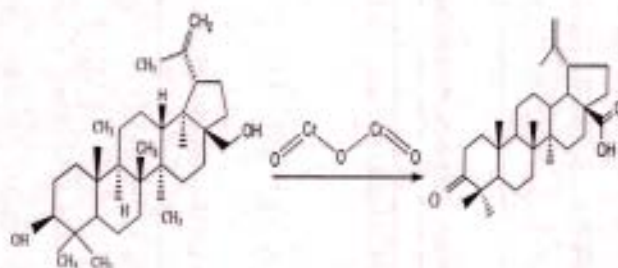
Now, the next step consisted the chemical conjugation of synthesized amino acid alkyl ester derivatives with betulonic acid so as to derivitize biologically potent derivatives

Procedure for the Conjugation of Amino Acid Alkyl Esters with Betulonic Acid to Give Potent Derivatives

Dissolved Betulonic acid, amino acid alkyl ester and triethyl amine in tetrahydrofuran at room temperature. To this solution mixture added DCC and DMAP, stirred this for 48 hrs. Filtered the precipitate (Dicyclohexyl urea). Evaporated the filtrate to will remove THF. After evaporation, remaining was dissolved in ether/ethyl acetate (2:1). 100 ml water and HCl poured in separating funnel. Organic layer collected then dried on MgSO₄.

Synthetic Scheme

1. Betulin oxidised by chromium oxide to Betulonic acid



2. Formation of amino acid alkyl esters conjugates of betulonic acid





3. Formation of amino acid salt conjugate of betulonic acid

Synthetic Scheme for the Amino Acid Alkyl Ester Conjugates of Betulonic Acid

I Glycine methyl ester of betulonic acid

II Glycine ethyl ester of betulonic acid

III Glycine propyl ester of betulonic acid



III BIOLOGICAL ACTIVITY

Antibacterial activity

These are chemotherapeutic agent that destroy bacteria or hinders its growth. Synthetic antibacterial compounds are classified into two significant classes, topical and systemic compounds.

Antimicrobial activity:

An antimicrobial agent is whatever that can kills microscopic organism or hinders its growth. Antibacterial synthetics are classified in three general classes like antibacterial medicaments, antiseptics and disinfectants. They are utilized in low fixations in or upon the bodies of organisms to forestall or treat explicit bacterial sickness without harming the host living being. Antimicrobial movement is examined depending upon the invitro action in unadulterated cultures. In-vitro vulnerability test are finished by the accompanying techniques:

- Tube dilution method
- Agar dissemination technique

• Tube dilution method:

The antimicrobial agent dilutions will be favored in growth medium with the end goal that the medication concentration achieves the desired clinical significance range. An equivalent amount of

broth consisting 10⁵-10⁶ microbes/ml will be then added on to every tube and also to a control tube in which no microbial antagonist is present. Noticeable turbidity will be inspected in the tubes for after an overnight incubation. This strategy is utilized for detecting susceptibility of microbes in liquid media.

• Agar diffusion method:

In this procedure by employing pouring method Petri dishes of agar will be made. Inoculation of the agar will be done with microorganisms. In agar dilution method for the two; aerobes and anaerobes diverse antibiotic concentrations will be utilized in to an agar culture. For 24 hours at 37 °C temperature the plates are brooded. The microbial antagonist disperses through the agar and forms an inhibition zone. The diameter of the zone can be determined and an assessment of the level of action of the microbial antagonist can be acquired.

Material and Methodology

The microbiological testing of the subordinates was finished by agar well diffusion method.

Standard drug--- Ciprofloxacin

Media--- Nutrient agar media were utilized for the reason, which contains the constituents as introduced in. (table-1)

S.NO	Constituents	Quantity Required
1.	Peptic Digest	5gm/liter
2.	Yeast Extract	1.5gm/liter
3.	Beef Extract	1.5gm/liter
4.	Sodium Chloride	5gm/liter
5.	Agar	15gm/liter
6.	Distilled Water	1 liter

Experimental procedure:

Agar-diffusion method was utilized for the determination of preliminary bacterial antagonist activities. The agar well diffusion test was carried out using nutrient agar medium, according to the system set out by Magaldi et al. 2004 and at 15 lbs pressure (121°C) this agar medium was autoclaved for 15 minutes and afterward it was cooled instantly to 50- 55°C in ice-bath. This medium was filled in petridishes to a static 4 mm depth; this is

proportional to round about 40mL in a 90mm plate. The culture was then inoculated on the surface of medium after the medium had solidified. These were performed in a laminar air flow. The germ free swab was utilized on the outer surface of the nutrient agar culture to guarantee a uniform distribution. Then the petridishes were settled for few minutes to ensure abundant moisture absorption. Germ free plug borer (7mm) was utilized for making agar wells, and the



concentrations of the 25, 50, 75, 100 and 200 µg/ml of the diluted stock solutions were set in each

wells (Indian Pharmacopoeia, 1996). The level of inhibition can be determined utilizing the equation:

$$\% \text{ Inhibition} = \frac{I (\text{diameter of inhibition zone in mm})}{90 (\text{diameter of Petri-plates in mm})}$$

Antibacterial activity

Test strains

For the current work, effectiveness of the test compounds was resolved against following bacterial strains:

Gram +ve bacterial strains

1-Bacillus pumilus

2-

Staphylococcus aureus Gram -ve bacterial strains-

1- Escherichia coli 2- Klebsiella pneumonia

IV. RESULT AND DISCUSSION

S.No	Compound Code	Conc. (µg/ml)	Inhibition zone in mm			
			S.aureus	B.pumilus	E.coli	K.pneumoniae
1.	AS-1	1000	17	16	15	18
2.	AS-2	1000	18	17	16	18
3.	AS-3	1000	19	17	19	20
4.	Ciprofloxacin	1000	30	35	38	35

The in-vitro antibacterial activities of newly synthesized compounds (AS1 to AS3) were carried out by Agar Diffusion Method counter the micro organisms viz. gram positive (Bacillus pumilus, Staphylococcus aureus) gram negative (Escherichia coli, Klebsiella pneumonia).

All three derivatives were screened for antibacterial activities at 1000µg/mL concentrations. The inhibition zone (in mm) was estimated for each compound accompanying ciprofloxacin as standard drug and results were presented in table 2

Results demonstrated that compound AS1 showed maximum activity (inhibition zone in mm) against Klebsiella pneumonia, least action against Escherichia coli.

Compound AS2 showed maximum activity (inhibition zone in mm) against Klebsiella pneumonia, Staphylococcus aureus and minimal activity against Escherichia coli.

Compound AS-3 showed maximum action (inhibition zone in mm) against Klebsiella pneumonia and minimum activity against Bacillus pumilus.

V. CONCLUSION

The basic purpose of the present research work seems to be served and appreciably fulfilled as encouraging results have been obtained both in terms of establishment of structural features as well as the spectrum of the biological activities of the synthesized compounds. The results of the antibacterial study revealed that all three compounds (AS1, AS2 and AS3) exhibited medium to good antibacterial activity when juxtaposed with the standard drug.



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Betulonic Acid- Potent Candidate for Lead Development for Various Chronic Diseases

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ABSTRACT

The present research work commenced with the collection of, **bhojpatra** (*Betula utilis*). *Betula utilis* (common name bhojpatra) is known for its beneficial effects and medicinal values. Its potent derivative, (BetA, 3-oxo-20(29)-lupen-28-oic acid), (betulonic acid) is a pentacyclic lupine type triterpene, widely distributed throughout the plant kingdom. It has valuable biological properties such as antiviral, antitumor, anti-inflammatory, antimicrobial, hepatoprotective, as well as immunostimulant activities but its effectiveness is diminished by its poor water solubility. Efforts have been divided in this project work to surpass this disadvantage by obtaining complexes with hydrophilic substances such as amino acid alkyl esters and making the betulonic acid. In order to manifest its significant pharmacological actions we started with its extraction process which included various steps following reported methods such as hot and cold extraction, Soxhlet extraction, column chromatographic techniques with different organic solvents such as methanol, ethanol, propanol, isopropanol, butanol, dichloromethane, etc. Initially, bhojpatra was subjected to hot and cold extraction with above mentioned solvents and as a result, betulin was converted to betulonic acid using John's oxidising agent. On the other hand, amino acid alkyl esters were prepared using amino acids- glycine and arginine. Betulonic acid was conjugated with the prepared amino acid alkyl esters to obtain betulonic acid derivatives with desirable pharmacological actions. The synthesized betulonic acid derivatives were subjected to biological screening to evaluate their antimicrobial and anti-inflammatory effects.

Keywords: Betulonic acid, Bhojpatra, Anti-inflammatory, Antibacterial

1. INTRODUCTION

Lupene triterpenoids of plant origination, for example, betulin and betulonic and betulonic acids show an assortment of organic movement and are fascinating as beginning materials for synthetic and biocatalytic changes. (Tolstikov G. A., Flekhter O. B., E. E. Shults, Baltin L. A. & Tolstikov A. G, 2005) (Alakurtti S., Makela T., Koskimies S. & Yli-Kauhaluoma J, 2006)

Betulin is a phytochemical isolated from *Betula utilis* (**bhojpatra**, name in Sanskrit-Bhurja). The bark of birch trees consists of 10-15% of betulin and it can be easily isolated using simple extraction procedures. The family of this plant is **Betulaceae**. *Betula utilis* found shoot up beside moraines on all sides of bhojbasa in, nearabout the proboscis of the gangotri. It is a medium sized tree that grows upto a height of 20m. Its bark is glossy, reddish-white or whitish, with horizontal even and regular, lenticels. The shape of leaves are tapering, elliptical and irregularly serrate. The flowers are found to be monoecious which are pollinated by wind. The seeds are found to be slender and winged, Loamy and clay soils are suitable for the plant, it prefers fully dried soil and can even shoot-up in heavy clay soil. The favourable pH for the plant to shoot-up is acidic, neutral as well as alkaline soils. It can spring-up in partial/dappled umbrage (light5) or in traust shade. It possess antiseptic, odoriferous, antifatulent and contraceptive effects. The phytoconstituents that the bark comprises are betulin, lupeol, acetyloleanolic acid, betulonic acid, a ketone-lupenone, β -sitosterol, methyl betulitriterpenoid. (Sharma Promila, Singh Saumya, Yadav Shivani & Thapliyal Ashish, 2012)



1.1. BETULONIC ACID—A POTENT LEADING BIOLOGICAL MOIETY

Betu-lonic acid (Beta, 3-oxo-20(29)-lupen-28-oic acid), are pentacyclic triterpenes of the lupane type, which were isolated from birch bark. Betulonic acid and its derivatives can serve as interesting structural plan of action for the advancement of new therapeutic agents because of its high accessibility and a wide scope of natural activities. It has significant organic properties, for example, antiviral, antitumor, calming, antimicrobial, hepatoprotective, just as immunostimulant exercises.

It tends to be gotten rather simply from betulin by oxidation with chromium trioxide and betulin forms up to 30% of the dry weight of the extractive (the bark of birch trees). In this method preparation of betu-lonic acid merge two multi-step processes: (1) extraction of birch bark go after by concentration and purification of crude betulin; and (2) oxidation of crude or pure betulin into betulonic acid go after by its purification. Mostly the production is fairly laborious and refined, including solvent type change at each resulting step of these processes. Hence, alcohols and halogenated hydrocarbons are regularly utilized for bark extraction is gotten by crystallization of the concentrates from methanolic and chloroformic solutions (Petrenko N.I., Elantseva N. V., Petukhova V. Z., Shakirov M. M., Shul'ta E. E. & Tolstikov G. A., 2002) (Kim Darrick S. H. L., Chen Zhidong, Nguyen van Tuyen, Pezzuto, John M., Qiu, Shengxiang & Lu, Zhi-Zhen, 1997)

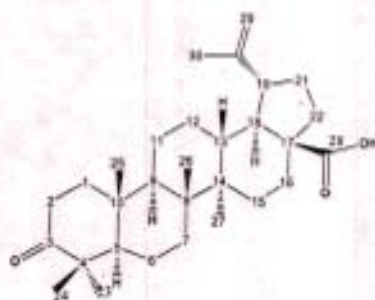


Fig.1- Chemical structure of betulonic acid

1.2. CONVENTIONAL UTILIZATION OF BETULA UTILIS

The bark of this tree was employed many decades back in India as sheet of paper for scribbling long holy writings and contents of sanskrit and another written works. Bhurja is name given to bhojpatra in sanskrit, sharing an analogy with other Indo-European terminology. The bark is commonly used as a material for packing, for building ceiling etc. Leaves of this tree are effective in cure of infections in urinary tract and stones present in bladder. The wood is employed for building work and for fodder. Some parts of the plant have also been used in local traditional medicines for the treatment of the fungal growth (bhurja-granthi). (Sharma Promila, Singh Saumya, Yadav Shivani & Thapliyal Ashish, 2012)



Fig 2-Birch tree



2. MATERIAL AND METHODS

2.1. EXTRACTION AND ISOLATION OF BETULIN

Present research project work is based on the approach of semi-synthetic work on natural products. Lot of research work have been conducted on bhojpatra adopting both natural and synthetic approaches. But here, we have consolidated both natural as well as synthetic work. In this reference few reported extraction and isolation procedures were carried out which are as follows:

- Initially 15 gm of bhojpatra was boiled in methanol for about 10-15 minutes in iodine flask along with few porcelain chips. Then its solid part was separated from solvent part. The solvent front was then concentrated to the maximum and kept overnight in fridge. This procedure was

found to be the simplest and provides with maximum yield of betulin in impurified form.

- When impurified betulin obtained, its recrystallisation process was carried out with butanol or isopropanol and water.
- TLC of the synthesized compounds was carried out in order to ascertain its purity.

Mobile phase for TLC: Ethyl acetate: benzene: formic acid (36:12:5)

Detecting agent: Anisaldehyde(0.5mL) + H₂SO₄(1 mL 97%) + Glacial acetic acid (50 mL)

Result



Less violet spot- Betulin
More violet spot- Betulonic acid

- There were chances that certain percentage of quantity would have remained within the bhojpatra after this procedure. So, for its extraction to maximum extent soxhlet apparatus was employed along with various solvents such as chloroform, DMF, methanol, ethanol, propanol, isopropanol, butanol, etc. The resultant yield from different organic solvents was calculated and compared.
- Soxhlet procedure was used to raise the yield of the product. This is referred to as hot process extraction.
- Along with these procedures, cold extraction process was also attempted which involved dipping of bhojpatra for 3-4 days in ethyl acetate: ethanol: water (4.5: 4.5: 1) for 10 ml.

2.2. PREPARATION OF BETULONIC ACID

2.2.1 PREPARATION OF JONE'S REAGENT

Jone's reagent was produced by dissolving chromium trioxide (70 gm, 0.70 moles) in 100 ml of water placed in a 500 ml beaker. The beaker was then kept in an ice bath. 18 ml of sulphuric acid (61 ml, 1.10 mole) and then 200 ml of water was further added on with constant manual stirring. The above mixture was then cooled to 0°C - 5°C.

2.2.2 PREPARATION OF BETULIN TO BETULONIC ACID

Jone's reagent was added drop by drop to a betulin (1 gm, 2.26 mmol) solution mixture in 50 ml acetone cooled to 0°C. Continuous stirring the above resulting mixture was done around 1.5 hrs at a temperature of 0° C, a further addition of 25 ml methanol was done, then for next 5 minutes the solution was stirred. 40 ml of water was further added. Vacuum was applied to remove the acetone and by using 40 ml of ethyl acetate the aqueous residue was extracted. The next step involved the separation of aqueous layers from the ethyl acetate layer. Washing of ethyl acetate layer was done first with 20 ml of water followed by washing with 15 ml of brine. Magnesium sulphate was employed for drying ethyl acetate layer, filtered and further removal of ethyl acetate layer was done under vacuum. Then column chromatography of residue was carried out using 60-200 mesh silica gel employing petroleum ether/ ethyl acetate (4:1) to produce betulonic acid (770 mg), whose melting point was detected to be in the range 247° C- 249°C. The reaction resulted in a 75% yield of betulonic acid.

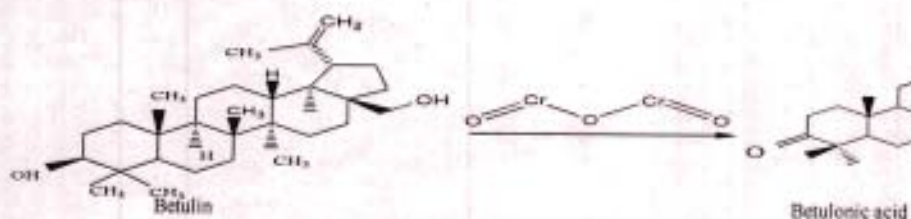


Fig.3-Conversion of betulin to betulonic acid

2.3. GENERAL PROCEDURE FOR PREPARATION OF AMINO ACID ALKYL ESTER HYDROCHLORIDES DERIVATIVES OF BETULONIC ACID

In a round bottom flask 0.1 mol amino acid was placed. Newly prepared distilled chlorotrimethylsilane (0.2 mol) was added steadily to this under continuous stirring with a magnetic stirrer. 100 ml of methanol was then added to the above mixture. The resulting solution was then mixed at 25°C. The resulting mixture was concentrated with the help of rotary evaporator as the reaction completed, to yield the product amino acid ester hydrochloride.

Following are the above mentioned method amino acid alkyl ester derivatives which were synthesized:

- Arginine methyl ester



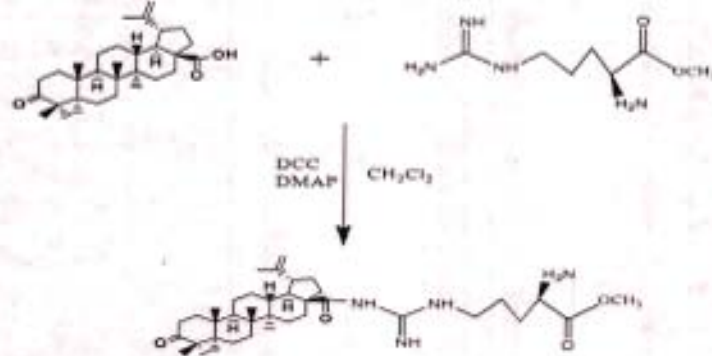
- Arginine ethyl ester
- Arginine propyl ester

Now, the next step consisted the chemical conjugation of synthesized amino acid alkyl ester derivatives with betulonic acid so as to derivitize biologically potent derivatives.

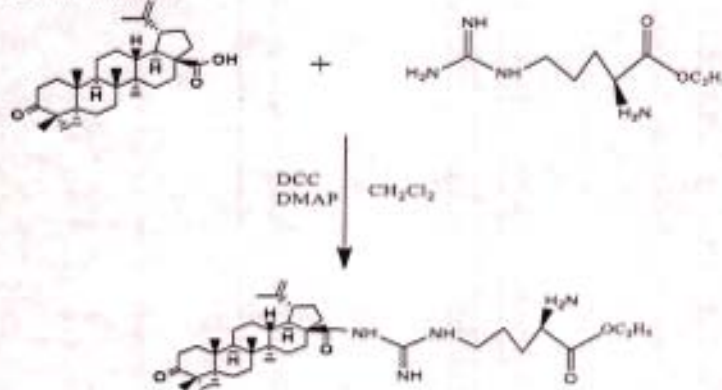
Dissolved Betulonic acid, amino acid alkyl ester and triethyl amine in tetrahydrofuran at room temperature. To this solution mixture added DCC and DMAP, stirred this for 48 hrs. Filtered the precipitate (Dicyclohexyl urea). Evaporated the filtrate to will remove THF. After evaporation, remaining was dissolved in ether/ethyl acetate (2:1). 100 ml water and HCl poured in separating funnel. Organic layer collected then dried on $MgSO_4$.

2.4. SYNTHETIC SCHEME FOR THE AMINO ACID ALKYL ESTER CONJUGATES OF BETULONIC ACID

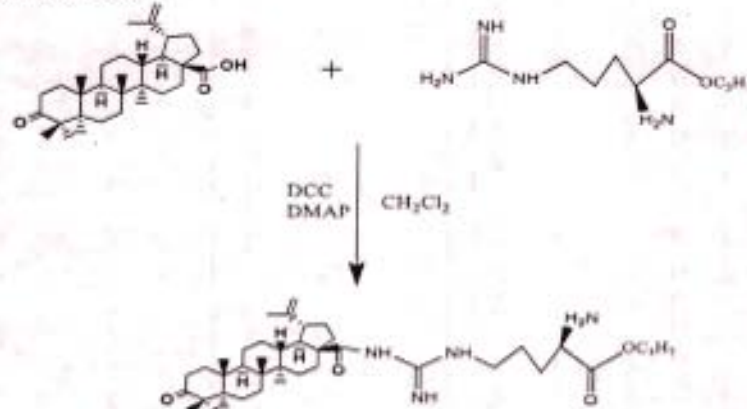
- Arginine methyl ester of betulonic acid



- Arginine ethyl ester of betulonic acid



- Arginine propyl ester of betulonic acid



3. BIOLOGICAL ACTIVITY

All the integrated compounds were screened for antimicrobial activity and anti-inflammation activity.

3.1. ANTIMICROBIAL ACTIVITY

Paul Ehrlich elevated the concept of chemotherapy to medicate microbial diseases; he predicted the growth of chemical therapeutics that would demolish microorganism without hurting the host. Pasteur established the actuality of antibiotics in 1877; air borne microscopic organisms restrained the improvement of *Bacillus anthracis* bacilli in urine. Sulpha medicaments came into significance in the last part of the 1930's; in 1929 Alexander Fleming found the 1st anti-biotic that was penicillin. Its initial clinical fundamentals were finished in 1940. Principal sulfonamide was Sulfa-pyridine. Waksman in 1940 and his comrate embraced a methodical hunt of acti-nomycetes although a wellspring of anti-biotics and locate streptomycin in 1944. This gathering of soil microbe's end up being a rich place of antibiotics and shortly antibiotic medication like Tetracyclines, Chloramphenicol, Erythromycin and numerous others followed. Many current antifungal have been available, two significant antibiotics; Amphotericin-B to manage with systemic mycosis and Griseofulvin to enhance attack on dermatophytes were presented close by 1960.

In the erstwhile 40 years significance has moved from looking new anti-infective producing antibiotics to creating semisynthetic subordinates of of more seasoned anti-microbials with considerably more engaging properties or clashing range action.

Anti-microbial medications are the greatest commitment of the current century to therapeutics. The importance is amplified in the developing countries where infective disease predominates. As a class they are one of the most oftentimes utilized as well as abused drugs.

3.1.1 Tube dilution method:

The antimicrobial agent dilutions will be favored in growth medium with the end goal that the medication concentration achieve the desired clinical significance range. An equivalent amount of broth consisting 10⁵-10⁶ microbes/ml will be then added on to every tube and also to a control tube in which no microbial antagonist is present. Noticeable turbidity will be inspected in the tubes for after an overnight incubation. This strategy is utilized for detecting susceptibility of microbes in liquid media.

3.1.2 Agar diffusion method:

In this procedure by employing pouring method Petri dishes of agar will be made. Inoculation of the agar will be done with microorganisms. In agar dilution method for the two, aerobes and anaerobes diverse antibiotic concentrations will be utilized in to an agar culture. For 24 hours at 37 °C temperature the plates are brooded. The microbial antagonist disperses through the agar and forms an inhibition zone. The diameter of the zone can be determined and an assessment of the level of action of the microbial antagonist can be acquired.

3.1.3 Media

Nutrient agar media were utilized for the reason, which contains the constituents as introduced in table 1.

Table 1 – Nutrient agar Media.

Constituents	Quantity Required
Peptic digest	5gm/liter
Yeast Extract	1.5gm/liter
Beef Extract	1.5gm/liter
Sodium Chloride	5gm/liter
Agar	15gm/liter
Distilled Water	1 liter

3.1.4 Experimental procedure:

Agar-diffusion method was utilized for the determination of preliminary bacterial antagonist activities. The agar well diffusion test was carried out using nutrient agar medium, according to the system set out by Magaldi *et al.* 2004 and at 15 lbs pressure (121°C) this agar medium was autoclaved for 15minutes and afterward it was cooled instantly to 50- 55°C in ice-bath. This medium was filled in petridishes to a static 4 mm depth; this is proportional to round about 40ml, in a 90mm plate. The culture was then inoculated on the surface of medium after the medium had solidified. These were performed in a laminar air flow. The germ free swab was utilized on the outer surface of the nutrient agar culture to guarantee a uniform distribution. Then the petridishes were settled for few minutes to ensure abundant moisture absorption. Germ free plug borer (7mm)was utilized for making agar wells, and the concentrations of the 25, 50, 75, 100 and 200 µg/ml of the diluted stock solutions were set in each wells(Indian Pharmacopoeia, 1996).



The level of inhibition can be determined utilizing the equation :

$$\% \text{ Inhibition} = \frac{I (\text{diameter of inhibition zone in mm})}{90 (\text{diameter of Petri-plates in mm})}$$

3.1.5 Test strains

For the current work, effectiveness of the test compounds was resolved against following bacterial strains:

Gram +ve bacterial strains-

- *Bacillus pumilus*
- *Staphylococcus aureus*

Gram -ve bacterial strains-

- *Escherichia coli*
- *Klebsiella pneumoniae*

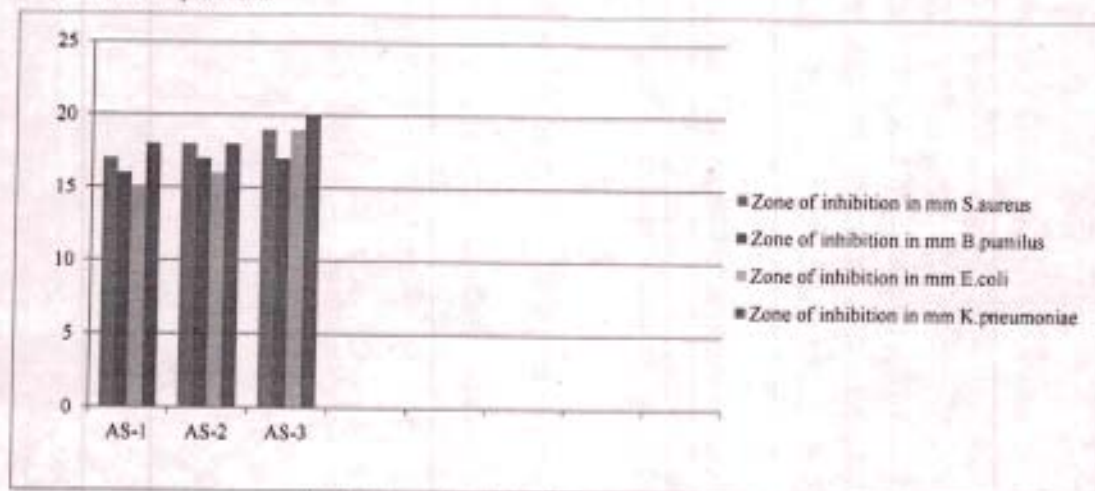


Fig.4- Antibacterial activity of synthesized compound

3.2 Anti-inflammatory activity

Inflammation antagonist action was completed via carrageenan induced paw oedema process in albino rats (120-160g). The anti-inflammatory evaluations of the incorporated compounds were executed using reported procedures (Winter et al, (1962). Diclofenac sodium was utilized as standard medication for correlation. Drugs and tested compounds were administered oral route by making 1% CMC suspension. Newly prepared aqueous suspension of carrageenan (1% w/v, 0.1 mL) was infused into the paw of each rat.

The investigation were done on the healthy adult albino rats (male/female) weighing between 120-160 g using carrageenan induced rat paw oedema method. They were fed on standard pellet diet and water.

3.2.1 Standard drug: Diclofenac sodium (10 mg/kg)

3.2.2 Equipment- Mercury displacement (plethysmograph).

3.3.3 Purpose and Rationale:

Among the strategies utilized for screening of inflammation antagonists, one of the most usually employed methods depends on the capacity of such specialists to restrain the edema build in the rear paw of the rodent after infusion of phlogistic agents. Brewer's yeast, formaldehydes, dextran, egg albumin, kaolin, aerosol, sulphated polysaccharides like carrageenan or naphthoyl heparamine have been utilized as phlogistic agents (irritants)

This impact can be quantified in few different ways. Typically the volume of the administered paw is estimated in advance and a after implementation of the aggravation and the paw volume by simple and less exact and by increasingly sophisticated electronically devised techniques. The estimation of the assessment is rarely dependent on the apparatus but much more on the irritant being selected.

Few irritant incite just a short enduring swelling while different irritant prompt the paw edema to offer more than 24 hours.

3.3.4 Procedure

- The animals were partitioned into groups, each group consist 6 rats. One group of animals designated to control and another one is for



standard drug (Diclofenac) respectively. Remaining groups dispensed to the test compounds.

- Diclofenac and sample compounds were administered by oral route by preparing 1% CMC suspension to groups (standard, control and test compound) respectively
- After 30 minutes, 0.1 mL of 1% newly prearranged suspension of carrageenan in 0.9% solution of NaCl was subcutaneously infused in the paw and amount was estimated.
- The foot volume was estimated again at 2 hrs and 4 hrs, the mean increment in the paw volume in one and all group was determined.
- By using water plethysmometer apparatus The paw volume was estimated
- The variation in volume gave the measure of edema created.
- The percentage of inhibition value determined by following equation.

$$\% \text{ Antiinflammatory action} = [1 - D_t/D_c] * 100$$

D_t = Paw volumes of oedema in test.

D_c = Paw volumes of oedema in control.

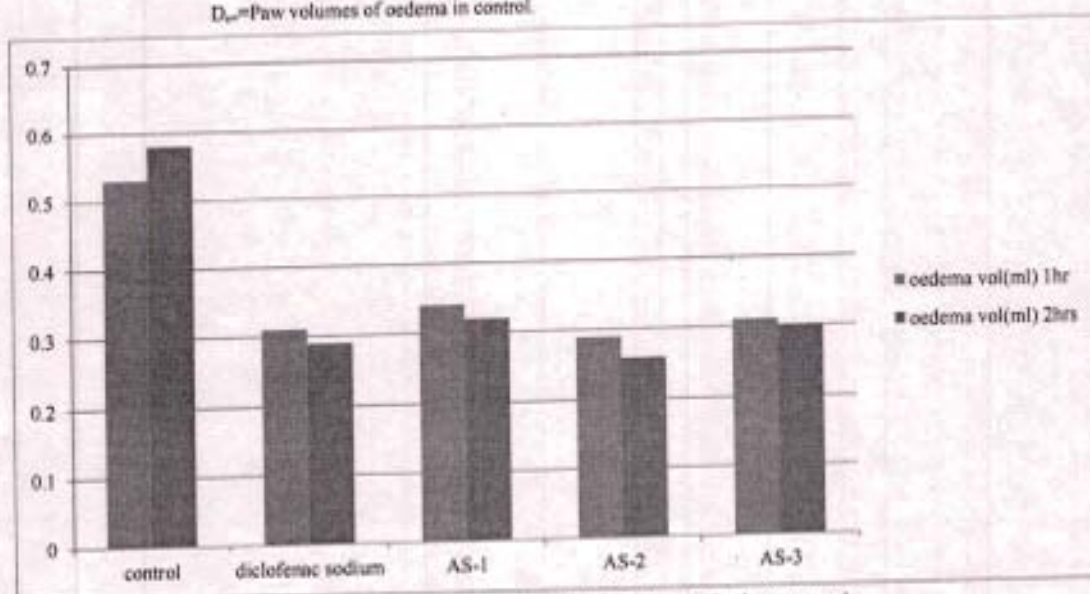


Fig.5- Anti-inflammatory screening (% inhibition) of synthesized compounds.

4. RESULTS AND DISCUSSION

4.1. Results of in-vitro Antibacterial screening

The *in-vitro* antibacterial activities of newly synthesized compounds (AS-1 to AS-5) were carried out by Agar Diffusion Method counter the micro organisms viz. gram positive (*Bacillus pumilus*, *Staphylococcus aureus*) gram negative (*Escherichia coli*, *Klebsiella pneumonia*). All three derivatives were screened for antibacterial activities at 1000µg/mL concentrations.

The inhibition zone (in mm) was estimated for each compound accompanying ciprofloxacin as standard drug and results were presented graphically in fig.4.

Results demonstrated that compound AS-1 showed maximum activity (inhibition zone in mm) against *Klebsiella pneumonia*, least action against *Escherichia coli*.

Compound AS-2 showed maximum activity (inhibition zone in mm) against *Klebsiella pneumonia*, *Staphylococcus aureus* and minimal activity against *Escherichia coli*.

Compound AS-3 showed maximum action (inhibition zone in mm) against *Klebsiella pneumonia* and minimum activity against *Bacillus pumilus*.

4.2. Results of anti-inflammatory screening

This result showed that the compounds possessing electron withdrawing group increase the action. Compound AS-1 and AS-2 revealed remarkable anti-inflammatory activity after both 1 hrs and 2 hrs. Diclofenac sodium (10 mg/Kg) was used as standard.

SUMMARY AND CONCLUSION

The basic purpose of the present research work seems to be served and appreciably fulfilled as encouraging results have been obtained both in terms of establishment of structural features as well as the spectrum of the biological activities of the synthesized compounds. Results of the biological screening have revealed that the compounds AS-1 and AS-2 were having moderate to good anti-inflammatory effect after 1hr & 2hrs at a dose of 10mg/Kg. The results of the antibacterial study revealed that all three compounds (AS-1, AS-2, AS-3) exhibited medium to good antibacterial activity when juxtaposed with the standard drug.

In nutshell, it may be concluded that the present research work may suggest new ideas and open new vistas for further research in the field of drug discovery and development particularly as far as structural manipulations in the synthetic medicinal chemistry is concerned.

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God is our refuge and strength, a very present help when we need it the most I thank him for guiding me through rough and torn paths, for always being less whisper away. We pray that he will always bless us, always show us the right path. We worship his love and care through which he has showered achievement and happiness upon us.

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