Neem Research Newsletter

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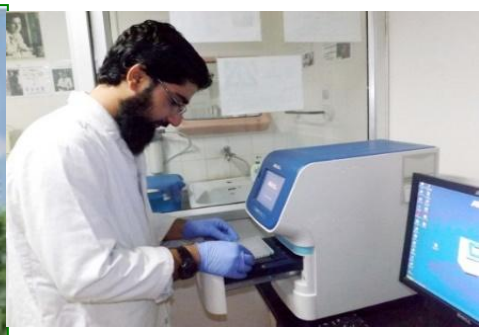

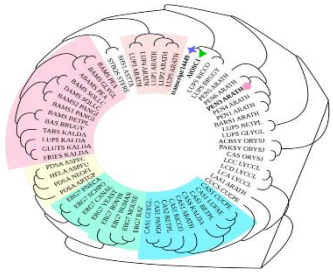

WORLD NEEM ORGANISATION (WNO)

From

The Editor's Desk……

In this issue of Neem Newsletter, we see the potential of neem in agriculture, environment, human health and in industry. Extracts of neem and gliricidia enhanced the yield and quality of leaf mustard by reducing the prevalence and feeding activity of harmful insect pests in a manner similar to synthetic insecticides. However, the botanical extracts preserved beneficial insects, did not significantly alter sensory attributes, especially taste and odor, and improved the visual appearance. Azadirachtin demonstrated lethality against the brown marmorated stink bug, *Halyomorpha halys*, an invasive pest causing major economic losses to crops. Silver nanoparticles synthesised using neem leaf extract was effective in mitigating salt stress on sunflower plants. Disposable electrochemical biosensor based on acetylcholinesterase showed excellent results, demonstrating the inhibition activity of azadirachtin and immense potential for pest control. Green production of silver nanoparticles using neem have outstanding qualities, such as better photocatalytic degradation of organic dyes like methylene blue, antibacterial efficacy towards multidrug-resistant pathogens, and biocompatibility for possible therapeutic applications. Basic fuel properties were analysed, and models developed for heterogeneous composite biodiesel using composite biodiesel/neem castor seed oil methyl ester. The lubricity potential of neem oil, tiger nut oil, and their blends in machining mild steel was examined. Neem oil surpassed tiger nut oil and conventional oils in minimizing cutting temperatures and enhancing surface quality. Moreover, an 80/20 blend of neem and tiger nut oils exhibited improved performance underscoring its potential as an effective cutting fluid. These findings advocate for the use of bio-based cutting fluids in machining operations, indicating environmental and economic advantages while promoting future research into alternative agro-based solutions. The potential of neem gum-based polymeric micelles was demonstrated as a cost-effective and environmentally friendly drug delivery platform. In dental practice, neem extract, showed promise as effective alternative to conventional irrigants for smear layer removal and antimicrobial activity. Ethanolic extract of neem leaves was found to be effective in treating acute hepatitis in mice. Yttrium oxide nanoparticles incorporated into nanofibrous mats consisting of polyurethane neem leaf extract and clindamycin hydrochloride exhibited enhanced antimicrobial activity, biofilm inhibition, and alleviation of diabetic wounds. In another study, neem stem bark active fraction and phytoconstituents were identified as a potential source to develop promising therapeutic agents against cervical cancer.

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Neem in Agriculture

Neem and Gliricidia Plant Leaf Extracts Improve Yield and Quality of Leaf Mustard by Managing Insect Pests' Abundance Without Harming Beneficial Insects and Some Sensory Attributes.

Kamanga RM, Bhikha S, Kamala FD, Mwale VM, Tembo Y, Ndakidemi PA.Insects. 2025 Feb 3;16(2):156. doi: 10.3390/insects16020156.PMID: 40003786

Production and consumption of vegetable crops has seen a sharp increase in the recent past owing to an increasing recognition of their nutraceutical benefits. In tandem, there has been unwarranted application of agrochemicals such as insecticides to enhance productivity and vegetable quality, at the cost of human health, and fundamental environmental and ecosystem functions and services. This study was conducted to evaluate the efficacy of neem and gliricidia botanical extracts in managing harmful insect pest populations in leaf mustard. Our results report that neem and gliricidia plant extracts enhance the yield and quality of leaf mustard by reducing the prevalence and feeding activity of harmful insect pests in a manner similar to synthetic insecticides. Some of the key insect pests reduced were *Lipaphis erysimi*, *Pieris oleracea*, *Phyllotreta Cruciferae*, *Melanoplus sanguinipes*, and *Murgantia histrionica*. However, compared to synthetic insecticides, neem and gliricidia plant extracts were able to preserve beneficial insects such as the *Coccinellidae* spp., *Trichogramma minutum*, *Araneae* spp., *Lepidoptera* spp., and *Blattodea* spp. Furthermore, plant extracts did not significantly alter sensory attributes, especially taste and odor, whereas the visual appearance of leaf mustard was greater in plants sprayed with neem and synthetic insecticides. Physiologically, plant extracts were also able to significantly lower leaf membrane damage as shown through the electrolyte leakage assay. Therefore, these plant extracts represent promising pesticidal plant materials and botanically active substances that can be leveraged to develop environmentally friendly commercial pest management products.

Laboratory and field efficacy of natural products against the invasive pest Halyomorpha halys and side effects on the biocontrol agent Trissolcus japonicus.

Chierici E, Marchetti E, Poccia A, Russo A, Giannuzzi VA, Governatori L, Zucchi L, Rondoni G, Conti E.Sci Rep. 2025 Feb 7;15(1):4622. doi: 10.1038/s41598-025-87325-9.PMID: 39920209

The brown marmorated stink bug, Halyomorpha halys (Hemiptera: Pentatomidae), is an invasive pest causing major economic losses to crops. Since its outbreaks in North America and Europe, H. halys has been controlled with synthetic pesticides. More sustainable methods have been proposed, including biocontrol and use of natural products. Here, we conducted laboratory and field investigations to evaluate organically registered products for their effectiveness against H. halys and their non-target effect on the egg parasitoid, Trissolcus japonicus (Hymenoptera: Scelionidae). In the laboratory, azadirachtin, orange oil, potassium salts of fatty acids, kaolin, basalt dust, diatomaceous earth, zeolite, sulphur formulations, calcium polysulfide, and mixtures of sulphurs plus

diatomaceous earth or zeolite demonstrated higher lethality against H. halys nymphs compared to control. Calcium polysulfide, azadirachtin and sulphur achieved more than 50% mortality. All treatments except azadirachtin and kaolin had negative effects on T. japonicus, with mortality exceeding 80% for calcium polysulfide and sulphur. Field experiments were conducted in 2021 and 2022 in pear orchards. Diatomaceous earth alone or alternated with sulphur or calcium polysulfide provided similar H. halys control, when compared to farm strategies based mostly on neonicotinoid (acetamiprid) treatments. Implications for H. halys control in integrated pest management are discussed.

Mitigation of salinity stress in sunflower plants (*Helianthus annuus* L.) through topical application of salicylic acid and silver nanoparticles.

Shahbaz M, Anwar T, Fatima S, Onursal N, Qureshi H, Qureshi WA, Ullah N, Soufan W, Zaman W.Physiol Mol Biol Plants. 2025 Jan;31(1):27-40. doi: 10.1007/s12298-024-01535-5. Epub 2024 Dec 30.PMID: 39901956

Salinity stress poses a significant threat to sunflower (*Helianthus annuus* L.) by impairing water and nutrient uptake, disrupting cellular functions, and increasing oxidative damage. This study investigates the impact of Salicylic acid (SA) and silver nanoparticles (AgNPs) on growth, biochemical parameters, and oxidative stress markers in salt-stressed sunflower plants. Experiments were conducted in a controlled greenhouse environment at the Islamia University of Bahawalpur, Pakistan, using sunflower seeds (Orisun 701). AgNPs were synthesized using neem leaf extract and characterized through SEM, FTIR, zeta potential analysis, and XRD. Treatments included foliar application of SA (10 mM) and AgNPs (40 ppm) under 100 mM sodium chloride-induced salt stress. Growth metrics, antioxidant enzyme activities, chlorophyll content, and oxidative stress markers (H_2O_2 and MDA levels) were measured to evaluate treatment effects. The SA and AgNP treatments improved sunflower growth under salt stress, with AgNPs showing a greater impact. SA increased shoot fresh weight by 16.4%, root fresh weight by 6.9%, and chlorophyll content by 12.7%, while AgNPs enhanced shoot fresh weight by 30.5%, root fresh weight by 11.6%, and total chlorophyll by 80%. AgNPs also significantly reduced H_2O_2 by 42.7% and MDA by 34.6%, indicating reduced oxidative damage. Cluster analysis further demonstrated the distinct physiological responses elicited by AgNPs compared to SA. SA and AgNPs enhance sunflower resilience to salinity, with AgNPs showing a particularly strong effect on chlorophyll content and oxidative stress markers. These findings highlight the potential of SA and AgNPs as effective treatments for salt stress, suggesting further research across different crops and environments.

Disposable electrochemical biosensor based on acetylcholinesterase for inhibition assays using a natural substance and plant extracts.

Dos Santos Araújo S, Fonseca WT, das Graças Fernandes da Silva MF, Forim MR, Fernandes JB, Censi Faria R.Anal Methods. 2025 Feb 28. doi: 10.1039/d4ay02084g. Online ahead of print.PMID: 40017448

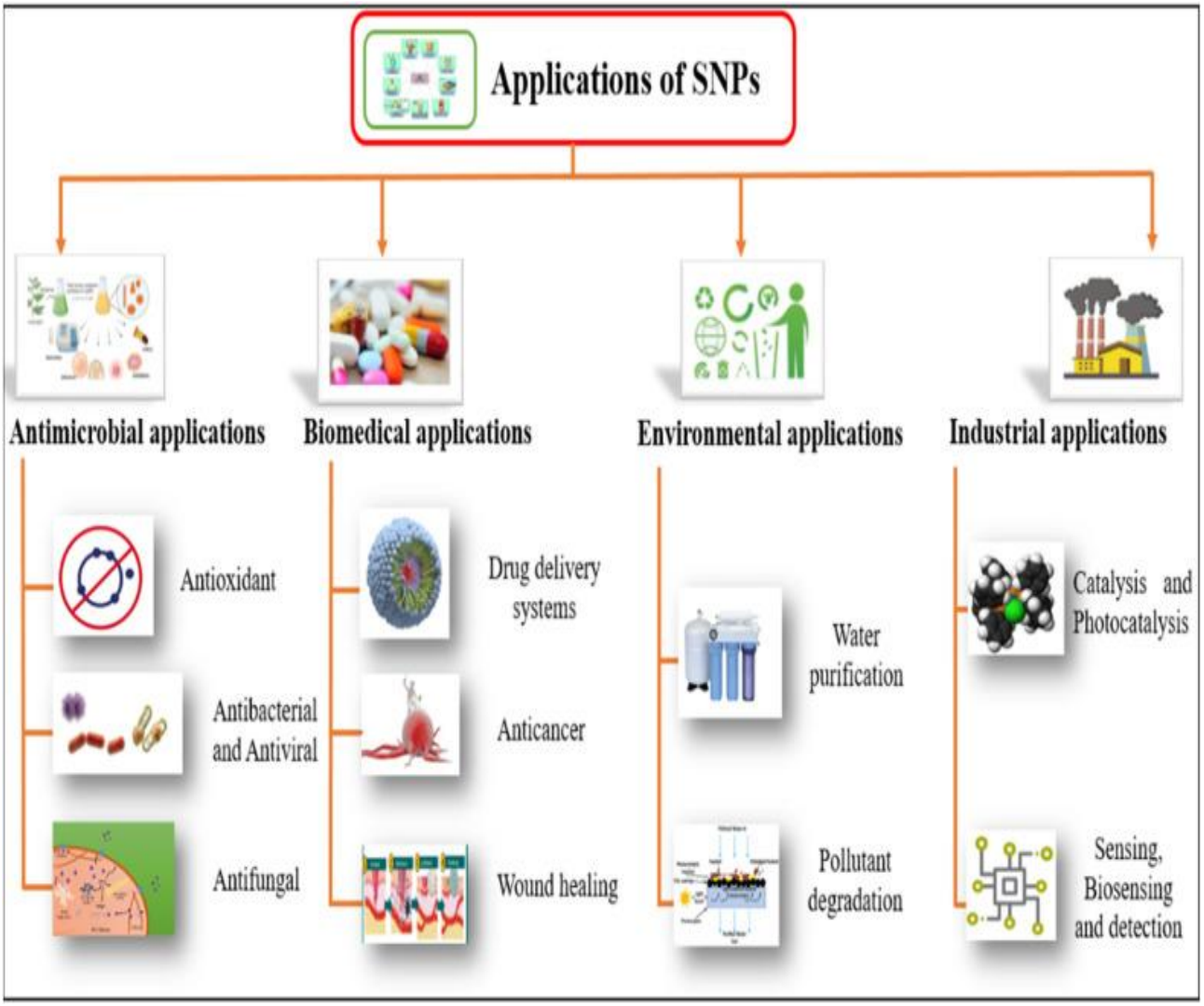
In general, insects are considered pests in agricultural areas, and their control is essential for high productivity of the cultivated areas. Control of these insects can be achieved by the inhibition of enzymes present in the insect's body. The enzyme acetylcholinesterase

(AChE) is present in the neuromuscular junctions of vertebrates and invertebrates, and it is an important target for pest control. Herein, we present the development of a disposable electrochemical biosensor based on AChE (Bio-AChE) to rapidly evaluate the presence of new potential inhibitors in crude extracts of plants. Bio-AChE was constructed by modifying the disposable screen-printed carbon electrode (SPCE) with glutathione-decorated gold nanoparticles on which AChE was covalently immobilized. Electrochemical studies confirm the effective immobilization of the enzyme, and the Bio-AChE was applied to assess the inhibitory activity of azadirachtin, obtained from *Azadirachta indica*, which is a well-known AChE inhibitor. The proposed biosensor showed excellent results, demonstrating the inhibition activity of azadirachtin against AChE. The crude extracts of *Picramnia riedelli*, *P. ciliata*, and *Toona ciliata* were evaluated with the Bio-AChE, and all showed inhibition percentage values of around 50%. The extracts were evaluated by ^{1}H NMR spectra, which identified classes of natural compounds that could be responsible for the inhibition activity. The proposed disposable Bio-AChE was shown to be a reliable method for simple and rapid screening of new inhibitors in plant extracts, opening an avenue for the screening of new natural products with potential for pest control.

Neem for Sustainable Environment & Green Synthesis

A review on green synthesis of silver nanoparticles (SNPs) using plant extracts: a multifaceted approach in photocatalysis, environmental remediation, and biomedicine.
Shahzadi S, Fatima S, Ul Ain Q, Shafiq Z, Janjua MRSA.RSC Adv. 2025 Feb 6;15(5):3858-3903. doi: 10.1039/d4ra07519f. eCollection 2025 Jan 29.PMID: 39917042

A sustainable and viable alternative for conventional chemical and physical approaches is the green production of silver nanoparticles (SNPs) using plant extracts. This review centers on the diverse applications of plant-mediated SNPs in biomedicine, environmental remediation, and photocatalysis. *Ocimum sanctum* (tulsi), *Curcuma longa* (turmeric), and *Azadirachta indica* (neem) and many others are plant extracts that have been used as stabilizing and reducing agents because of their extensive phytochemical profiles. The resulting SNPs have outstanding qualities, such as better photocatalytic degradation of organic dyes like methylene blue, antibacterial efficacy towards multidrug-resistant pathogens, biocompatibility for possible therapeutic applications, and regulated magnitude (10-50 nm), enhanced rigidity, and tunable surface plasmon resonance. Significant effects of plant extract type, amount, and synthesis parameters on the physical and functional characteristics of SNPs are revealed by key findings. Along with highlighting important issues and potential paths forward, this review also underlines the necessity of scalable production, thorough toxicity evaluations, and investigating the incorporation of SNPs into commercial applications. This work highlights how plant-based SNPs can be used to address global environmental and biological concerns by straddling the division between sustainable chemistry and nanotechnology.

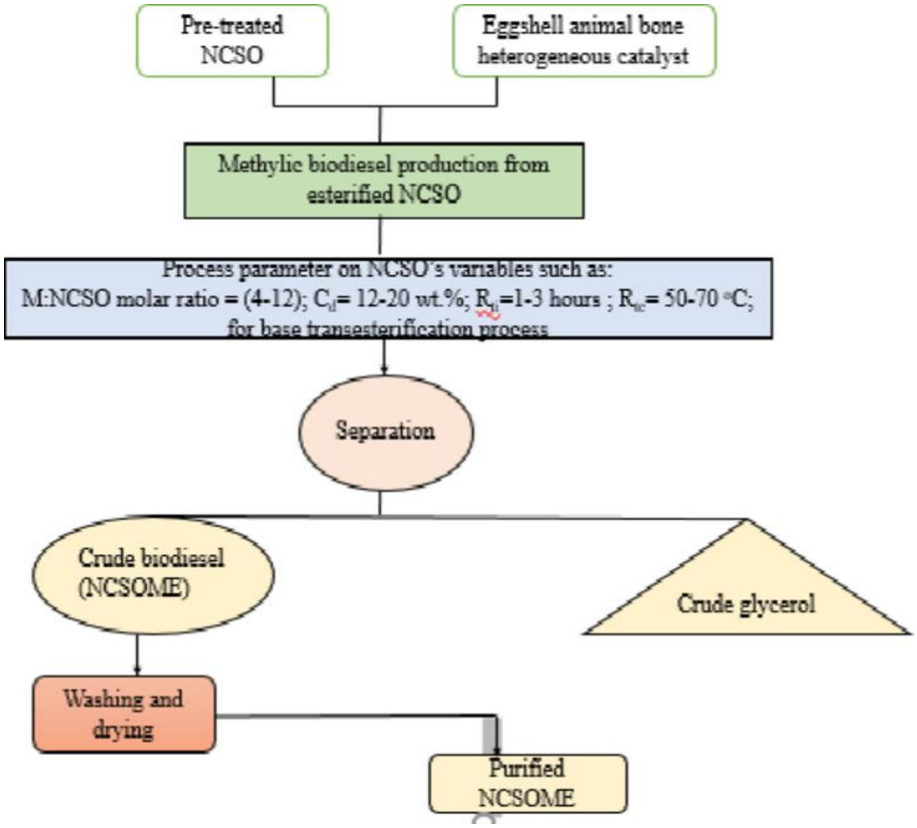


ANN-ANFIS model for optimising methylic composite biodiesel from **neem** and castor oil and predicting emissions of the biodiesel blend.

Zhu CZ, Samuel OD, Taheri-Garavand A, Elboughdiri N, Paramasivam P, Hussain F, Enweremadu CC, Ayanie AG.Sci Rep. 2025 Feb 15;15(1):5638. doi: 10.1038/s41598-025-88901-9.PMID: 39955378

Researchers and stakeholders have shown interest in heterogeneous composite biodiesel (HCB) due to its enhanced fuel properties and environmental friendliness (EF). The lack of high viscosity datasets for parent hybrid oils has hindered their commercialisation. Reliable models are lacking to optimise the transesterification parameters for developing HCB, and the scarcity of predictive models has affected climate researchers and environmental experts. In this study, basic fuel properties were analysed, and models were developed models for the yield of HCB and kinematic viscosity (KV) for composite biodiesel/neem castor seed oil methyl ester (NCSOME) using Artificial Neural Network (ANN) and Adaptive Neuro Fuzzy Inference System (ANFIS). Statistical indices such as computed coefficient of determination (R^2), root-mean-square-error (RMSE), standard error of

prediction (SEP), mean average error (MAE), and average absolute deviation (AAD) were used to evaluate the effectiveness of the techniques. Emission models for NCSOME-diesel blends were also established. The study investigated the impact of optimised fuel types/NCSOME-diesel (10-30 vol%), ZnO nanoparticle dosage (400-800 ppm), engine speed (1100-1700 rpm), and engine load (10-30%) on emission characteristics and environmental friendliness indices (EFI) such as carbon monoxide (CO), Oxides of Nitrogen (NOx), and Unburnt Hydrocarbon (UHC) using Response Surface Methodology (RSM). The ANFIS model demonstrated superior performance in terms of R^2, RMSE, SEP, MAE, and AAD compared to the ANN model in predicting yield and KV of HCB. The optimal emission levels for CO (49.26 ppm), NO_x (0.5171 ppm), and UHC (2.783) were achieved with a fuel type of 23.4%, nanoparticle dosage of 685.432 ppm, engine speed of 1329.2 rpm, and engine load of 10% to ensure cleaner EFI. The hybrid ANFIS and ANN models can effectively predict and model fuel-related characteristics and improve the HCB process, while the RSM model can be a valuable tool for climate and environmental stakeholders in accurate forecasting and promoting a cleaner environment. The valuable datasets can also provide reliable information for strategic planning in the biodiesel and automotive industries.



Neem for Industrial Applications

Lubricity potentials of Azadirachta indica (**neem**) oil and Cyperus esculentus (tiger nut) oil extracts and their blends in machining of mild steel material.

Ekengwu IE, Okoli IG, Okafor OC, Ezenwa ON, Ogu JC.Heliyon. 2025 Jan 16;11(2):e42059. doi: 10.1016/j.heliyon.2025.e42059. eCollection 2025 Jan 30.PMID: 39906805

Friction amongst the cutting tool and workpiece in metal machining produces heat that reduces tool life and workpiece integrity. Consequently, non-biodegradable soluble mineral oil is predominantly used as a lubricant to enhance machining operations. Nevertheless, recent investigations focus on environmentally friendly biodegradable oils for lubrication. Therefore, this study examines the lubricity potential of neem oil, tiger nut oil, and their blends in machining mild steel. It also evaluates the performance characteristics of individual bio-oils and their blends against conventional soluble mineral oil and dry-drilling methods. Neem and tiger nut oils were extracted using pressing and solvent methods, followed by an analysis of their physiochemical properties. The experimental design utilized the I-Optimal custom design and simplex lattice design (SLD) for the individual and blended oils respectively. Response Surface Methodology (RSM) was applied for optimization, with feed rate, oil type, and spindle speed as independent variables, and cutting temperature, surface finish, depth of cut, chip thickness, chip thickness ratio, cutting speed, and material removal rate as response variables. The optimal cutting conditions were predicted at a spindle speed of 695 rpm, feed rate of approximately 0.4735, and neem oil being the cutting fluid. The predicted response values were cutting temperature - 33.5 °C, surface roughness - 2.65 μm, depth of cut - 41.4825 mm, chip thickness - 0.18951 mm, chip thickness ratio - 269.586, cutting speed - 17.4695 m/min, and material removal rate - 2.38025E-05. Results indicated neem oil surpassed tiger nut oil and conventional oils in minimizing cutting temperatures and enhancing surface quality, achieving a desirability value of 0.85428 under optimal conditions. Moreover, an 80/20 blend of neem and tiger nut oils exhibited improved performance, attaining a desirability value of 0.992, underscoring its potential as an effective cutting fluid. The findings advocate for the use of bio-based cutting fluids in machining operations, indicating environmental and economic advantages while promoting future research into alternative agro-based solutions. However, limitations regarding material applicability and the necessity for further investigation into the micro-structural effects of cutting fluids on diverse engineering materials are acknowledged.

pH-sensitive polymeric micelles of polyvinyl acetate grafted **neem** gum amphiphilic graft copolymer for curcumin delivery.
Sharma S, Lal UR, Bal T.Int J Biol Macromol. 2025 Feb 5;303:140574. doi: 10.1016/j.ijbiomac.2025.140574. Online ahead of print.PMID: 39920929

The increasing demand for efficient drug delivery systems to address the challenges of hydrophobic therapeutic agents, such as poor solubility and bioavailability, has driven research into polymeric micelles. In this study, polymeric micelles were formulated using polyvinyl acetate grafted neem gum amphiphilic graft copolymer (NG-g-PVAc), synthesized via a microwave-assisted technique, to encapsulate and deliver curcumin, a hydrophobic model drug with significant therapeutic potential. Curcumin-loaded polymeric micelles (CUR-PMs) were prepared using a co-solvent evaporation method, achieving a high encapsulation efficiency of 95.42 ± 2.22 % and a critical micelle concentration (CMC) of 0.027 mg/mL, indicating stability in aqueous environments. Physicochemical characterization, including particle size analysis, XRD, and TEM, confirmed the formation of well-dispersed, spherical micelles. In vitro release studies revealed a pH-sensitive and sustained release profile over 8 days, with enhanced drug release at pH 7.4, simulating physiological conditions. CUR-PMs demonstrated superior antibacterial activity against gram-positive bacteria, suggesting potential in wound-healing applications, and exhibited significantly higher cytotoxicity against Hep-G2 cells compared to free curcumin, highlighting improved therapeutic efficacy. Toxicological studies in zebrafish and murine models confirmed the safety and biocompatibility of CUR-PMs, supporting their suitability for biomedical applications. This work highlights the potential of neem gum-based polymeric micelles as a cost-effective and environmentally friendly drug delivery platform. However, limitations such as scalability and long-term stability under varying storage conditions require further investigation to facilitate clinical translation.

Evaluation of Smear Layer Removal and Antimicrobial Efficacy of Intracanal Herbal Irrigants.
Alelyani AA.J Pharm Bioallied Sci. 2024 Dec;16(Suppl 4):S3139-S3141. doi: 10.4103/jpbs.jpbs_617_24. Epub 2024 Sep 20.PMID: 39926932

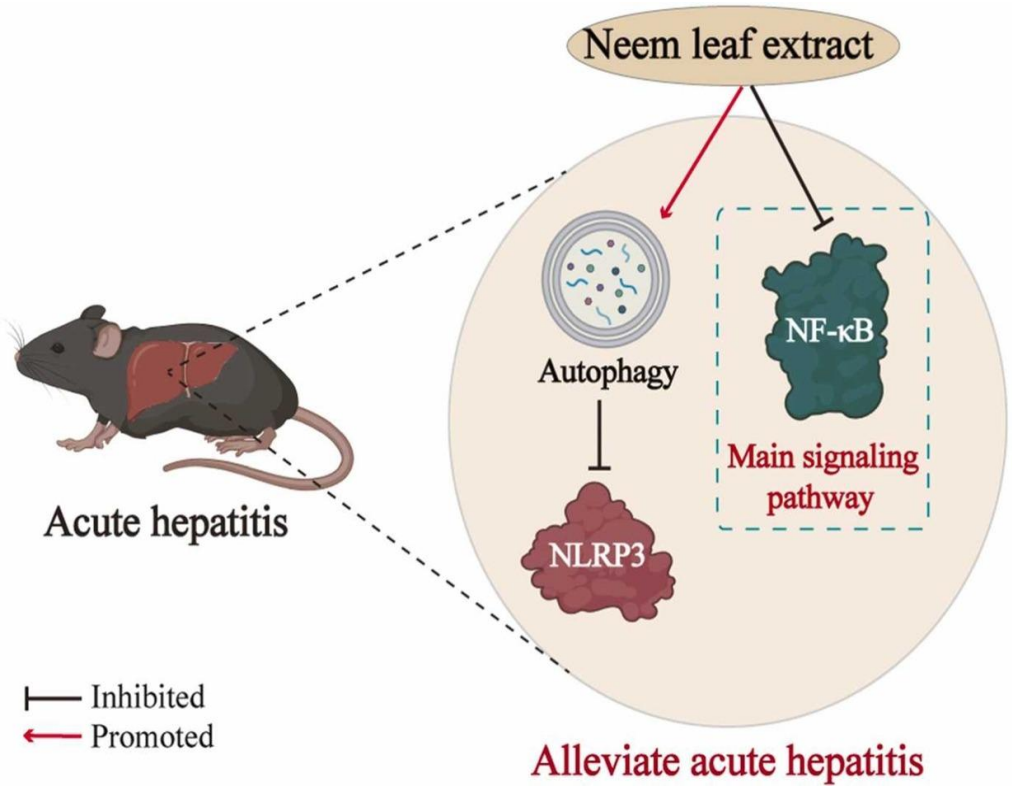
Background: The removal of the smear layer and antimicrobial efficacy are critical factors in endodontic treatment. Conventional chemical irrigants like sodium hypochlorite (NaOCl) and ethylenediaminetetraacetic acid (EDTA) have proven effective but can have adverse effects. **Materials and methods:** A total of 60 extracted single-rooted human teeth were randomly assigned to four groups of 15 each. Group 1 was irrigated with 5.25% NaOCl, group 2 with 17% EDTA, group 3 with 10% neem extract, and group 4 with 5% turmeric extract. Smear layer removal was assessed using scanning electron microscopy (SEM) at the coronal, middle, and apical thirds of the root canals. Antimicrobial efficacy was tested against *Enterococcus faecalis* using the agar diffusion method, and the zones of inhibition were measured in millimeters. **Results:** Neem extract showed an average smear layer

removal score of 2.5 (±0.3) compared to 1.8 (±0.2) for NaOCl, 1.9 (±0.3) for EDTA, and 3.2 (±0.4) for turmeric extract. Neem and turmeric extracts demonstrated significant antimicrobial activity with mean inhibition zones of 16 mm (±1.2) and 14 mm (±1.1), respectively, compared to 18 mm (±1.5) for NaOCl and 15 mm (±1.3) for EDTA. **Conclusion:** Herbal irrigants, particularly neem extract, show promise as effective alternatives to conventional irrigants for smear layer removal and antimicrobial activity. Their biocompatibility and comparable efficacy suggest potential for use in endodontic practice.

Neem leaf extract alleviates LPS/D-GalN induced acute hepatitis in mice through its anti-inflammatory effects and activation of autophagy.

Jin M, Mengfan LV, Yu H, Cheng J, Zhang Y, Zhai Y, Feng H.Mol Immunol. 2025 Feb 24;180:33-43. doi: 10.1016/j.molimm.2025.02.015. Online ahead of print.PMID: 39999524

Acute hepatitis, characterized by rapid onset and high mortality, can result from infections, toxins, and other factors. However, current treatment options have significant side effects, necessitating further research into alternative therapies. This study investigated the extraction method of neem extract and found that its ethanolic extract effectively reduced mortality and decreased ALT and AST levels in mice serum, improving liver pathology. HPLC analysis identified azadirachtin and nimbolide in the extract. It also downregulated NF-κB, NLRP3, and p62 levels, while upregulating Lc3B and Atg5 levels. Experiments in Atg5 knockout mice showed that the absence of Atg5 weakened the extract's efficacy in reducing liver damage and inflammation and affected the extent of NLRP3 protein downregulation. However, it did not affect the extract's ability to reduce NF-κB. Overall, the ethanolic extract of neem leaves primarily modulates the inflammatory response through the NF-κB signaling pathway. The extract's efficacy in reducing NLRP3 is associated with autophagy. These discoveries offer a new theoretical basis for the role of neem in treating acute hepatitis.



Synthesis of Yttria Nanoparticle-Loaded Electrospun Nanofibers for Enhanced Antimicrobial Activity, Biofilm Inhibition, and Alleviation of Diabetic Wounds.

Ghosh A, Bhattacharya T, Mandal D, Dutta K, Dey S, Saha K, Chattopadhyay D.ACS Appl Bio Mater. 2025 Feb 26. doi: 10.1021/acsabm.4c01818. Online ahead of print.PMID: 40009776

Diabetes-related sores and ulcers are quite common around the world and can cause complicated disruptions to both patient compliance and socioeconomic structure. Diabetic wounds take longer to heal due to pathophysiological causes, persistent infections, and increasingly severe medical problems. Nanoparticles (NPs) derived from nanotechnology have drawn interest due to their revolutionary potential in understanding the biological milieu and offering therapeutic strategies for wound healing. In this regard, the potential of yttrium oxide nanoparticles (YNPs) has been studied extensively to understand their efficacy in diabetic wound healing. Yttrium oxide nanoparticles having size in the range of 2-10 nm were prepared and incorporated into nanofibrous mats consisting of polyurethane as the matrix polymer, and leaf extract of *Azadirachta indica* and clindamycin hydrochloride as additive conventional antidiabetic and antibacterial agents to form S3. Physicochemical characterization tests confirmed the formation of nanofibers having average diameters in the range of 320-470 nm, respectively. The study demonstrated that S3 shows an enhanced zone of inhibition against *E. coli* (29 mm), *S. aureus* (32 mm), and *P. aeruginosa* (30 mm). Moreover, the nanofibrous mats also prevented microbial penetration and biofilm formation, as observed from MTT, CV, and confocal microscopy images. *In vivo* wound healing study conducted on diabetic mice revealed that S3 exhibited high wound contraction after 9 days of treatment. Additionally, the fabricated mat lowered plasma glucose levels, hepatotoxicity, and oxidative stress biomarkers. Therefore, it can be concluded that YNP-loaded nanofibrous composite mats have a strong potential in alleviating diabetic wounds.

Limonoid-rich fraction from Azadirachta indica A. Juss. (**neem**) stem bark triggers ROS-independent ER stress and induces apoptosis in 2D cultured cervical cancer cells and 3D cervical tumor spheroids.

Kumar S, Das B, Maurya G, Dey S, Gupta P, Sarma JD.BMC Cancer. 2025 Feb 25;25(1):334. doi: 10.1186/s12885-025-13601-6.PMID: 40000992

Background: The existing anticancer drugs in clinical practice show poor efficacy in cervical cancer patients and are associated with multiple side effects. Our previous study demonstrated the strong antineoplastic activity of crude extract prepared from the stem bark of Azadirachta indica (Neem) against cervical cancer. However, the active phytoconstituents of neem stem bark extract and its underlying anticancer mechanism are yet to be investigated. Thus, the present study aimed to identify the active fraction from crude neem stem bark extract to further dissect its anticancer mechanism and determine the active components. **Methods:** Dichloromethane (DCM) extract from neem stem bark was prepared and fractionated using thin-layer chromatography. The fractions obtained were screened against HeLa and ME-180 cervical cancer cell lines to identify the most active fraction, which was then selected for further studies. Clonogenic assay, cell cycle

analysis, apoptosis assay, and reactive oxygen species (ROS) assay were performed to determine the cytotoxicity of the active fraction. Gene expression was analyzed using real-time PCR and western blot to determine the mechanism. Additionally, the HeLa cells-derived 3D spheroid model was used to determine the antitumor efficacy of the active fraction. Electrospray ionization-mass spectrometry, Fourier-transform infrared spectroscopy, and proton nuclear magnetic resonance were used to identify the phytoconstituents of the fraction. **Results:** Initial screening revealed fraction 2 (F2) as the most active fraction. Additionally, F2 showed the least cytotoxic effect on normal human fibroblast cells. Mechanistically, F2 induced cell cycle arrest and apoptosis in cervical cancer cells. F2 increased ROS levels, induced ER stress, and activated cell survival pathway. Treatment with N-acetyl cysteine revealed that F2 induced ROS-independent ER stress and apoptosis. 3D spheroid viability and growth delay experiments demonstrated the strong antitumor potential of F2. Finally, six compounds, including one flavonoid (nicotiflorin) and five limonoids, were identified in the F2 fraction. **Conclusion:** This is the first study to identify the active fraction and its phytoconstituents from neem stem bark and demonstrate the anticancer mechanism against cervical cancer. Our study highlights the importance of investigating neem stem bark-derived limonoids and nicotiflorin as a potential source to develop new anticancer therapeutic agents.