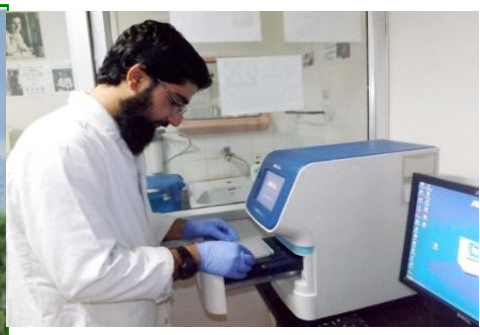
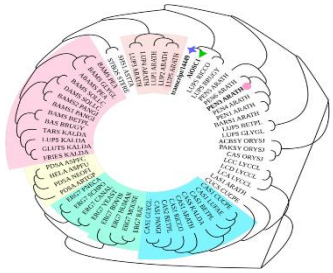
Neem Research Newsletter

Volume 4, Issue 5, 2024





WORLD NEEM ORGANISATION (WNO)

From

The Editor's Desk……

This edition of the Newsletter brings some interesting research findings in diverse areas. Experimental evidence suggests that vermicast made from neem leaf litter may serve as a potent combination of a biofertilizer and a pesticide. Neem leaf extract was shown to have curative potential against bacterial infection in fish. The effect of neem oil biodiesel on the structural integrity of carbon steel alloy was investigated. Biocompatible-neem gum/Polyvinyl alcohol composite polymers were shown to exhibit superior dielectric strength and versatility, surpassing traditional inorganic ceramic dielectrics in advanced electronics and pulsed power systems. Neem leaf extract based green-synthesized ZnO nanoparticles coated on spent tea waste activated carbon was demonstrated to be useful for pharmaceuticals and personal care products removal. Andrographolide from neem could serve as a potential lead compound for the development of an anti-schistosomal drug. The mechanisms underlying the anti-aging and anti-oxidative properties of neem leaf extract have been unveiled providing insights for pharmacological interventions. Neem leaf glycoprotein immunotherapy was demonstrated to be useful in targeting therapy-elusive cancer stem cells without evoking toxicity. The neem limonoid gedunin was found to induce cell death in a rat model of gastric carcinogenesis and in glioblastoma cell lines.

S. Nagini

Core Founding Member, WNO
Chief Scientific Coordinator &
Regional Director, South India

Neem in Agriculture & Aquaculture

A Comprehensive Perception of Biological Control Potential of Endophytes and Quality Refinement of Lagenaria siceraria.

Rehan N, Farhat H, Shafique HA, Shaheen S.

Curr Microbiol. 2024 May 21;81(7):184. doi: 10.1007/s00284-024-03706-9.PMID: 38771325

Agriculture and livestock management practices known as organic farming rely more on internal processes than external inputs. Natural environments depend heavily on diversity, and organic farming incorporates both the stated purpose of fostering diversity as well as the use of diversity as a management tool. A more complete understanding of agriculture in terms of agro-ecology has begun to be questioned by the traditional reductionist approach to the study of agriculture. Therefore it is necessary to be aware more about the significance of microbes in processes including soil growth, plant nourishment, and the eradication of plant disease, pest, and weeds. In this study, fluorescent Pseudomonas strain (EFP56) and Trichoderma harzianum were studied for antifungal and antibacterial activity against four common root rot fungi and four common laboratory bacteria in vitro experiments. Furthermore, soil-borne disease surveillance and nutritional quality of Lagenaria siceraria, fluorescent Pseudomonas strain (EFP56) and Trichoderma harzianum were combined with neem cake and cotton cake to check their efficacy. Through the application of organic soil amendments in combination with biocontrol agents improved the quality of vegetables and their nutritional value by raising their polyphenol, carbohydrate, and protein content as well as enhancing antioxidant scavenging status. The experiments were conducted in pots and in fields to confirm their efficacy rate. The final outcomes also revealed greater induction of defense system, disease lessening and enriched fruit quality. Consortium of neem cake and cotton cake with bio-stimulants can regulate biotic as well as abiotic stress.

Assessment of microbial flora and pesticidal effect of vermicast generated from Azadirachta indica (neem) for developing a biofertilizer-cum-pesticide as a single package.

Thamizharasan A, Aishwarya M, Mohan V, Krishnamoorthi S, Gajalakshmi S.

Microb Pathog. 2024 May 15:106690. doi: 10.1016/j.micpath.2024.106690. Online ahead of print.PMID: 38759935

The soil comprising organic matter, nutrients, serve as substrate for plant growth and various organisms. In areas where there are large plantations, there is a huge leaf litter fall. The leaf litter upon decomposition releases nutrients and helps in nutrient recycling, for which the soil engineers such as earthworms, ants and termites are important key players. In this context, this study was conducted to assess the characteristics of the vermicast obtained by vermicomposting neem leaf litter in terms of microbial flora, plant growth promoting properties and antagonistic activities of the vermicast against phytopathogens. Vermicomposting of neem leaf litter was done using two epigeic earthworm species Eisenia fetida and Eudrilus eugeniae. The vermicast exhibited antagonistic potential against plant pathogens. Out of the four vermiwash infusions studied

the 75% formulation reduced the disease incidence against mealybug by 82% in the tree Neolamarkia cadamba. The result of the study suggests that vermicast made from neem leaf litter may be a potent combination of a biofertilizer and a pesticide.

Morpho-physiological studies of sandalwood-host interaction under individual and interactive water and salt stress.

Sharma A, Kumar A, Chahal S, Verma K, Chauhan K, Kumar R, Mann A.

Biol Futur. 2024 May 13. doi: 10.1007/s42977-024-00220-8. Online ahead of print.PMID: 38739202

To find out the possibilities of growing white sandalwood in sub-tropical regions of India where farmers facing the problem of water deficit and salinity stress, a RBD experiment was conducted. Sandalwood grown alone and with five selected hosts (Alternanthera sp., Neem, Shisham, Dek and Agarwood) on the basis of prior study under water deficit, salinity stress and combined water deficit and salinity stress. Sandalwood plants were harvested after 180 days of imposing stress treatments. Morphological traits (plant height, collar diameter, shoot fresh and dry biomass) showed significant reduction under water deficit and salinity stress, which were further aggravated under combined water deficit and salinity stress. Studied plant water traits, ionic balance and gas exchange attributes were also reduced by these stresses. While among studied host, Shisham and Dek identified as the best host species under water deficit, salinity and interactive stress by maintaining ion homeostasis, osmotic adjustments and plant water regulation. Results depicted that sandalwood plants cultivated alone were not able to survive under salinity and combined stress conditions and showed poor growth under water deficit and control conditions. Different indices were also calculated based on morpho-physiological and ionic traits and also indicated that sandalwood grown with Dalbergia sissoo and Melia dubia showed higher drought, salt and stress tolerance potential, which made sandalwood adaptable under these stresses. Therefore, the present study signifies the importance of host especially D. sissoo and M. dubia which might be excellent long-term host species for sandalwood cultivation under sub-tropical conditions to thrive under changing environments.

The therapeutic role of Azadirachta indica leaves ethanolic extract against detrimental effects of Aeromonas veronii infection in Nile tilapia, Oreochromis niloticus.

Khalifa HA, Sharawy E, Younis EM, Abdelwarith AA, Ibrahim RE, Amer SA, Davies SJ, Abo-Elmaaty AMA.

Fish Physiol Biochem. 2024 May 25. doi: 10.1007/s10695-024-01349-y. Online ahead of print.PMID: 38795269

Bacterial pathogens cause high fish mortalities and in turn economic losses in fish farms. Innovative strategies should be applied to control bacterial infections instead of antibiotics to avoid the resistance problem. Consequently, the present investigation studied the curative potential of Azadirachta indica leaves ethanolic extract (AILEE) on Aeromonas veronii infection in Oreochromis niloticus. A preliminary trial was assessed to evaluate the curative dose of AILEE which was found to be 2.5 mg/L. One hundred and sixty fish were

divided into equal four groups in four replications, where group 1 and group 2 were non-challenged and treated with 0- and 2.5-mg/L AILEE, respectively. Group 3 and group 4 were challenged with A. veronii and treated with 0- and 2.5-mg/L AILEE, respectively for 10 days. A. veronii infection produced severe clinical manifestations and a high mortality rate in the infected fish. Furthermore, the infected fish exhibited a significant rise in the hepatorenal indices (aspartate aminotransferase, alanine aminotransferase, and creatinine), the oxidant biomarker (malondialdehyde), and the stress indicators (glucose and cortisol). A significant reduction in the protein profile and antioxidant/immune parameters (catalase, immunoglobulin M, lysozyme, nitric oxide, and phagocytic activity) was observed in the infected fish. Water application of the infected group to 2.5-mg/L AILEE notably ameliorated the hepatorenal indices, the oxidant biomarker, and the stress indicators. Furthermore, AILEE improved the antioxidant/immune indices. Water application of 2.5-mg/L AILEE could be useful against A. veronii infection in O. niloticus culture.

Neem for Sustainable Environment & Green Synthesis

Effect of neem oil biodiesel on the surface and structural integrity of carbon steel alloy: Chromatographic, spectroscopic, and morphological investigations.

Adama KK, Ukhurebor KE, Pal K, Hossain I.

Int J Biol Macromol. 2024 May 7;269(Pt 2):132199. doi: 10.1016/j.ijbiomac.2024.132199. Online ahead of print.PMID: 38723824

This study explores the impacts of neem oil biodiesel (BD), which was produced and characterized using GC-MS, FTIR, and UV-Vis spectroscopic techniques to elucidate pure and corrosion-product neem oil BD at room temperature (25 °C) and different immersion durations of 0, 28, 42, and 56 days. The OM and SEM were also employed to study the surface, structural integrity, and interphase interaction between the BD and the carbon steel (C1020) before and after immersion for different durations. The dominant fatty acid (FA) group in both pure and corrosion-product neem oil BD was C_{18}, with a total composition of 72.3 %, hence determining the nature of the BD interaction with the carbon steel. The study revealed that carbon steel (C1020) was susceptible to attacks by neem oil BD, and the duration of immersion had substantial influence on the surface morphology and structural integrity of the steel. It is therefore anticipated that this study will significantly advance the field of alternative fuel research.

Green and efficient extraction of phenolic compounds from Neem leaves using deep eutectic solvents based ultrasonic-assisted extraction.

Kaur K, Schmitt-Kopplin P, Malik AK.

Food Chem. 2024 Apr 27;451:139500. doi: 10.1016/j.foodchem.2024.139500. Online ahead of print.PMID: 38696941

Deep eutectic solvent (DES) combined with ultrasonic-assisted extraction was employed as an environmentally friendly technique for extracting antioxidant phenolic compounds from Neem leaves in place of organic solvents. Choline chloride-Ethylene glycol (1:2) with

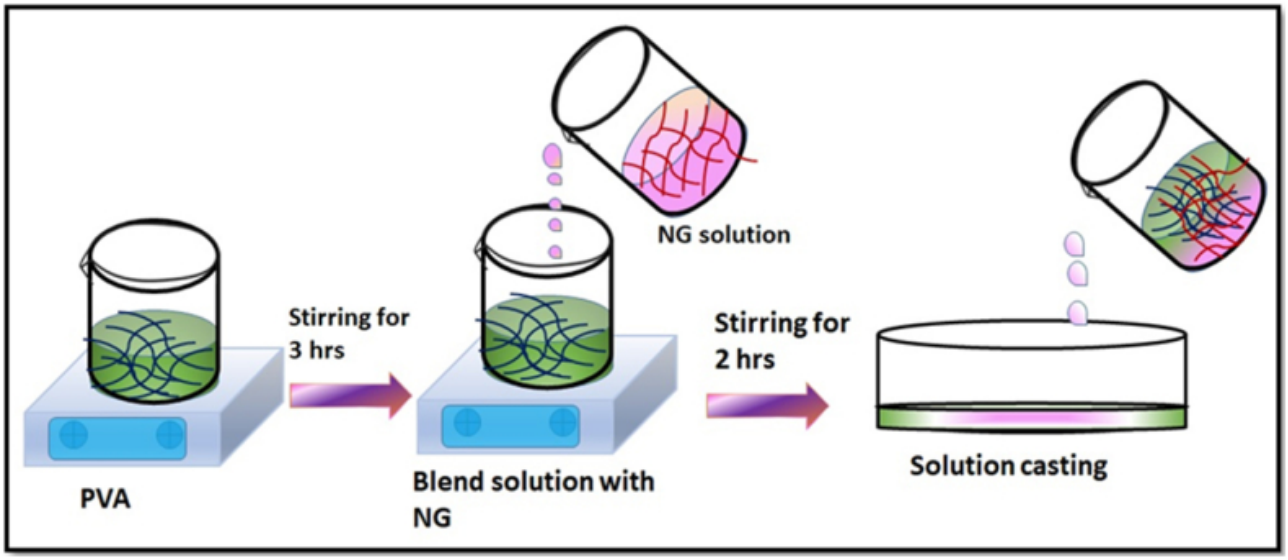
40% V/V water content (DES-1) was investigated as a potential total phenolic content extractant (38.2 ± 1.2 mg GAE/g DW, where GAE: gallic acid equivalent, DW: dry weight). The optimal operational parameters assessed using single-factor experiments to maximize the total phenolic compounds content were as follows: extraction time of 30 min, 40% V/V water content, liquid-solid ratio of 15:1, and room temperature. Additionally, the in-vitro antioxidant experiments (2,2-diphenyl-1- picrylhydrazyl radical scavenging assay and ferric reducing antioxidant power assay) demonstrated the DES-1-based extract of Neem leaves as a potent antioxidant agent, compared to traditional solvents. Moreover, microscopic morphological analysis supported the effectiveness of DES-1 for the noticeable alteration in the fiber surface structure of Neem leaves after extraction which benefited in the release of polyphenols from these leaves. Eventually, the mass analysis of the extract disclosed the presence of eleven polyphenols in the extract. The Green Analytical Procedure Index revealed the greenness of the extraction method.

Biocompatible neem gum-modified polyvinyl alcohol composite as dielectric material for flexible energy devices.

Parangusan K, Subramaniam V, Babu A, Venkatesh PS, Vijayalakshmi S, Ponnamma D.

Heliyon. 2024 Mar 20;10(7):e28379. doi: 10.1016/j.heliyon.2024.e28379. eCollection 2024 Apr 15.PMID: 38689987

In our pursuit of a flexible energy storage solution, we have developed biocompatible (bc)-NG/PVA composite polymers by combining neem tree gum (NG) with polyvinyl alcohol (PVA). This innovative bio-inspired approach harnesses NG's unique properties for both the bio-electrolyte and bio-electrode components. The resulting bc-NG/PVA composites exhibit superior dielectric strength and versatility, surpassing traditional inorganic ceramic dielectrics in advanced electronics and pulsed power systems. Our study investigates the dielectric characteristics, conductivities, electric modulus, and impedance parameters of Pure PVA and NG-doped PVA composites. Adding 5 % NG to PVA significantly boosts its conductivity from 10^{-8} S cm^{-1} to 10^{-4} S cm^{-1}, while the dielectric constant of PVA/5 % NG composite jumps to 104.5 compared to pure PVA. These improvements position the composite films of 5 % NG added PVA as promising materials for diverse applications. The heightened performance of these NG-blended PVA composite materials underscores their potential as a valuable resource for flexible energy storage solutions.

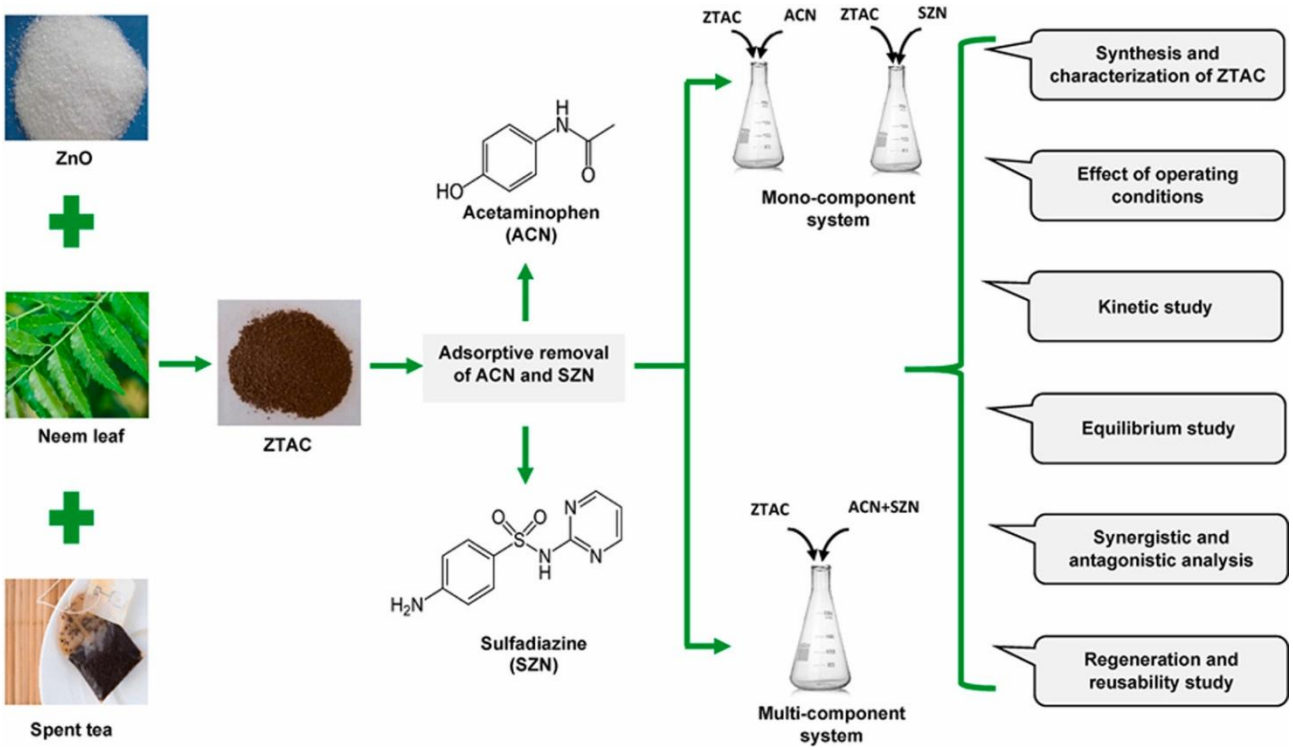


Azadirachta indica leaf extract based green-synthesized ZnO nanoparticles coated on spent tea waste activated carbon for pharmaceuticals and personal care products removal.

Sanjeev NO, Vallabha MS, Valsan AE.

Environ Res. 2024 May 3;252(Pt 3):119047. doi: 10.1016/j.envres.2024.119047. Online ahead of print.PMID: 38704006

Pharmaceuticals and personal care products (PPCPs) are emerging contaminants in aqueous systems, posing threat to both human health and environment. In prior research, predominant focus has been on examining various adsorbents for removing PPCPs from single-pollutant systems. However, no study has delved into simultaneous adsorption of PPCPs multi-pollutant mixture. This study evaluates performance of Azadirachta indica leaf extract-based green-synthesized ZnO nanoparticles coated on spent tea waste activated carbon (ZTAC) for removing sulfadiazine (SZN) and acetaminophen (ACN). Adsorption investigations were conducted in single-component (ACN/SZN) and binary-component (ACN + SZN) systems. The synthesized ZTAC was characterized using SEM, XRD, FTIR, EDX, porosimetry and pH_{pzc} analysis. The study examines impact of time (1-60 min), dose (0.2-4 g/L), pH (2-12) and PPCPs concentration (1-100 mg/L) on ACN and SZN removal. Various kinetic and isotherm models were employed to elucidate mechanisms involved in sorption of PPCPs. Furthermore, synergistic and antagonistic aspects of sorption process in multi-component system were investigated. ZTAC, characterized by its crystalline nature and surface area of 980.85 m^2/g, exhibited maximum adsorption capacity of 47.39 mg/g for ACN and 34.01 mg/g for SZN under optimal conditions of 15 min, 3 g/L and pH 7. Langmuir isotherm and pseudo-second-order kinetic model best-fitted the experimental data indicating chemisorption mechanism. Removal of ACN and SZN on ZTAC demonstrated synergistic nature, signifying cooperative adsorption. Overall, valorization of ZTAC offers effective and efficient adsorbent for elimination of PPCPs from wastewater.



Neem for Human Health

In Silico Comparison of Bioactive Compounds Characterized from Azadirachta indica with an FDA-Approved Drug against Schistosomal Agents: New Insight into Schistosomiasis Treatment.

Oyinloye BE, Shamaki DE, Agbebi EA, Onikanni SA, Ubah CS, Aruleba RT, Dao TNP, Owolabi OV, Idowu OT, Mathenjwa-Goqo MS, Esan DT, Ajiboye BO, Omotuyi OI.

Molecules. 2024 Apr 23;29(9):1909. doi: 10.3390/molecules29091909.PMID: 38731401

The burden of human schistosomiasis, a known but neglected tropical disease in Sub-Saharan Africa, has been worrisome in recent years. It is becoming increasingly difficult to tackle schistosomiasis with praziquantel, a drug known to be effective against all *Schistosoma species*, due to reports of reduced efficacy and resistance. Therefore, this study seeks to investigate the antischistosomal potential of phytochemicals from *Azadirachta indica* against proteins that have been implicated as druggable targets for the treatment of schistosomiasis using computational techniques. In this study, sixty-three (63) previously isolated and characterized phytochemicals from *A. indica* were identified from the literature and retrieved from the PubChem database. In silico screening was conducted to assess the inhibitory potential of these phytochemicals against three receptors (*Schistosoma mansoni* Thioredoxin glutathione reductase, dihydroorotate dehydrogenase, and Arginase) that may serve as therapeutic targets for schistosomiasis treatment. Molecular docking, ADMET prediction, ligand interaction, MMGBSA, and molecular dynamics simulation of the hit compounds were conducted using the Schrodinger molecular drug discovery suite. The results show that Andrographolide possesses a satisfactory pharmacokinetic profile, does not violate the Lipinski rule of five, binds with favourable affinity with the receptors, and interacts with key amino acids at the active site. Importantly, its interaction with dihydroorotate dehydrogenase, an enzyme responsible for the catalysis of the de novo pyrimidine nucleotide biosynthetic pathway rate-limiting step, shows a glide score and MMGBSA of -10.19 and -45.75 Kcal/mol, respectively. In addition, the MD simulation shows its stability at the active site of the receptor. Overall, this study revealed that Andrographolide from *Azadirachta indica* could serve as a potential lead compound for the development of an anti-schistosomal drug.

Synergistic antimicrobial action of chitosan-neem extracts nanoformulation as a promising strategy for overcoming multi-drug resistant bacteria.

Almowallad SJ, Alqahtani LS.

Int J Biol Macromol. 2024 May 24:132337. doi: 10.1016/j.ijbiomac.2024.132337. Online ahead of print.PMID: 38797302

The objective of the present study was to analyze and identify the phytochemical components found in neem leaf extracts using gas chromatography-mass spectrometry (GC-MS) and Fourier-transform infrared spectroscopy (FTIR) methods. The samples were acquired using ethyl acetate (EA) and petroleum ether (PE) solvents. Moreover, the extracts were assessed for their antibacterial and antioxidant features. In addition, chitosan nanoparticles (Cs NPs) containing neem extracts were synthesized and

evaluated for their antibacterial properties, explicitly targeting multi-drug resistant (MDR) bacteria. The neem extracts were analyzed using GC-MS, which identified components such as hydrocarbons, phenolic compounds, terpenoids, alkaloids, and glycosides. The PE extract showed significant antibacterial activity against a range of bacteria. In addition, the PE extract exhibited significant antioxidant activity, exceeding both the EA extract and vitamin C. Both extracts exhibited notable antibiofilm activity, significantly impeding the production of biofilm. The Cs NPs, loaded with neem extracts, exhibited significant antibacterial action against multidrug-resistant (MDR) microorganisms. The Cs NPs/EA materials had the greatest zone of inhibition (ZOI) values of 24 ± 2.95 mm against Pseudomonas aeruginosa. Similarly, the Cs NPs/PE materials exhibited a zone of inhibition (ZOI) measurement of 22 ± 3.14 mm against P. aeruginosa. This work highlights the various biochemical components of neem extracts, their strong abilities to combat bacteria and oxidative stress, and the possibility of Cs NPs containing neem extracts as effective treatments for antibiotic-resistant bacterial strains.

Neem Leaf Extract Exhibits Anti-Aging and Antioxidant Effects from Yeast to Human Cells.

Dang J, Zhang G, Li J, He L, Ding Y, Cai J, Cheng G, Yang Y, Liu Z, Fan J, Du L, Liu K.

Nutrients. 2024 May 16;16(10):1506. doi: 10.3390/nu16101506.PMID: 38794743

Neem leaves have long been used in traditional medicine for promoting longevity. zHowever, the precise mechanisms underlying their anti-aging effects remain elusive. In this study, we investigated the impact of neem leaf extract (NLE) extracted from a 50% ethanol solution on the chronological lifespan of Saccharomyces cerevisiae, revealing an extension in lifespan, heightened oxidative stress resistance, and a reduction in reactive oxygen species. To discern the active compounds in NLE, LC/MS and the GNPS platform were employed. The majority of identified active compounds were found to be flavonoids. Subsequently, compound-target pharmacological networks were constructed using the STP and STITCH platforms for both S. cerevisiae and Homo sapiens. GOMF and KEGG enrichment analyses of the predicted targets revealed that "oxidoreductase activity" was among the top enriched terms in both yeast and human cells. These suggested a potential regulation of oxidative stress response (OSR) by NLE. RNA-seq analysis of NLE-treated yeast corroborated the anti-oxidative effect, with "oxidoreductase activity" and "oxidation-reduction process" ranking high in enriched GO terms. Notably, CTT1, encoding catalase, emerged as the most significantly up-regulated gene within the "oxidoreductase activity" cluster. In a *ctt1* null mutant, the enhanced oxidative stress resistance and extended lifespan induced by NLE were nullified. For human cells, NLE pretreatment demonstrated a decrease in reactive oxygen species levels and senescence-associated β-galactosidase activity in HeLa cells, indicative of anti-aging and anti-oxidative effects. This study unveils the anti-aging and anti-oxidative properties of NLE while delving into their mechanisms, providing novel insights for pharmacological interventions in aging using phytochemicals.

Computational design, docking, and molecular dynamics simulation study of RNA helicase inhibitors of dengue virus.

Satpathy R, Acharya S, Behera R.

J Vector Borne Dis. 2024 May 6. doi: 10.4103/JVBD.JVBD_188_23. Online ahead of print.PMID: 38712711

Background and objectives: RNA viruses are complex pathogens in terms of their genetic makeup, mutation frequency, and transmission modes. They contain the RNA helicase enzyme, which plays a crucial role in the viral genome replication process. This work aims to develop and screen a potential molecule that could function as a dengue virus (DENV) RNA helicase inhibitor.**Methods:** The present study was performed by taking 26 potential derivatives of gedunin phytochemicals from the PubChem database as ligands. The binding study of the compounds were analyzed by in silico docking method considering DENV RNA helicase enzyme as the receptor. **Results:** After a thorough analysis of the docking scores, toxicity, and physicochemical properties, compound tetrahydrogedunin was obtained as the best. Based on tetrahydrogedunin molecular structure, 100 drug-like molecules were designed using the Data Warrior tool. After the screening process for drug-likeness and ADMET properties, the derivative number 42 was considered as the promising. Further comparative docking of derivative 42 and a standard inhibitor molecule ST-610 with DENV RNA helicase enzyme showed binding affinity as -10.0 kcal/mol and -9.6 kcal/mol, respectively. The favorable interaction between DENV RNA helicase and derivative 42 was further validated by 50 nanoseconds molecular dynamics simulation and MM-GBSA analysis.**Interpretation conclusion:** Since the antiviral activity of derivative 42 has not been reported till date, the compound was predicted as a novel therapeutic molecule that can act against the dengue virus (DENV) RNA helicase enzyme.

A Comparative Evaluation of Antimicrobial and Cytotoxic Efficacy of Biosynthesized Silver Nanoparticles and Chemically Synthesized Silver Nanoparticles Against Enterococcus faecalis: An In Vitro Study.

Chandran N, Ramesh S, Shanmugam R, S J.

Cureus. 2024 Apr 16;16(4):e58428. doi: 10.7759/cureus.58428. eCollection 2024 Apr.PMID: 38765427

Introduction Effective root canal cleaning and sealing are essential for a successful endodontic procedure. For the purpose of disinfecting root canals, both herbal and non-herbal medications are recommended. This study aimed to analyze the antimicrobial and cytotoxic properties of biosynthesized silver nanoparticles (AgNPs) synthesized from *Azadirachta indica*/neem and chemically synthesized AgNPs from trisodium citrate (TSC) against oral pathogens to be further used as an irrigant in endodontic treatment. Materials and methods To synthesize *A. indica* AgNPs, powdered fresh *A. indica* leaves were weighed, added to double distilled water, heated for 30 minutes, and then combined with silver nitrate solution. TSC was also used to create TSC AgNPs. X-ray diffraction (XRD), scanning electron microscopy (SEM), ocular observation, and the ultraviolet-visible light (UV-vis) spectrum were used to characterize the AgNPs. Studies were conducted on the extract's characteristics, including its cytotoxicity and antibacterial activity. Results The

hue shift and peak on the UV-vis spectrophotometer were signs that AgNPs were forming. The XRD pattern showed that the sample included crystalline AgNPs, mostly spherical ones. By using SEM, the presence of AgNPs was also verified. AgNPs that were synthesized showed antimicrobial efficacy against *Enterococcus faecalis*. Compared to chemically synthesized AgNPs, *A. indica* AgNPs showed lower minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values, a bigger zone of inhibition (ZOI), and less cytotoxic action. Conclusion This study demonstrates the minimal cytotoxicity and antibacterial activity of *A. indica* AgNPs against *E. faecalis*. This suggests that they might also be employed as root canal cleaners. Before experimenting with animals or cell lines in clinical trials for endodontic treatment, further research should be done.

Feasibility Insights of the Green-Assisted Calcium-Phosphate Coating on Biodegradable Zinc Alloys for Biomedical Application: In Vitro and In Vivo Studies.

Palai D, De A, Prasad PS, Roy T, Mukherjee S, Dhara S, Das S, Das K.

ACS Appl Mater Interfaces. 2024 May 15;16(19):24274-24294. doi: 10.1021/acsami.4c02540. Epub 2024 May 3.PMID: 38699930

In the field of bone tissue engineering, recently developed Zn alloy scaffolds are considered potential candidates for biodegradable implants for bone regeneration and defect reconstruction. However, the clinical success of these alloys is limited due to their insufficient surface bioactivities. Further, the higher concentration of Zn^{2+} produced during degradation promotes antibacterial activity, but deteriorates osteogenic properties. This study fabricated an *Azadirachta indica* (neem)-assisted brushite-hydroxyapatite (HAp) coating on the recently developed Zn-2Cu-0.5Mg alloy to tackle the above dilemma. The microstructure, degradation behavior, antibacterial activity, and hemocompatibility, along with in vitro and in vivo cytocompatibility of the coated alloys, are systematically investigated. Microstructural analysis reveals flower-like morphology with uniformly grown flakes for neem-assisted deposition. The neem-assisted deposition significantly improves the adhesion strength from 12.7 to 18.8 MPa, enhancing the mechanical integrity. The potentiodynamic polarization study shows that the neem-assisted deposition decreases the degradation rate, with the lowest degradation rate of 0.027 mm/yr for the ZHN2 sample. In addition, the biomineralization process shows the apatite formation on the deposited coating after 21 days of immersion. In vitro cytotoxicity assay exhibits the maximum cell viability of 117% for neem-assisted coated alloy in 30% extract after 5d and the improved cytocompatibility which is due to the controlled release of Zn^{2+} ions. Meanwhile, neem-assisted coated alloy increases the ZOI by 32 and 24% for Gram-positive and Gram-negative bacteria, respectively. Acceptable hemolysis (<5%) and anticoagulation parameters demonstrate a promising hemocompatibility of the coated alloy. In vivo implantation illustrates a slight inflammatory response and vascularization after 2 weeks of subcutaneous implantation, and neo-bone formation in the defect areas of the rat femur. Micro-CT and histology studies demonstrate better osseointegration with satisfactory biosafety response for the neem-assisted coated alloy as compared to that without neem-assisted deposition. Hence, this neem-assisted brushite-Hap coating strategy elucidates a new perspective on the surface modification of biodegradable implants for the treatment of bone defects.

Insights into Nimbolide molecular crosstalk and its anticancer properties.

Shaheen S, Khalid S, Aaliya K, Gul A, Hafeez A, Armaghan M, Almarhoon ZM, Calina D, Khan K, Sharifi-Rad J.

Med Oncol. 2024 May 18;41(6):158. doi: 10.1007/s12032-024-02379-5.PMID: 38761317

Nimbolide, one of the main ingredients constituent of Azadirachta indica (neem) leaf extract, has garnered attention for its potential as an anticancer agent. Its efficacy against various cancers and chemopreventive action has been demonstrated through numerous in vivo and in vitro studies. This updated review aims to comprehensively explore the chemopreventive and anticancer properties of nimbolide, emphasizing its molecular mechanisms of action and potential therapeutic applications in oncology. The review synthesizes evidence from various studies that examine nimbolide's roles in apoptosis induction, anti-proliferation, cell death, metastasis inhibition, angiogenesis suppression, and modulation of carcinogen-metabolizing enzymes. Nimbolide exhibits multifaceted anticancer activities, including the modulation of multiple cell signaling pathways related to inflammation, invasion, survival, growth, metastasis, and angiogenesis. However, its pharmacological development is still in the early stages, mainly due to limited pharmacokinetic and comprehensive long-term toxicological studies. Nimbolide shows promising anticancer and chemopreventive properties, but there is need for systematic preclinical pharmacokinetic and toxicological research. Such studies are essential for establishing safe dosage ranges for first-in-human clinical trials and further advancing nimbolide's development as a therapeutic agent against various cancers. The review highlights the potential of nimbolide in cancer treatment and underscores the importance of rigorous preclinical evaluation to realize its full therapeutic potential.

Neem Leaf Glycoprotein disrupts exhausted CD8+ T cell-mediated cancer stem cell aggression.

Chakravarti M, Bera S, Dhar S, Sarkar A, Choudhury PR, Ganguly N, Das J, Sultana J, Guha A, Biswas S, Das T, Hajra S, Banerjee S, Baral R, Bose A.

Mol Cancer Res. 2024 May 14. doi: 10.1158/1541-7786.MCR-23-0993. Online ahead of print.PMID: 38743057

Targeting exhausted CD8+T cell (TEX) induced aggravated cancer stem cells (CSC) holds immense therapeutic potential. In this regard, immunomodulation via Neem Leaf Glycoprotein (NLGP), a plant-derived glycoprotein immunomodulator is explored. Since former reports have proven immune-dependent tumor restriction of NLGP across multiple tumor models, we hypothesized that NLGP might reprogram and rectify TEX to target CSCs successfully. Here we report that NLGP's therapeutic administration significantly reduced TEX -associated CSC virulence in in vivo B16-F10 melanoma tumor model. Similar trend was observed in in vitro generated TEX and B16-F10/MCF7 co-culture setups. NLGP rewired CSCs by downregulating clonogenicity, multidrug resistance phenotypes and PDL1, OCT4, SOX2 expression. Cell cycle analysis revealed that NLGP-educated TEX efficiently pushed CSCs out of quiescent-phase (G0G1) into synthesis-phase (S), supported by hyper-phosphorylation of G0G1-S transitory cyclins and Rb-

proteins. This rendered quiescent CSCs susceptible to s-phase targeting chemotherapeutic drugs like 5-Fluorouracil (5FU). Consequently combinatorial treatment of NLGP and 5FU brought optimal CSC targeting efficiency with increase in apoptotic bodies and pro-apoptotic BID expression. Notably a strong nephron-protective effect of NLGP was also observed, which prevented 5FU associated toxicity. Furthermore, Dectin-1 mediated NLGP uptake and subsequent alteration of Notch1 and mTOR axis was deciphered as the involved signalling network. This observation unveiled Dectin-1 as a potent immunotherapeutic drug-target to counter T cell exhaustion. Cumulatively, NLGP immunotherapy alleviated exhausted CD8+T cell induced CSC aggravation. Implications: Our study recommends that NLGP-immunotherapy can be utilized to counter ramifications of T cell exhaustion and to target therapy elusive aggressive CSCs without evoking toxicity.

Gedunin induces apoptosis and inhibits HMBG1/PI3K/AKT signaling pathways in a rat model of gastric carcinogenesis induced by methylnitronitrosoguanidine.

Zhang W, Dong Y, Sun C.

Arch Med Sci. 2024 Mar 30;20(2):691-697. doi: 10.5114/aoms/183651. eCollection 2024.PMID: 38757023

Introduction: This study aimed to evaluate the anti-cancer effects of gedunin, a natural compound, in a rat model of gastric carcinogenesis induced by MNNG. **Methods:** Fifty-four rats were randomly assigned to six groups for a 60-day study on the effects of MNNG and gedunin. Groups 1-4 received 200 mg/kg MNNG (1, 10, or 100 mg/kg), and group 5 had only 100 mg/kg gedunin. **Results:** Gedunin at low doses exhibited anti-cancer and protective properties against MNNG-induced damage, including reduced inflammation, and apoptosis.**Conclusions:** Gedunin demonstrates a U-shaped dose-response, with low doses offering protection and high doses promoting tumor growth.

Gedunin modulates cellular growth and apoptosis in glioblastoma cell lines.

Stouffer M, Wandling E, Dickson L, Lin S, Duan H, Powe E, Jean-Louis D, Tiwari AK, Amos S.

Cancer Rep (Hoboken). 2024 May;7(5):e2051. doi: 10.1002/cnr2.2051.PMID: 38702989

Background: Glioblastomas are characterized by aggressive behavior. Surgery, radiotherapy, and alkylating agents, including temozolomide are the most common treatment options for glioblastoma. Often, conventional therapies fail to treat these tumors since they develop drug resistance. There is a need for newer agents to combat this deadly tumor. Natural products such as gedunin have shown efficacy in several human diseases. A comprehensive study of gedunin, an heat shock protein (HSP)90 inhibitor, has not been thoroughly investigated in glioblastoma cell lines with different genetic modifications. **Aims:** A key objective of this study was to determine how gedunin affects the biological and signaling mechanisms in glioblastoma cells, and to determine how those mechanisms affect the proliferation and apoptosis of glioblastoma cells. **Methods:** The viability potentials of gedunin were tested using MTT, cell counts, and wound healing assays. Gedunin's effects on glioma cells were further validated using LDH and colony formation assays. In addition, we investigated the survival and apoptotic molecular

signaling targets perturbed by gedunin using Western blot analysis and flow cytometry. **Results:** Our results show that there was a reduction in cell viability and inhibition of wound healing in the cells tested. Western blot analysis of the gene expression data revealed genes such as EGFR and mTOR/Akt/NF kappa B to be associated with gedunin sensitivity. Gedunin treatment induced apoptosis by cleaving poly ADP-ribose polymerase, activating caspases, and downregulating BCL-xL. Based on these results, gedunin suppressed cell growth and HSP client proteins, resulting in apoptosis in glioblastoma cell lines. **Conclusion:** Our data provide in vitro support for the anticancer activity of gedunin in glioma cells by downregulating cancer survival proteins.

The integration of multidisciplinary approaches revealed PTGES3 as a novel drug target for breast cancer treatment.

Yin Q, Ma H, Dong Y, Zhang S, Wang J, Liang J, Mao L, Zeng L, Xiong X, Chen X, Wang J, Zheng X.

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Background: The main challenge in personalized treatment of breast cancer (BC) is how to integrate massive amounts of computing resources and data. This study aimed to identify a novel molecular target that might be effective for BC prognosis and for targeted therapy by using network-based multidisciplinary approaches. **Methods:** Differentially expressed genes (DEGs) were first identified based on ESTIMATE analysis. A risk model in the TCGA-BRCA cohort was constructed using the risk score of six DEGs and validated in external and clinical in-house cohorts. Subsequently, independent prognostic factors in the internal and external cohorts were evaluated. Cell viability CCK-8 and wound healing assays were performed after PTGES3 siRNA was transiently transfected into the BC cell lines. Drug prediction and molecular docking between PTGES3 and drugs were further analyzed. Cell viability and PTGES3 expression in two BC cell lines after drug treatment were also investigated.**Results:** A novel six-gene signature (including APOOL, BNIP3, F2RL2, HINT3, PTGES3 and RTN3) was used to establish a prognostic risk stratification model. The risk score was an independent prognostic factor that was more accurate than clinicopathological risk factors alone in predicting overall survival (OS) in BC patients. A high risk score favored tumor stage/grade but not OS. PTGES3 had the highest hazard ratio among the six genes in the signature, and its mRNA and protein levels significantly increased in BC cell lines. PTGES3 knockdown significantly inhibited BC cell proliferation and migration. Three drugs (gedunin, genistein and diethylstilbestrol) were confirmed to target PTGES3, and genistein and diethylstilbestrol demonstrated stronger binding affinities than did gedunin. Genistein and diethylstilbestrol significantly inhibited BC cell proliferation and reduced the protein and mRNA levels of PTGES3.**Conclusions:** PTGES3 was found to be a novel drug target in a robust six-gene prognostic signature that may serve as a potential therapeutic strategy for BC.