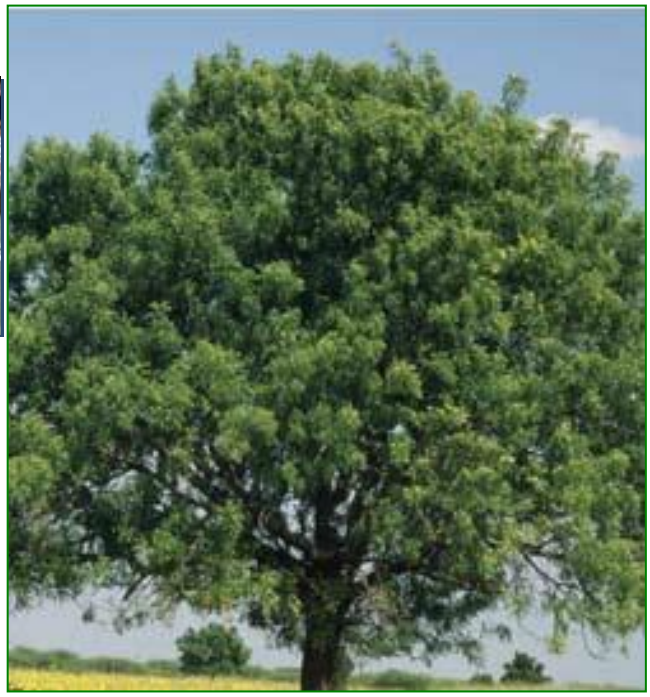
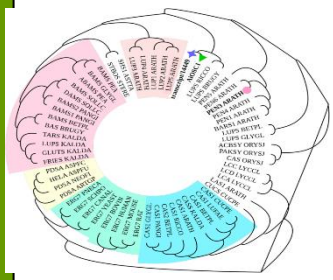
Neem Research Newsletter

Volume 3, Issue 8, 2023





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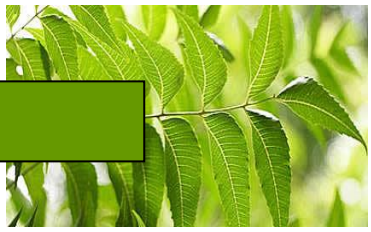
"Neem, the Earth's Saviour" ©



WORLD NEEM ORGANISATION (WNO)

From The Editor's Desk.....

Several interesting research findings on neem continue to be reported underscoring the immense potential of this evergreen tree. Neem oil as nano-additive with carbon nano tubes improved performance and reduced emissions in diesel engines. Scientists developed a simple and efficient method for the green synthesis of highly fluorescent carbon dots (CDs) from neem leaves. Silver nanoparticles synthesized using neem leaves exhibited antibacterial properties. Neem leaf extract was shown to be effective as endodontic irrigant during root canal treatment. The use of neem for the treatment of malaria in Ghana and Western Uganda has been highlighted. Tri-Yannarose, a Thai traditional herbal medicine formula composed of *Areca catechu*, *Azadirachta indica*, and *Tinospora crispa* was found to possess potent antioxidant activity indicating its potential use in the treatment of oxidative stress disorders. Studies have revealed that neem leaf extracts can be used as an effective drug to manage inflammation. Deacetyl epoxyazadiradione ameliorated Bisphenol-PA-induced neurotoxicity by mitigating inflammation. Nimbin and its analog from neem seeds were found to suppress the migration of osteosarcoma cells and induce cell death. The anticancer potential of limonoids from the neem tree has been comprehensively reviewed. Topical application of neem oil was demonstrated to be effective in controlling blood sucking lice in goats.

S. Nagini

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

Nanobiopesticides: Are they the future of phytosanitary treatments in modern agriculture?

Machado S, Pereira R, Sousa RMOF.

Sci Total Environ. 2023 Aug 17:166401. doi: 10.1016/j.scitotenv.2023.166401. PMID: 37597566

The world's population is continuously increasing; therefore, food availability will be one of the major concerns of our future. In addition to that, many practices and products used, such as pesticides and fertilizers have been shown harmful to the environment and human health and are assumed as being one of the main factors responsible for the loss of biodiversity. Also, climate change could agravate the problem since it causes unpredictable variation of local and regional climate conditions, which frequently favor the growth of diseases, pathogens and pest growth. The use of natural products, like essential oils, plant extracts, or substances of microbial-origin in combination with nanotechnology is one suitable way to outgrow this problem. The most often employed natural products in research studies to date include pyrethrum extract, neem oil, and various essential oils, which when enclosed shown increased resistance to environmental factors. They also demonstrated insecticidal, antibacterial, and fungicidal properties. However, in order to truly determine if these products, despite being natural, would be hazardous or not, testing in non-target organisms, which are rare, must start to become a common practice. Therefore, this review aims to present the existing literature concerning nanoformulations of biopesticides and a standard definition for nanobiopesticides, their synthesis methods and their possible ecotoxicological impacts, while discussing the regulatory aspects regarding their authorization and commercialization. As a result of this, you will find a critical analysis in this reading. The most obvious findings are that i) there are insufficient reliable ecotoxicological data for risk assessment purposes and to establish safety doses; and ii) the requirements for registration and authorization of these new products are not as straightforward as those for synthetic chemicals and take a lot of time, which is a major challenge/limitation in terms of the goals set by the Farm to Fork initiative.

Assessing the potential of biopesticides to control cabbage stem flea beetle Psylliodes chrysocephala.

Price CSV, Campbell H, Pope TW.

Pest Manag Sci. 2023 Aug 25. doi: 10.1002/ps.7746. PMID: 37622417

Background: Cabbage stem flea beetle (CSFB) is an economically important pest of oilseed rape crops in Europe that was effectively controlled by neonicotinoid insecticide seed treatments until they were banned by the European Union in 2013. Since then, CSFB has been a difficult pest to control effectively, in part due to many populations having developed resistance to pyrethroids, the only authorized insecticides used to control this pest in many countries. Alternative solutions are therefore necessary, such as biopesticides. We tested an entomopathogenic fungus, three entomopathogenic bacteria isolates, two fatty acids and azadirachtin against CSFB adults under laboratory conditions. We also tested the efficacy

of the pyrethroid insecticide lambda-cyhalothrin. **Results:** Fatty acids were effective with up to 100% CSFB mortality after 24 hours. The entomopathogenic fungus Beauveria bassiana resulted in up to 56% mortality 14 days after treatment. Entomopathogenic bacteria formulations and azadirachtin were not effective (< 50% and <40% mortality, respectively). Results from a bioassay using lambda cyhalothrin indicated that the CSFB used in this study were resistant to this insecticide. **Conclusion:** Entomopathogenic fungi and fatty acids could potentially be used to control CSFB as part of an Integrated Pest Management (IPM) programme. This study is the first to investigate the efficacy of different biopesticides to control CSFB under laboratory conditions. As such, these biopesticides require further testing to optimise formulation, application methods and to assess impact on non-target organisms. Finally, efficacy under field conditions must be determined to understand the influence of environmental variables.

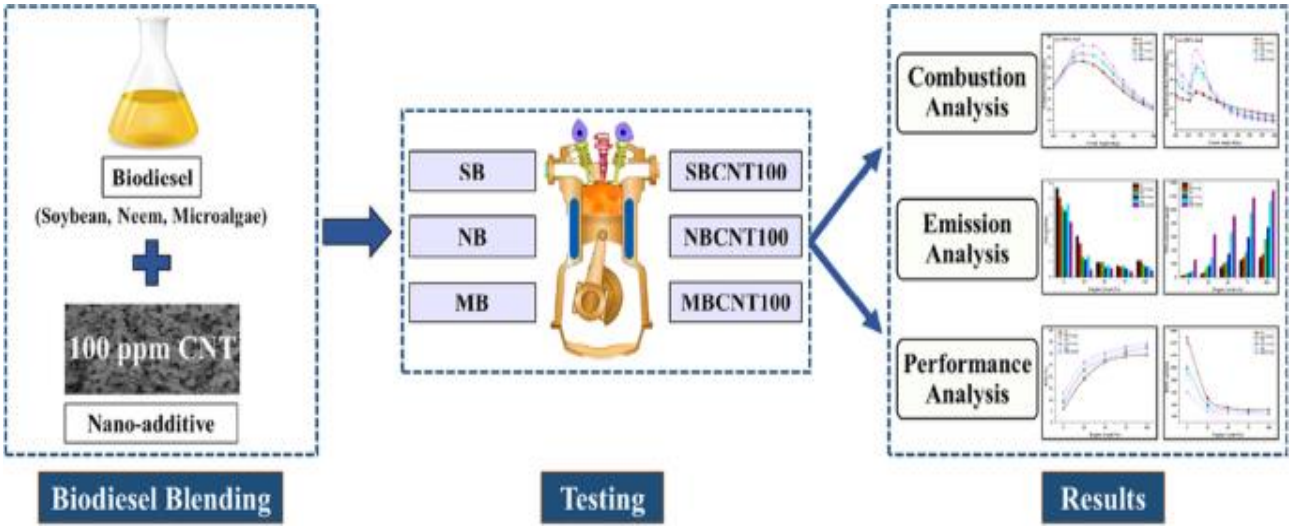
Neem for Sustainable Environment & Green Synthesis

Comparative combustion, emission, and performance analysis of a diesel engine using carbon nanotube (CNT) blended with three different generations of biodiesel.

Rajpoot AS, Saini G, Chelladurai HM, Shukla AK, Choudhary T.

Environ Sci Pollut Res Int. 2023 Aug 3. doi: 10.1007/s11356-023-28965-0. PMID: 37535288

Nano-additives are being employed in successive generations of biodiesels to increase the performance characteristics and output of diesel engines. In this study, the impact of mixing carbon nanotubes (CNT) with three different generations of biodiesel in a diesel engine is assessed. With 100 ppm of CNT nanoparticles mixed together, pure biodiesels made from first-generation oil (soybean), second-generation oil (neem), and third-generation oil (Nannochloropsis oculata microalgae) are used for the analysis. With an engine load ranging from 0 to 100%, a one-cylinder, four-stroke, direct injection diesel engine is employed. The engine has a water-cooling system, a compression ratio of 17.5:1, and a fuel injection angle of 23° before TDC. The evaluated engines' improved performance and lower emissions serve as proof of the outcomes. The results are evidenced by the lower emissions and higher performance of the tested engines. The biodiesel containing CNT nanoparticles enhanced the cylinder pressure by 0.8-10.69%, the heat release rate (HRR) by 6.38-21.69%, and the brake thermal efficiency (BTE) by 0.32-1.62%. Subsequently, it reduced the brake-specific fuel consumption (BSFC) by 2.53-8.13%, the brake-specific energy consumption (BSEC) by 1.07-3.77%, the smoke opacity (BSN) by 6.26-12.85%, the particulate matter (PM) emissions by 11.04-18.33%, and the carbon dioxide (CO_2) emissions by 2.53-8.14% at full engine load. However, an increase in 13.62-18.37% nitrogen emissions (NOx) emissions is also observed with the addition of CNT at 100% load. The investigation supports the use of CNT nano-additives in diesel engines for improved performance and reduced emissions.



Biogenic synthesis of highly fluorescent carbon dots using Azadirachta indica leaves: An eco-friendly approach with enhanced photocatalytic degradation efficiency towards Malachite green.

Vijeata A, Chaudhary GR, Chaudhary S, Umar A.

Chemosphere. 2023 Aug 26:139946. doi: 10.1016/j.chemosphere.2023.139946. PMID: 37640216

A simpler and efficient method has been developed for the green synthesis of highly fluorescent carbon dots (CDs) from Azadirachta Indica leaves. The surface morphology of developed CDs has shown the existence of spherical particles in the size range of 3-8 nm with superior biocompatibility and high quantum yield value i.e. 42.3%. The particles exhibited a highly fluorescent and crystalline nature along with a bandgap value of 4.02 eV. The prepared CDs served as a factorial design for the sensing and degradation of Malachite green among other dyes. The main perspective of the current finding is that the designed catalyst exhibits excellent sensing results towards Malachite green with a limit of detection i.e. 0.144 μM in the concentration range of 0-50 μM. Moreover, the UV triggered results of photocatalysis illustrated a good dye removal efficacy by developed CDs with an average of 90.73, 98.25, 52 and 6.13% degradation in Methylene blue (MB), Malachite green (MG), Rhodamine 6G (Rh 6G) and Methyl orange (MO) upon 70 min of irradiation with mercury lamp. Additionally, the proton NMR, FTIR and FESEM results of the recycled samples also confirm the complete degradation of MG dye with the application of N-CDs.

Neem for Human Health

Green synthesis of silver nanoparticles mediated Azadirachta indica extract and study of their characterization, molecular docking, and antibacterial activity.

Gawai AA, Kharat AR, Chorge SS, Dhawale SA.

J Mol Recognit. 2023 Aug 18:e3051. doi: 10.1002/jmr.3051. PMID: 37594180

The green production of silver nanoparticles (AgNPs) produces AgNPs with minimum influence on the environment by using plant components such as alkaloids, carbohydrates, lipids, enzymes, flavonoids, terpenoids, and polyphenols as reducing agents. In the present investigation, Azadirachta indica leaf extract was used to form AgNPs from a 1 mM silver nitrate solution. The plan proved to be incredibly straightforward, cost-effective, and effective. The production of the nanoparticles was observed visually, where the colorless fluid turns into a brown-colored solution. Further research was carried out using x-ray diffraction, Fourier-transform infrared analysis, scanning electron microscopy, and transmission electron microscopy (TEM) in addition to UV-visible spectroscopy. The size range of AgNPs determined by TEM was 10-30 nm. When the diffusion technique was employed to demonstrate the antibacterial effect of AgNPs on various pathogens, the zones of inhibition for Staphylococcus aureus, Bacillus cereus, and Escherichia coli, when 50 g of AgNPs were used were 16, 12, and 17 mm, respectively. By examining the leakage of reducing sugars and proteins, the mechanism by which nanoparticle antibacterial properties were explored, showed that AgNPs were capable of lowering membrane permeability.

Phenotypic Analysis, Molecular Characterization, and Antibiogram of Caries-Causing Bacteria Isolated from Dental Patients.

Farva K, Sattar H, Ullah H, Raziq A, Mehmood MD, Tareen AK, Sultan IN, Zohra Q, Khan MW.

Microorganisms. 2023 Jul 31;11(8):1952. doi: 10.3390/microorganisms11081952.PMID: 37630520

Dental caries is a biofilm-mediated, sugar-driven, multifactorial, dynamic disease that results in the phasic demineralization and remineralization of dental hard tissues. Despite scientific advances in cariology, dental caries remains a severe global concern. The aim of this study was to determine the optimization of microbial and molecular techniques for the detection of cariogenic pathogens in dental caries patients, the prevalence of cariogenic bacteria on the basis of socioeconomic, climatological, and hygienic factors, and in vitro evaluation of the antimicrobial activity of selected synthetic antibiotics and herbal extracts. In this study, oral samples were collected from 900 patients for bacterial strain screening on a biochemical and molecular basis. Plant extracts, such as ginger, garlic, neem, tulsi, amla, and aloe vera, were used to check the antimicrobial activity against the isolated strains. Synthetic antimicrobial agents, such as penicillin, amoxicillin, erythromycin, clindamycin, metronidazole, doxycycline, ceftazidime, levofloxacin, and ciprofloxacin, were also used to access the antimicrobial activity. Among 900 patients, 63% were males and 37% were

females, patients aged between 36 and 58 (45.7%) years were prone to disease, and the most common symptom was toothache (61%). For oral diseases, 21% used herbs, 36% used antibiotics, and 48% were self-medicated, owing to sweets consumption (60.66%) and fizzy drinks and fast food (51.56%). *Staphylococcus mutans* (29.11%) and *Streptococcus sobrinus* (28.11%) were found as the most abundant strains. Seven bacterial strains were successfully screened and predicted to be closely related to genera *S. sobrinus*, *S. mutans*, *Actinomyces naeslundii*, *Lactobacillus acidophilus*, *Eubacterium nodatum*, *Propionibacterium acidifaciens*, and *Treponema Pallidum*. Among plant extracts, the maximum zone of inhibition was recorded by ginger (22.36 mm) and amla (20.01 mm), while among synthetic antibiotics, ciprofloxacin and levofloxacin were most effective against all microbes. This study concluded that phyto extracts of ginger and amla were considered suitable alternatives to synthetic antibiotics to treat dental diseases.

Comparative Evaluation of Smear Layer Removal Efficacy of Neem Leaf Extract, Propolis, and Orange Oil when used as Endodontic Irrigants: An *in vitro* Scanning Electron Microscopic Study.

Setia R, Bajaj N, Bhola M, Brar GS.

Contemp Clin Dent. 2023 Apr-Jun;14(2):128-134. doi: 10.4103/ccd.ccd_611_21. PMID: 37547437

Introduction: In root canal treatment, chemical debridement of the root canal with the help of irrigants is important due to the complex internal anatomy of the teeth. Biomechanical root canal preparation produces a smear layer. It covers the dentinal tubules, which may interfere with the penetration of various irrigants and intracanal medicaments, so the smear layer has to be removed. The potential side effects and certain limitations of chemical irrigants for smear layer removal have led to a shift toward herbal alternatives. **Objective:** The aim of the present study was to evaluate and compare the smear layer removal efficacy of Neem leaf extract, Propolis, and Orange oil as endodontic irrigants using the scanning electron microscope. **Materials and methods:** Ninety samples were taken and then randomly (simple random sampling method) divided into three experimental groups (n = 30) depending upon different endodontic irrigants used: Group 1-Neem leaf extract, Group 2-Orange oil and Group 3-Propolis and smear layer removal evaluation was done under the scanning electron microscope Scanning Electron Microscope (SEM) at all the levels, i.e., coronal, middle, and apical. **Results:** The collected data were analyzed using the Chi-square test. Group 1 (Neem leaf extract) showed the highest smear layer removal efficacy, which was followed by Group 2 (Orange oil) and the least efficacy of smear layer removal was shown by Group 3 (Propolis) at coronal, middle, and apical levels. **Conclusion:** Group 1 (Neem leaf extract) performed significantly better in removing the smear layer at coronal, middle, and apical levels as compared to the other two groups.

Ethnomedicinal plants used for malaria treatment in Rukungiri District, Western Uganda.

Gumisiriza H, Olet EA, Mukasa P, Lejju JB, Omara T.
Trop Med Health. 2023 Aug 30;51(1):49. doi: 10.1186/s41182-023-00541-9. PMID: 37644587

Background: Malaria remains a major global health challenge and a serious cause of morbidity and mortality in sub-Saharan Africa. In Uganda, limited access to medical facilities has perpetuated the reliance of indigenous communities on herbal medicine for the prevention and management of malaria. This study was undertaken to document ethnobotanical knowledge on medicinal plants prescribed for managing malaria in Rukungiri District, a meso-endemic malaria region of Western Uganda. **Methods:** An ethnobotanical survey was carried out between May 2022 and December 2022 in Bwambara Sub-County, Rukungiri District, Western Uganda using semi-structured questionnaire. A total of 125 respondents (81 females and 44 males) were randomly selected and seven (7) key informants were engaged in open interviews. In all cases, awareness of herbalists on malaria, treatment-seeking behaviour and herbal treatment practices were obtained. The ethnobotanical data were analyzed using descriptive statistics, informant consensus factor and preference ranking. **Results:** The study identified 48 medicinal plants belonging to 47 genera and 23 families used in the treatment of malaria and its symptoms in the study area. The most frequently cited species were Vernonia amygdalina, Aloe vera and Azadirachta indica. Leaves (74%) was the most used plant organ, mostly for preparation of decoctions (41.8%) and infusions (23.6%) which are administered orally (89.6%) or used for bathing (10.4%). **Conclusions:** Indigenous knowledge of medicinal plants used as prophylaxis and for treatment of malaria still exist among the local communities of Bwambara Sub-County. However, there is a need to investigate the antimalarial efficacy, phytochemical composition and safety of species (such as Digitaria abyssinica and Berkheya barbata) with high percentage use values to validate their use.

Herbs Used in Antimalarial Medicines: A Study in the Greater Accra Region of Ghana.

Nortey NND, Korsah S, Tagoe M, Apenteng JA, Owusu FA, Oppong J, Attah AE, Allotey S.
Evid Based Complement Alternat Med. 2023 Aug 19;2023:6697078. doi: 10.1155/2023/6697078. eCollection 2023.PMID: 37636997

Methods: Pharmacy shops were randomly scouted and products were observed. The active ingredients were documented and their frequencies were determined.**Results:** Forty-four (44) plant species belonging to twenty-eight (28) families were recorded for the treatment of malaria in the survey. The predominant families were the Leguminosae and Meliaceae families. *Cryptolepis sanguinolenta (Ghanaian quinine* or yellow dye root) and *Azadirachta indica (*neem tree) were the most cited plants. *Cryptolepis* and neem tree were used 17 and 15 times, respectively, in the finished herbal products for treating malaria. *Conclusion. Cryptolepis sanguinolenta* and *Azadirachta indica (*neem tree) are important herbs for the treatment of malaria in Ghana. Locally manufactured herbal antimalarials are important for the treatment of malaria in urban and rural communities in Ghana.

Antimalarial Activities of a Therapeutic Combination of Azadirachta indica, Mangifera indica and Morinda lucida Leaves: A Molecular View of its Activity on Plasmodium falciparum Proteins.

Abdulai SI, Ishola AA, Bewaji CO.

Acta Parasitol. 2023 Jul 21. doi: 10.1007/s11686-023-00698-7. PMID: 37474844

Background: The search for new antimalarial drugs remains elusive prompting research into antimalarial combinations from medicinal plants due to their cheapness, efficacy and availability. Azadirachta indica (AI), Morinda lucida (ML) and Mangifera indica (MI) have all been reported as potent antimalarial plants. **Purpose:** This study evaluated the efficacy of an antimalarial combination therapeutics prepared from leaves of AI, ML and MI using in vitro, in vivo and molecular methods. **Methods:** Refined extracts of the plants combination was made by partitioning the aqueous extract of plants combinations (AI + MI, AI + ML, MI + ML, AI + MI + ML) using methanol and ethyl acetate consecutively. The resulting ethyl acetate partitioned fraction was evaluated for its antimalarial activity. Molecular docking and molecular dynamics simulation were employed to determine the possible mechanism of action of the constituent of the most active combination against four important P. falciparum proteins. **Results:** The result revealed that the refined extract from combinations AI + ML and MI + ML at 16 mg/kg bodyweight have the highest chemo-suppressive effect of 90.7% and 91.0% respectively compared to chloroquine's 100% at 10 mg/kg. Also, refined extract from MI + ML combination improved PCV levels significantly ($p < 0.05$) compared to controls. Molecular docking revealed oleanolic acid and ursolic acid as multiple inhibitors of plasmepsin II, hiso-aspartic protease, falcipain-2 and P. falciparum Eonyl acyl-carrier protein reductase with relative stability during 100 ns of simulation. **Conclusion:** The study unveiled the potentials of ML and MI as good candidates for antimalarial combination therapy and further established their use together as revealed in folklore medicine.

Exploring the multi-gene regulatory molecular mechanism of Saudi Arabian flora against epilepsy based on data mining, network pharmacology and docking analysis.

Falah Alshehri F, Alzahrani FM, Alkhoshaiban A, Saad Al Shehri Z.

Saudi Pharm J. 2023 Sep;31(9):101732. doi: 10.1016/j.jsps.2023.101732. Epub 2023 Aug 5.PMID: 37638220

Epilepsy is a chronic neurological disorder marked by recurrent seizures, significantly affecting the population in Saudi Arabia across all age demographics. The global prevalence of active epilepsy is around 6.38/1,000 persons and in the Arabian region, the median prevalence of active epilepsy is 4.4/1,000 persons. However, over 75% of individuals are untreated. Consequently, the development of therapeutic strategies with increased efficacy and safety profiles is essential to improve the survival rate among epilepsy patients. The current study integrates network pharmacology along with Bioinformatics approaches to explore the potential molecular mechanisms of local flora of Saudi Arabia including *Solanum incanum, Abrus precatorius, Withania somnifera,* and *Azadirachta indica* in epilepsy treatment. In the preliminary phase, data related to the bioactive components of the local plants and the associated target genes of both these plants and epilepsy were gathered

from scientific literature and open-source databases. This data was then analyzed to identify common targets between the plants and ovarian cancer. Based on these common targets, a protein-protein interaction (PPI) network was constructed utilizing the STRING database, which was subsequently incorporated into the Cytoscape software for identification of hub genes based on their degree of connectivity. Lastly, an interplay network depicting the associations between the compounds and the overlapping genes was formulated via Cytoscape, to study the potential network pharmacology implications of these active compounds in relation to ovarian cancer. Following that, a compound-target protein-pathway network was constructed which uncovered that namely abrectorin, genistin, (+)-catechin, precatorine, (+)-ascorbic acid, licoflavanone, skrofulein, stigmasterone, 5,7-Dihydroxy-4'-methoxy-8,3'-di-C-prenylflavanone could potentially be used as antagonists for the therapeutic management of epilepsy by targeting TNF and TP53 proteins. Furthermore, the implementation of molecular docking reinforces the binding affinity of the compound, indicating a robust stability of the forecasted compounds at the docked site. This research lays both a theoretical and experimental groundwork for more profound investigations and establishes a practical method for the strategic employment of active compounds in the development of anti-epileptic therapeutics.

Health risk assessment of toxic metal(loids) (As, Cd, Pb, Cr, and Co) via consumption of medicinal herbs marketed in Malawi.

Mlangeni AT.

Toxicol Rep. 2023 Jul 14;11:145-152. doi: 10.1016/j.toxrep.2023.07.004. eCollection 2023 Dec. PMID: 37538931

This study aimed to assess the potential health risks associated with consuming three commonly consumed medicinal herbs in Malawi: *Azadirachta indica*, *Mondia whitei*, and *Moringa oleifera*. The concentrations of five metal(loids) (As, Cd, Pb, Cr, and Co) were determined using inductively coupled plasma mass spectrometry, while their safety was assessed by comparing the measured values with the legislated maximum contaminant levels (MCL)and reported metal(loids) concentrations in other countries. The results indicated significant variations of metal(loids) concentrations amongst the studied medicinal herbs, with *Azadirachta indica* containing the highest mean As (0.078 ± 0.010 mg kg^{-1}) and Cd (0.049 ± 0.05 mg kg^{-1}) concentrations and *Mondia whitei* and *Moringa oleifera* contained the highest mean Co (1.01 ± 0.05 mg kg^{-1}) and Cr (1.42 ± 1.18 mg kg^{-1}) concentrations, respectively. However, the mean concentrations of As, Cd, Pb, Cr, and Co fell below the MCL set by World Health Organization (WHO), Alimentarius Commissions, and European Commission. The estimated daily intake (EDI) for each metal(loid) was less than 1, indicating that the studied medicinal herbs do not pose serious health risks to non-regular consumers. The study also emphasizes the importance of assessing the potential risks associated with consuming medicinal herbs contaminated with heavy metals or metalloids, as it can seriously threaten human health.

Evaluation of Chemical Compositions and the Antioxidant and Cytotoxic Properties of the Aqueous Extract of Tri-Yannarose Recipe (*Areca catechu*, *Azadirachta indica*, and *Tinospora crispa*).

Sanpinit S, Wetchakul P, Chonsut P, Prommee N, Punsawad C, Han J, Net-Anong S.

Antioxidants (Basel). 2023 Jul 15;12(7):1428. doi: 10.3390/antiox12071428. PMID: 37507966

Tri-Yannarose is a Thai traditional herbal medicine formula composed of *Areca catechu*, *Azadirachta indica*, and *Tinospora crispa*. It possesses antipyretic, diuretic, expectorant, and appetite-stimulating effects. This study aimed to evaluate the antioxidant activities, cytotoxicity, and chemical constituents of an aqueous extract following a Tri-Yannarose recipe and its plant ingredients. The phytochemical analysis was performed using LC-QTOF-MS. Antioxidant activities were determined using DPPH, ABTS, TPC, TFC, FRAP, NBT, MCA, and ORAC assays. Cytotoxicity was investigated using a methyl thiazol tetrazolium (MTT) assay. In addition, the relationship between the chemical composition of Tri-Yannarose and antioxidant activities was investigated by examining the structure-activity relationship (SAR). The results of the LC-QTOF-MS analysis revealed trigonelline, succinic acid, citric acid, and other chemical constituents. The aqueous extract of the recipe showed significant scavenging effects against ABTS and DPPH radicals, with IC_{50} values of 1054.843 ± 151.330 and 747.210 ± 44.173 µg/mL, respectively. The TPC of the recipe was 92.685 mg of gallic acid equivalent/g of extract and the TFC was 14.160 mg of catechin equivalent/g of extract. All extracts demonstrated lower toxicity in the Vero cell line according to the MTT assay. In addition, the SAR analysis indicated that prenyl arabinosyl-(1-6)-glucoside and quinic acid were the primary antioxidant compounds in the Tri-Yannarose extract. In conclusion, this study demonstrates that Tri-Yannarose and its plant ingredients have potent antioxidant activities with low toxicity. These results support the application of the Tri-Yannarose recipe for the management of a range of disorders related to oxidative stress.

Addressing Oral Health Disparities of a Tribal Population Through a Combined Implementation of Focus Group Discussion, Mobile Technology Networking, and Creating a Supportive Environment: A Prospective Study.

Kumari M, Sharma S, Raj A, Jha A, Shivakumar S, Kumar A.

Cureus. 2023 Jul 1;15(7):e41266. doi: 10.7759/cureus.41266. eCollection 2023 Jul. PMID: 37533614

Background and objective: Oral health disparities generally exist among tribal populations, prompting creative solutions to tackle these challenges. By using a combined implementation strategy of including focus group discussion (FGD), mobile technology networking (MTN), and creating a supportive environment, this study aims to assess and bring positive changes in oral health in these populations. **Methods:** The current study employed a mixed-method approach on a sample of 100 tribal volunteers. Qualitative assessment included FGD conducted regularly for three months based on themes such as oral hygiene habits, access to oral health, technology in oral health, the relationship of oral health to general health, and the role of diet in oral health. Quantitative evaluation included

recording of the oral hygiene index-simplified and gingival index to measure gingival status. Messages on oral health were routinely posted to mobile phones to reinforce oral health education. Appropriate use of indigenous oral hygiene aids (neem and datun) was also taught during the discussion session. Clinical examinations were compared before and after FGD. Data were analyzed using IBM SPSS Statistics for Windows, Version 25 (Released 2017; IBM Corp., Armonk, New York, United States). A paired 't' test was used to find significant differences in gingival status at $p<0.05$. **Results:** The FGD sessions deduced observations such as limited access to dental care, inadequate oral hygiene practices such as usage of neem sticks and twigs, and lack of oral health awareness. The implementation of MTN facilitated the dissemination of oral health information and enhanced communication between community members and healthcare providers. The gingival index score significantly improved from pre-FGD to post-FGD with a mean difference of 0.41700 significant at $p=0.000$. Oral hygiene of the target population shifted from "Fair" oral hygiene status to "Good" oral hygiene status. **Conclusion:** The combined implementation of FGD, MTN, and creation of a supportive environment demonstrated promising results in addressing oral health disparities among the tribal population. The interventions led to improved gingival status and better utilization of oral hygiene practices. These findings highlight the importance of tailored interventions, community engagement, and mobile technology in addressing oral health disparities in tribal populations. Ongoing support, sustainability, and further research are necessary to ensure the long-term impact and effectiveness of these interventions.

Pharmacoinformatics-Based Approach for Uncovering the Quorum-Quenching Activity of Phytocompounds against the Oral Pathogen, *Streptococcus mutans*.

Marimuthu SCV, Murugesan J, Babkiewicz E, Maszczyk P, Sankaranarayanan M, Thangamariappan E, Rosy JC, Ram Kumar Pandian S, Kunjiappan S, Balakrishnan V, Sundar K.

Molecules. 2023 Jul 19;28(14):5514. doi: 10.3390/molecules28145514.PMID: 37513386

Streptococcus mutans, a gram-positive oral pathogen, is the primary causative agent of dental caries. Biofilm formation, a critical characteristic of *S. mutans*, is regulated by quorum sensing (QS). This study aimed to utilize pharmacoinformatics techniques to screen and identify effective phytochemicals that can target specific proteins involved in the quorum sensing pathway of *S. mutans*. A computational approach involving homology modeling, model validation, molecular docking, and molecular dynamics (MD) simulation was employed. The 3D structures of the quorum sensing target proteins, namely SecA, SMU1784c, OppC, YidC2, CiaR, SpaR, and LepC, were modeled using SWISS-MODEL and validated using a Ramachandran plot. Metabolites from *Azadirachta indica* (Neem), *Morinda citrifolia* (Noni), and *Salvadora persica* (Miswak) were docked against these proteins using AutoDockTools. MD simulations were conducted to assess stable interactions between the highest-scoring ligands and the target proteins. Additionally, the ADMET properties of the ligands were evaluated using SwissADME and pkCSM tools. The results demonstrated that campesterol, meliantrol, stigmasterol, isofucosterol, and ursolic acid exhibited the strongest binding affinity for CiaR, LepC, OppC, SpaR, and Yidc2, respectively. Furthermore, citrostadienol showed the highest binding affinity for both SMU1784c and SecA. Notably, specific amino acid residues, including ASP86, ARG182,

ILE179, GLU143, ASP237, PRO101, and VAL84 from CiaR, LepC, OppC, SecA, SMU1784c, SpaR, and YidC2, respectively, exhibited significant interactions with their respective ligands. While the docking study indicated favorable binding energies, the MD simulations and ADMET studies underscored the substantial binding affinity and stability of the ligands with the target proteins. However, further in vitro studies are necessary to validate the efficacy of these top hits against *S. mutans*.

Revolutionizing the effect of Azadirachta indica extracts on edema induced changes in C-reactive protein and interleukin-6 in albino rats: in silico and in vivo approach.

Ammara A, Sobia A, Nureen Z, Sohail A, Abid S, Aziz T, Nahaa MA, Rewaa SJ, Ahellah MJ, Nouf SAA, Nehad AS, Manal YS, Amnah AA, Majid A, Abdulhakeem SA, Anas SD, Saad A.

Eur Rev Med Pharmacol Sci. 2023 Jul;27(13):5951-5963. doi: 10.26355/eurrev_202307_32947.PMID: 37458623

Objective: The aim of the present study is to determine the in vivo and in silico anti-inflammatory effect of Azadirachta indica (A. indica) in carrageenan-induced rats and its blood biomarkers. A. indica (Neem) is a widely used medicinal plant across the world, especially in Pakistan. Neem leaves have been traditionally used for the synthesis of drugs and treatment of a wide variety of diseases. **Materials and methods:** In this study, sixty albino rats (160-200 g) were divided into 4 groups: control (group I), standard (group II), ethanolic and aqueous (group III and IV) at doses of 50, 100, 200 and 400 mg/kg. **Results:** Ethanolic and aqueous extracts showed maximum inhibition in paw size at the 5th hour (400 mg/kg). Similarly, biomarkers measured, including Interleukin-6 and C-reactive protein, exhibited significant anti-inflammatory activity at the highest dose of 400 mg/kg in both experimental groups but were more distinct in the group treated with ethanolic extracts. Correlation between C-reactive protein (CRP) and inter-leukin-6 (IL-6) showed positive correlation in group III, while negative in group IV. Similarly, positive and negative correlations were observed between CRP biomarkers and paw size in group III and IV, and the same results were also shown in the case of IL-6 and paw size. In molecular docking, the binding energy value of protein CRP and IL-1β with the identified ligands quercetin and nimbosterol showed (-8.2 kcal/mol and -7.7 kcal/mol) the best binding affinity as compared to standard drug diclofenac with -7.0 kcal/mol binding energy respectively. **Conclusions:** In conclusion, in silico and in vivo analysis revealed that the extracts of A. indica leaves can be used as an effective drug to manage inflammation.

Triterpenes as Potential Drug Candidates for Rheumatoid Arthritis Treatment.

Faustino C, Pinheiro L, Duarte N.

Life (Basel). 2023 Jul 5;13(7):1514. doi: 10.3390/life13071514.PMID: 37511889

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease characterized by joint inflammation, swelling and pain. Although RA mainly affects the joints, the disease can also have systemic implications. The presence of autoantibodies, such as anti-cyclic citrullinated peptide antibodies and rheumatoid factors, is a hallmark of the disease. RA is a significant cause of disability worldwide associated with advancing age, genetic predisposition, infectious agents, obesity and smoking, among other risk factors. Currently, RA treatment depends on anti-inflammatory and disease-modifying anti-rheumatic drugs

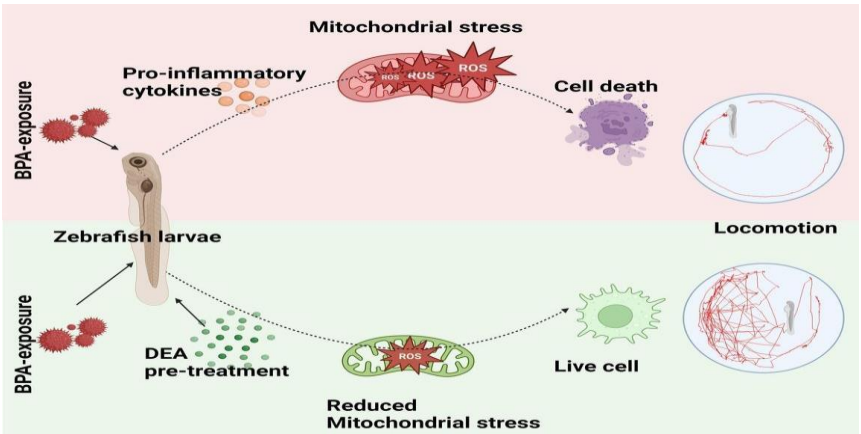
intended to reduce joint inflammation and chronic pain, preventing or slowing down joint damage and disease progression. However, these drugs are associated with severe side effects upon long-term use, including immunosuppression and development of opportunistic infections. Natural products, namely triterpenes with anti-inflammatory properties, have shown relevant anti-arthritic activity in several animal models of RA without undesirable side effects. Therefore, this review covers the recent studies (2017-2022) on triterpenes as safe and promising drug candidates for the treatment of RA. These bioactive compounds were able to produce a reduction in several RA activity indices and immunological markers. Celastrol, betulinic acid, nimbolide and some ginsenosides stand out as the most relevant drug candidates for RA treatment.

Deacetyl epoxyazadiradione ameliorates BPA-induced neurotoxicity by mitigating ROS and inflammatory markers in N9 cells and zebrafish larvae.

Murugan R, Haridevamuthu B, Kumar RS, Almutairi BO, Arokiyaraj S, Arockiaraj J.

Comp Biochem Physiol C Toxicol Pharmacol. 2023 Jun 30:109692. doi: 10.1016/j.cbpc.2023.109692. PMID: 37394128

Bisphenol A (BPA) leaches from plastic products have become a major inevitable concern among the research society. Human exposure to BPA leads to deleterious effects on multiple organs by the induced hyper inflammatory and oxidative stress responses. Due to the compromised antioxidant mechanism, the brain environment was highly susceptible and required special concern to ameliorate the effects of BPA. Hence, this study investigates the potential of neem-derived semi natural deacetyl epoxyazadiradione (DEA) against the oxidative stress and inflammatory response induced by BPA exposure in N9 cells and zebrafish larvae. The results from the in vitro analyses showed a decrease in cell viability in the MTT assay and a decline in mitochondrial damage in BPA-exposed N9 cells. Further in vivo, results revealed that pre-treatment of DEA to zebrafish larvae has significantly reduced the level of superoxide anion and increased the production of antioxidant enzymes such as SOD, CAT, GST, GPx and GR. We also found a significant decrease in the production of nitric oxide ($p < 0.0001$) and iNOS gene expression at 150 µM concentration. Further, DEA pre-treatment improved the behaviour of zebrafish larvae by ameliorating the production of the AChE enzyme. In conclusion, DEA protected zebrafish larvae from BPA toxicity by ameliorating oxidative stress and inflammatory responses.



Nimbin (N1) and analog N3 from the neem seeds suppress the migration of osteosarcoma MG-63 cells and arrest the cells in a quiescent state mediated via activation of the caspase-modulated apoptotic pathway.

Sudhakaran G, Velayutham M, Aljarba NH, A
l-Hazani TM, Arokiyaraj S, Guru A, Arockiaraj J.

Mol Biol Rep. 2023 Jul 14. doi: 10.1007/s11033-023-08627-7. PMID: 37450077

Background: Natural products are considered effective sources for new therapeutic research and development. The numerous therapeutic properties of natural substances in traditional medicine compel us to investigate the anti-cancer properties of Nimbin (N1) and its semi-natural analog Nimbic acid (N3) from Azadirachta indica against MG-63 Osteosarcoma cells. **Materials and methods:** The therapeutic efficacy of N1 and N3 were screened for their toxicity and cytotoxic activity using L6 myotubes, zebrafish larvae and MG-63 osteosarcoma cells. The mitochondrial membrane potential was evaluated using the Rhodamine 123 stain. Further, the nuclear and cellular damage was distinguished using Hoechst and Acridine orange/EtBr stain. The mechanism of cell cycle progression, cellular proliferation and caspase cascade activation was screened using scratch assay, flow cytometry, and mRNA expression analysis. **Results:** The Nimbin and analogue N3 were found to be non-toxic to normal L6 cells (Rat skeletal muscles), exhibited cytotoxicity in MG-63 cells, and were exposed to be an active inhibitor of cell proliferation and migration. Analogs N1 and N3 induced negative mitochondrial membrane potential when stained with Rhodamine 123, leading to nuclear damage and apoptosis stimulation using AO/EtBr and Hoechst. Further, N1 and N3 induced cell cycle arrest in G0/G1 phase in flow cytometry using PI staining and induced apoptosis by activating the caspase cascade and upregulated Caspase 3 and caspase 9. **Conclusion:** The study demonstrated cytotoxic activity against MG-63 osteosarcoma cells while being non-toxic to normal L6 cells. These compounds inhibited cell proliferation and migration, induced mitochondrial dysfunction, nuclear damage, and apoptosis stimulation. Furthermore, N1 and N3 caused cell cycle arrest and activated the caspase cascade, ultimately leading to apoptosis. These findings indicate that N1 and N3 hold promise as potential candidates used alone or combined with existing drugs for further investigation and development as anti-cancer agents.

Limonoids from neem (Azadirachta Indica A. Juss.) are potential anticancer drug candidates.

Nagini S, Palrasu M, Bishayee A.

Med Res Rev. 2023 Aug 17. doi: 10.1002/med.21988. PMID: 37589457

Neem (Azadirachta indica A. Juss.), a versatile evergreen tree recognized for its ethnopharmacological value, is a rich source of limonoids of the triterpenoid class, endowed with potent medicinal properties. Extracts of neem have been documented to display anticancer effects in diverse malignant cell lines as well as in preclinical animal models that has largely been attributed to the constituent limonoids. Of late, neem limonoids have become the cynosure of research attention as potential candidate agents for cancer prevention and therapy. Among the various limonoids found in neem, azadirachtin, epoxyazadiradione, gedunin, and nimbolide, have been extensively investigated for anticancer activity. Azadirachtin, a potent biodegradable pesticide, exhibits profound

antiproliferative effects by preventing mitotic spindle formation and cell division. The antiproliferative activity of gedunin has been demonstrated to be mediated primarily via inhibition of heat shock protein90 and its client proteins. Epoxyazadiradione inhibits pro-inflammatory and kinase-driven signaling pathways to block tumorigenesis. Nimbolide, the most potent cytotoxic neem limonoid, inhibits the growth of cancer cells by regulating the phosphorylation of keystone kinases that drive oncogenic signaling besides modulating the epigenome. There is overwhelming evidence to indicate that neem limonoids exert anticancer effects by preventing the acquisition of hallmark traits of cancer, such as cell proliferation, apoptosis evasion, inflammation, invasion, angiogenesis, and drug resistance. Neem limonoids are value additions to the armamentarium of natural compounds that target aberrant oncogenic signaling to inhibit cancer development and progression.

Neem in Veterinary Science & Medicine

Efficacy and Safety of Neem Oil for the Topical Treatment of Bloodsucking Lice *Linognathus stenopsis* in Goats under Field Conditions.

Cotticelli A, Matera R, Piscopo N, Bosco A, Claps S, Del Serrone P, Zoratti A, Castaldo E, Veneziano V, Rufrano D, Neglia G, Buono F.

Animals (Basel). 2023 Aug 7;13(15):2541. doi: 10.3390/ani13152541.PMID: 37570349

The aim of the present study was to evaluate the efficacy and safety of neem oil on caprine pediculosis and on kids' growth performances. The neem (*Azadirachta indica*) belongs to the Meliaceae family, and in Eastern countries it is mainly considered for the insecticidal activities of the kernel oil. The neem seeds contain bioactive principles, such as azadirachtin A, salannin, nimbin, and nimbolide. The trial was carried out on 24 kids, 120 days old, maintained in open yards. Animals were divided in 4 homogeneous groups (n = 6 animals/group) based on age, louse count, body condition score (BCS) and live body weight: Control Group (C, saline NaCl, 0.9%), Neem Group 1 (NO-100, 100 mL of neem oil per 10 kg), Neem Group 2 (NO-200, 200 mL/10 kg), Neem Group 3 (NO-300, 300 mL/10 kg). The treatments were performed by spraying the insecticide on the goat's body. The study lasted 56 days, and weekly, the kids underwent louse count, BCS and body weight determination, and FAMACHA score. Data were analyzed by ANOVA for repeated measures. The species of lice identified was *Linognathus stenopsis*. Kids belonging to NO-200 and NO-300 showed a stronger reduction of louse count throughout the study (>95%). The daily weight gain recorded was significantly higher ($p < 0.05$) in NO-300 than C. No differences were found for BCS and FAMACHA scores. The results of this trial showed that the administration of neem oil to control caprine pediculosis caused by sucking lice represents an alternative to synthetic compounds.