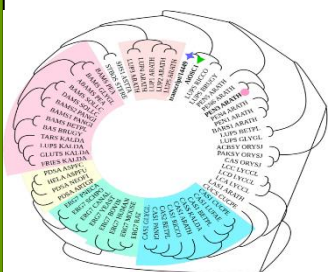


Neem Research Newsletter

Volume 2, Issue 1, 2022



WORLD NEEM ORGANISATION (WNO)



From
The Editor's Desk.....

It gives me great pleasure to present the first issue of the Neem Newsletter for the year 2022 on behalf of the World Neem Organisation (WNO). This issue provides abstracts of research papers published from January 1 to April 30 2022 on the impact of neem in diverse areas such as agriculture, environment, animal health, and human health besides basic sciences. Illustrations are provided wherever available as well as PMIDs for those readers who wish to delve more deeply. The first volume of the Newsletter was brought out in 2021 in two issues. Considering the voluminous data generated on neem, it was decided to bring out quarterly issues from 2022. It is heartening to note that over 50 papers were published in the first quarter of the year underscoring the potential of neem in different domains. In particular, scientists have begun to unravel the myriad health benefits of neem both against communicable and non-communicable diseases. I feel it is appropriate at this juncture to increase the frequency of the newsletter depending on the publication rate. Feedback/suggestions are most welcome.

S. Nagini

Core Founding Member, WNO
Chief Scientific Coordinator &

Regional Director, South India



Neem in Agriculture

Effect of slow release nitrogenous fertilizers and biochar on growth, physiology, yield, and nitrogen use efficiency of sunflower under arid climate.

Waqar M, Habib-Ur-Rahman M, Hasnain MU, Iqbal S, Ghaffar A, Iqbal R, Hussain MI, Sabagh AE.

Environ Sci Pollut Res Int. 2022 Mar 9. PMID: 35262889

Sunflower plants need nitrogen consistently and in higher amount for optimum growth and development. However, nitrogen use efficiency (NUE) of sunflower crop is low due to various nitrogen (N) losses. Therefore, it is necessary to evaluate the advanced strategies to minimize N losses and also improve sunflower productivity under arid climatic conditions. A field trial was conducted with four slow release nitrogenous fertilizers [SRNF (bacterial, neem, and sulfur-coated urea and N loaded biochar)] and three N levels (100% = 148 kg N ha⁻¹, 80% = 118 kg N ha⁻¹, and 60% = 89 kg N ha⁻¹) of recommended application (100%) for sunflower crop under arid climatic conditions. Results showed that neem-coated urea at 148 kg N ha⁻¹ significantly enhanced crop growth rate (CGR) (19.16 g m⁻² d⁻¹) at 60-75 days after sowing (DAS); leaf area index (2.12, 3.62, 5.97, and 3.00) at 45, 60, 75, and 90 DAS; and total dry matter (14.27, 26.29, 122.67, 410, and 604.33 g m⁻²) at 30, 45, 60, 75, and 90 DAS. Furthermore, higher values of net leaf photosynthetic rate (25.2 μmol m⁻² s⁻¹), transpiration rate (3.66 mmol s⁻¹), and leaf stomatal conductance (0.39 mol m⁻² s⁻¹) were recorded for the same treatment. Similarly, neem-coated urea produced maximum achene yield (2322 kg ha⁻¹), biological yield (9000 kg ha⁻¹), and harvest index (25.8%) of the sunflower crop. Among various N fertilizers, neem-coated urea showed maximum NUE (20.20 kg achene yield kg⁻¹ N applied) in comparison to other slow release N fertilizers. Similarly, nitrogen increment N₆₀ showed maximum NUE (22.40 kg grain yield kg⁻¹ N applied) in comparison to N₈₀ and N₁₀₀. In conclusion, neem-coated urea with 100% and 80% of recommended N would be recommended for farmers to get better sunflower productivity with sustainable production and to reduce the environmental nitrogen losses.

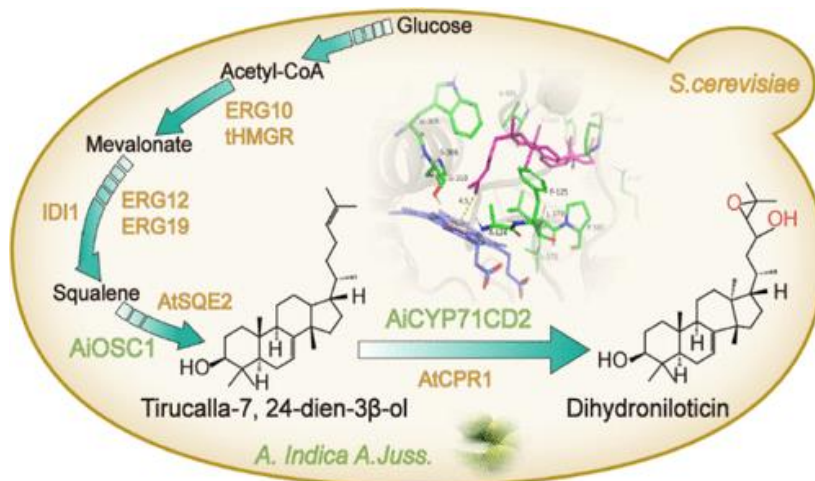
Metabolic Engineering of *Saccharomyces cerevisiae* for de Novo Dihydroniloticin Production Using Novel CYP450 from Neem (*Azadirachta indica*).

Hou K, Yu W, Wang X, Liu J, Liu Y, Liu J, Su X, Zhang X, Xue Q, Wang C.

J Agric Food Chem. 2022 Mar 8. PMID: 35258300

Azadirachtin, a limonoid isolated from the neem tree, has attracted considerable interest due to its excellent performance in pest control. Studies have also reported pharmaceutical activities of dihydroniloticin, an intermediate in azadirachtin biosynthesis, but these pharmaceutical activities could not be validated due to the limited supply. In this study, *AiCYP71CD2* was first identified as involved in azadirachtin biosynthesis in neem by expressing it in *Nicotiana benthamiana* and yeast (*Saccharomyces cerevisiae*). Homology modeling and molecular docking analysis revealed that *AiCYP71CD2* may exhibit a higher ability in catalyzing tirucalla-7,24-dien-3β-ol into dihydroniloticin compared with

MaCYP71CD2 from *Melia azedarach* L. G310 was identified as the critical residue responsible for the higher catalytic ability of AiCYP71CD2. Condon-Optimized AiCYP71CD2 greatly improved the catalytic efficiency in yeast. *De novo* dihydroniloticin production using the novel AiCYP71CD2 was achieved by constructing the *S. cerevisiae* DI-3 strain, and the titer could reach up to 405 mg/L in a fermentor, which was an alternative source for dihydroniloticin.



Copaiba oil and Neem extract can be a potential alternative for the behavioral control of *Sitophilus zeamais*.

Martins JC, Silva ÉM, Silva RS, Ferreira SR, Picanço MC.

Braz J Biol. 2022 Mar 7;84:e254628. PMID: 35239787

Insects' ethology is an important factor when it is desired to carry out pest management. This knowledge makes it possible to manipulate behavioral activities, repel, or attract insects according to needs and interests. The maize weevil *Sitophilus zeamais* (Mots., 1855) (Coleoptera: Curculionidae), one of the main stored grain pests, has been the target of studies of behavioral changes studies through natural substances due to its resistance to different insecticidal classes. Thus, this study aimed to evaluate the effect of sublethal concentrations of neem extract and copaiba oil on the locomotor behavior of *S. zeamais*. The behavioral characteristic considered were walking activity, the frequency of contact of insects with the treated grain mass, and the time spent for this behavior. The walking activity of the *S. zeamais* increased with exposure to Neem extract and Copaiba oil. In general, the Neem extract and Copaiba oil-induced more contact with grain mass than the control, suggesting an attractive effect on the insect, however more significant for the Neem oil. The insect's behavior was altered, presenting a specific path due to Copaiba oil and Neem extract stimuli. These results indicate that Copaiba oil and Neem extract can be a potential alternative for controlling *S. zeamais* on stored products since changes in this pests' behavior can reduce qualitative and quantitative grain damage. Thus, the development of products based on Copaiba oil and Neem extract may be helpful for storage pest management.

Nutrient enhancement potentials of moringa (*Moringa oleifera*), neem (*Azadirachta indica*), and pawpaw (*Carica papaya*) fortified composts in contaminated soils.

Taiwo AM, Oladotun OR, Gbadebo AM, Alegbeleye WO, Hassan TM.
Environ Monit Assess. 2022 Mar 2;194(3):237. PMID: 35235049

This study assessed the nutrient enhancement potentials of moringa (*Moringa oleifera*), neem (*Azadirachta indica*), and pawpaw (*Carica papaya*) fortified composts in contaminated soils. The composts were formulated from poultry manure; leaves of moringa, neem, and pawpaw; and sawdust for a period of 8 weeks. Contaminated soil samples were collected from a dumpsite in Abeokuta, Ogun State. The contaminated soils were treated with the stabilized composts for a period of 4 weeks. Castor oil plants were introduced to assess the nutrient quality of the composts. Soil and compost parameters (pH, electrical conductivity (EC), organic carbon (OC), total nitrogen (TN), C/N ratio, P, K, Na, Mg, and Ca) were determined using the standard procedures. Data collected were evaluated for simple descriptive and inferential statistics. Results showed non-significance ($p > 0.05$) of C/N ratios that ranged between 12.75 ± 2.43 and 12.96 ± 1.49 . The pH values of moringa- and neem-fortified composts were slightly acidic, while the pawpaw-formulated compost was slightly alkaline. The levels of TN and OC were three times higher in moringa- and pawpaw-fortified composts than in the neem compost. Introduction of the composts to the contaminated soils decreased the soil pH but increased the nutrient quality parameters such as TN (33-50%), OC (56-77%), P (7-20%), Na (89-91%), K (12-25%), and Mg (10-13%). The three compost types increased the physiological properties of the castor oil plants. The study indicated the promising potentials of the three composts for nutrient enhancement of soil.

Lateralization of Courtship Traits Impacts Pentatomid Male Mating Success-Evidence from Field Observations.

Romano D, Benelli G, Stefanini C.

Insects. 2022 Feb 5;13(2):172. PMID: 35206745

Lateralization has been documented in many insect species, but limited information on courtship and mating lateralization in wild conditions is available. We conducted field investigation on the courtship and mating behavior of the neem bug, *Halys dentatus*, a polyphagous insect mainly infesting *Azadirachta indica*, with particular attention to lateralization of mating displays. We investigated the presence of population-level behavioral asymmetries during *H. dentatus* sexual interactions and their influence on male mating success. Two lateralized traits were found: left or right-biased male approaches to the female and left or right-biased male turning displays. Males approaching females from their left side were mainly right-biased in the 180° turning display, and males that approached females from their right side were mainly left-biased. Right-biased males by turning 180° to carry out end-to-end genital contact, performed a lower number of copulation attempts, thus starting copula earlier than left-biased males. Mating success was higher when males approached the left side of females during sexual interactions. A higher number of successful mating interactions was observed in right-biased males when turning 180°. Our results add useful knowledge on the reproductive behavior of *H. dentatus* in the field, with potential applications for identifying useful benchmarks to monitor the quality of individuals mass-reared for pest control purposes over time.

Influence of patch geometry, post-bark-extraction-treatment on bark recovery and standardizing number of sprouts for bark harvest from coppices in *Cinnamomum zeylanicum* blume: implications for sustainable harvesting.

Hanumantha M, Vasudeva R.

Environ Monit Assess. 2022 Feb 23;194(3):214. PMID: 35199293

Though *Cinnamomum zeylanicum* is a very important commercial aromatic bark yielding and oldest known tree spice of India, there are hardly any studies to understand the nature of wound healing and bark recovery. Further, optimal number of sprouts to be retained under coppice system to maximize the bark yield per tree in *C. zeylanicum* is not yet standardized. The present investigation was carried out to understand the influence of patch geometry and application of post-bark-extraction-protection treatments on bark regeneration in mature trees as well as to standardize an optimal number of sprouts to be retained under coppice system to maximize the bark yield per tree. In general, wound healing in *C. zeylanicum* occurred from the edge of the blaze and was quicker in the narrower patch than the broader patch; application of Bordeaux paste or neem seed kernel extract on the blazed area immediately after the bark extraction, resulted in significantly higher mean percent bark recovery and higher bark oil content than control. Allowing seven coppice sprouts per stem resulted in higher dry mass of bark per plant and higher bark oil than other treatments under coppice system. The results of the study would enable formulation of management strategies specifically for sustainable bark harvesting in *Cinnamomum* species.

Effects of Various Polymeric Films on the Pericarp Microstructure and Storability of Longan (cv. Shixia) Fruit Treated with Propyl Disulfide Essential Oil from the Neem (*Azadirachta indica*) Plant.

Khan MR, Huang C, Ullah R, Ullah H, Qazi IM, Nawaz T, Adnan M, Khan A, Su H, Ren L.

Polymers (Basel). 2022 Jan 28;14(3):536. PMID: 35160524

Plant extracts represent a rich repository of metabolites with antioxidant and antimicrobial properties. Neem (*Azadirachta indica*) is a medicinal plant considered the tree of the 21st century. In this study, we investigated the antioxidant and antimicrobial effects of propyl disulfide (PD), a major volatile compound in neem seed, against the pericarp browning (BI), microbial decay incidence (DI), and water loss of longan fruit. Fresh longan cv. Shixia samples were packaged in oriented polypropylene (OPP) and polyethylene (PE) packages of different thicknesses (20, 40, and 60 μm). Sterile gauze was fixed inside the packages and 500 μL of PD was placed on them to avoid the direct contact of PD with fruit samples. Packages were sealed immediately to minimize vaporization and stored at 12 ± 1 $^{\circ}\text{C}$ for 18 days. Fruit samples packaged in open net packages served as controls. The results showed that fruit treated with PD in OPP and PE packages significantly prevented losses of water, DI, and BI compared to control treatment. PD also maintained the color, TSS values, TA values, pH values, high peel firmness, high TPC content, and high TFC content, and reduced the activity levels of PPO and POD. Scanning electron microscope (SEM) analysis indicated that the exocarp, mesocarp, and endocarp of longan peel were smooth, uniform, and compact with no free space compared to control, where cracks, a damaged and loose structure, and a lot of fungal mycelia were found. The shortest shelf life of 9 days was observed in control as compared to 18 days in OPP-20 and OPP-40; 15 days in OPP-60,

PE-20, and PE-40; and 12 days in PE-60 packaging films. Therefore, PD as a natural antioxidant and antimicrobial agent, in combination with OPP-20 and OPP-40 polymeric films, could successfully be applied commercially to extend the postharvest shelf life of longan.

The combination of organic and inorganic fertilizers influence the weed growth, productivity and soil fertility of monsoon rice.

Ghosh D, Brahmachari K, Skalický M, Roy D, Das A, Sarkar S, Moulick D, Brestič M, Hejnak V, Vachova P, Hassan MM, Hossain A.

PLoS One. 2022 Jan 27;17(1):e0262586. PMID: 35085310

Synthetic fertilizer and herbicides encompass the largest share in nutrient and weed management on food grain crops that create serious environmental issues. Integrated nutrient and non-chemical weed management approaches may help to reduce the chemical load in the environment, maintaining higher weed control efficiency and yield. A field experiment was conducted for two consecutive monsoon seasons during 2015 and 2016 in farm fields to develop a profitable and sustainable rice production system through integrated nutrient and weed management practices. A varied combination of nutrients either alone or integrated with chemical and non-chemical weed management were tested on transplanted rice in a factorial randomized block design with three replications. The results showed that the integration of concentrated organic manures with chemical fertilizer effectively inhibited weed growth and nutrient removal. Integration of nutrient and weed management practices significantly enhanced 9% biomass growth, 10% yield of the rice crop along with 3-7% higher nutrient uptake. Brassicaceous seed meal (BSM) and neem cake also had some influence on weed suppression and economic return. Thus, the integrated nutrient and weed management practices in rice cultivation might be an effective way to achieve economic sustainability and efficient rice cultivation in eastern India. Shortages of farmyard manure and vermicompost could be supplemented by BSM and neem cake in the integrated module.

***Galleria mellonella* (greater wax moth) as an eco-friendly in vivo approach for the assessment of the acute toxicity of medicinal plants: Application to some plants from Cameroon.**

Arsene MMJ, Viktorovna PI, Davares AKL.

Open Vet J. 2021 Oct-Dec;11(4):651-661. PMID: 35070860

Background: The evaluation of medicinal plants' toxicity is a prerequisite prior to their usage. The vertebrate models used for this purpose are often the object of ethical consideration. Though invertebrate models including *Galleria mellonella* (*GM*) have demonstrated the ability to be used to assess the toxicity of various products. To the authors' knowledge, *GM* has never been exploited to determine the toxicity of medicinal plants.

Aim: The aim of this study was to demonstrate the potential of *GM* larvae as a simple, inexpensive, and rapid model for the evaluation of the toxicity of herbal medicines.

Method: In this study, the toxicity of aqueous and ethanolic (80%, v/v) extracts of seven well known plants from Cameroon namely leaves of *Cymbopogon citratus* (DC.) Stapf, *Moringa oleifera* Lam and *Vernonia amygdalina* Delile; barks of *Cinchona officinalis* and *Enantia chlorantha* Oliv; barks and seeds of *Garcinia lucida* Vesque and leaves and seeds of *Azadirachta indica* (Neem) was evaluated using the larval form of *GM*. The median lethal

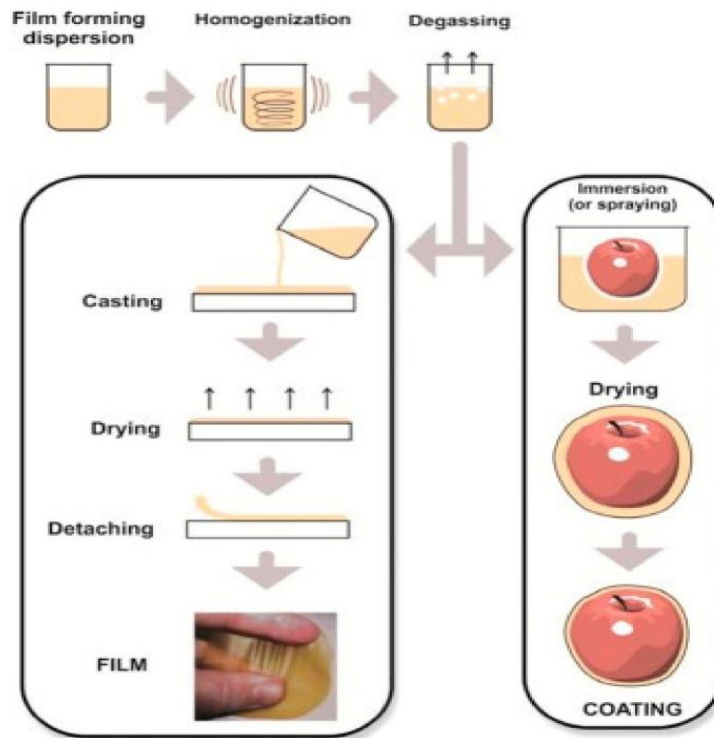
doses (LD₅₀), 90% (LD₉₀), and 100% (LD₁₀₀) were determined using the spline cubic survival curves and equations from the data obtained on the survival rate of *GM* 24 hours after the injection with the extracts. **Results:** We found that distilled water extracted a more important mass of phytochemical compounds (7.4%-21.2%) compared to ethanolic solution (5.8%-12.4%). LD varied depending on the plant materials and ethanolic extracts (hydroalcoholic extract, (HAE)) were more toxic to *GM* than aqueous ones. The LD₅₀ (mg/ml) of the tested extracts varied from 4.87 [3.90 g/kg body weight (bw)] to >200 (> 166.67 g/kg bw), the LD₉₀ (mg/ml) from 25.00 (18.52 g/kg bw) to >200 (> 181.82 g/kg bw) and LD₁₀₀ (mg/ml) from 45.00 (40.91 g/kg bw) to > 200 (>181.82 g/kg bw). The HAE of *A. indica* seed and *C. officinalis* bark exhibited the highest toxicity with LD₅₀ (g/kg bw) of 3.90 and 4.81, respectively. **Conclusion:** The results obtained in this study suggest that *GM* can be used as a sensitive, reliable, and robust eco-friendly model to gauge the toxicity of medicinal plants. Thus, avoid the sacrifice of vertebrate models often used for this purpose to limit ethical concerns.

The Development of a Green Innovative Bioactive Film for Industrial Application as a New Emerging Technology to Protect the Quality of Fruits.

Ahmed W, Azmat R, Khojah E, Ahmed R, Qayyum A, Shah AN, Abbas A, Moin S, Samra BN.

Molecules. 2022 Jan 13;27(2):486. PMID: 35056801

Today, the most significant challenge encountered by food manufacturers is degradation in the food quality during storage, which is countered by expensive packing, which causes enormous monetary and environmental costs. Edible packaging is a potential alternative for protecting food quality and improving shelf life by delaying microbial growth and providing moisture and gas barrier properties. For the first time, the current article reports the preparation of the new films from Diterpenoids and Secomeliacins isolated from *Melia azedarach* (Dharek) *Azadirachta indica* plants to protect the quality of fruits. After evaluating these films, their mechanical, specific respirational, coating crystal elongation, elastic, water vapor transmission rate (WVTR), film thickness, and nanoindentation test properties are applied to apple fruit for several storage periods: 0, 3, 6, 9 days. The fruits were evaluated for postharvest quality by screening several essential phytochemical, physiological responses under film coating and storage conditions. It was observed that prepared films were highly active during storage periods. Coated fruits showed improved quality due to the protection of the film, which lowered the transmission rate and enhanced the diffusion rate, followed by an increase in the shelf life. The coating crystals were higher in Film-5 and lower activity in untreated films. Application of films through dipping was a simple technique at a laboratory scale, whereas extrusion and spraying were preferred on a commercial scale. The phytochemicals screening of treated fruits during the storage period showed that a maximum of eight important bioactive compounds were present in fruits after the treatment of films. It was resolved that new active films (1-5) were helpful in the effective maintenance of fruit quality and all essential compounds during storage periods. It was concluded that these films could be helpful for fruits growers and the processing industry to maintain fruit quality during the storage period as a new emerging technology.



Evaluation of the Effect of Fungatol and Gamma-T-ol on the Emergence and Adult Parasitoid Survival of Mummies of Cotton Aphids Parasitized by *Aphidius colemani*.
Hassan E, Mostafiz MM, Iramu ET, George D, Lee KY.

Insects. 2021 Dec 29;13(1):38. PMID: 35055883

Beneficial insects play a major role in controlling pest populations. In sustainable agricultural production systems, control methods compatible with integrated pest management (IPM) are preferred over broad-spectrum pesticides. EOs from aromatic plants may provide a new and safe alternative to synthetic chemicals. In this research, the efficacy of Fungatol, Gamma-T-ol, Fungatol plus neem, and Gamma-T-ol plus neem was evaluated against *Aphidius colemani* Viereck (Hymenoptera: Braconidae; Aphidiidae), the parasitoid of the cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae). Under laboratory and greenhouse conditions, five different concentrations of each formulation were applied to parasitized mummies and adult parasitoids. Results for parasitoid emergence from aphid mummies sprayed with different concentrations of Fungatol, Gamma-T-ol, Fungatol plus neem, and Gamma-T-ol plus neem in the laboratory and glasshouse showed that the formulations did not adversely affect adult emergence as rates above 60% were observed. For residual toxicity tests done by exposing adult parasitoids to a fresh, dry biopesticide film sprayed on glass plates, less than 20% mortality was observed after 48 h of exposure. Adult longevity tests revealed that the highest concentrations of some of the formulations evaluated were slightly toxic to *A. colemani*. According to the IOBC rating, our results indicated that most of the tested concentrations for each formulation were harmless to *A. colemani*. Based on the above results, it may be proposed that the formulations evaluated in this study are potential botanical pesticide candidates for incorporation into an IPM program.

Potential of vermicompost extract in enhancing the biomass and bioactive components along with mitigation of *Meloidogyne incognita*-induced stress in tomato.

Tikoria R, Kaur A, Ohri P.

Environ Sci Pollut Res Int. 2022 Mar 24. PMID: 35332451

Increasing inorganic fertilizer and pesticide use has been linked to increased health risks for humans and cattle, as well as substantial water and soil contamination. In recent years, vermicomposting has shown to be a viable alternative to chemical pesticides. Vermicompost and vermicompost products such as extract and leachate assist plants in a number of ways. According to recent studies, vermicompost extract (VCE), when used as a supplement, is thought to work as a growth and stress tolerance booster for plants. These liquid supplements also help to suppress a range of pests, such as root knot nematodes. In the present study, neem- and cattle dung-based vermicompost extracts of different concentrations (0, 20, 40, 60, 80 and 100%) were prepared and used for their application against nematode infection in tomato seedlings under laboratory conditions. Apart from its antagonistic action against *Meloidogyne incognita*, the influence of VCE on plant growth was investigated by analyzing its morphological characteristics in tomato seedlings infected and uninfected with *M. incognita*. Seeds were pre-soaked in VCE for the seed priming process before being allowed for germination. After 10 days of nematode inoculation, biochemical parameters like protein content, activity of antioxidative enzymes, non-enzymatic antioxidants, stress indices, photosynthetic pigments, proline content and secondary metabolites were also analyzed. The results revealed that neem-based VCE was fatal to second-stage juveniles, with an 82% mortality rate following exposure to the highest dose. When eggs were exposed to 100% VCE, 33.8% of hatching was suppressed, indicating that VCE had an antagonistic effect on nematode egg hatching. Further, all the morphological and biochemical parameters were significantly enhanced in VCE-treated tomato seedlings as compared to untreated seedlings. Stress indices were also found to be significantly lowered by the VCE treatments in the infected plants. The effect of VCE on seedling growth and physiology was shown to be concentration dependent. As a result, the current findings show that VCE has the potential to be used as a plant growth accelerator as well as an environmentally friendly biocontrol agent against nematode pathogenesis in tomato plants.

Leaf extract of neem (*Azadirachta indica*) alleviates adverse effects of drought in quinoa (*Chenopodium quinoa* Willd.) plants through alterations in biochemical attributes and antioxidants.

Naz H, Aisha Akram N, Ashraf M, Ingo Hefft D, Latief Jan B.

Saudi J Biol Sci. 2022 Mar;29(3):1367-1374. PMID: 35280556

The influence of varying concentrations (0, 1, 3, 4, 5, 6, 8, and 10 % v/v) of neem (*Azadirachta indica*) leaf extract on drought stressed (40 % field capacity) quinoa (*Chenopodium quinoa* Willd.) plants was assessed. During the current study two cultivars of quinoa (V7 and V9) were used. This study revealed that water stress adversely affects the fresh and dry weight of shoots and roots as well as chlorophyll pigments (*a* and *b*) of both quinoa cultivars. In contrast, drought stress enhanced glycinebetaine (GB), free proline, phenolic content, hydrogen peroxide (H₂O₂), activities of superoxide dismutase (SOD),

peroxidase (POD), and catalase (CAT) enzymes, and relative membrane permeability (RMP). However, application of neem leaf extract improved the accumulation of key osmoprotectants like proline, GB and activities of enzymatic antioxidants. Our findings showed 5 % neem leaf extract is an effective treatment in counteracting the oxidative damage caused by water stress, thereby improving overall plant growth. Of both cultivars of quinoa, the response of cv. V9 to stress as well as foliar applied neem was relatively more promising.

Polyhydroxylated sesquiterpenes and ergostane glycosides produced by the endophytic fungus *Xylaria* sp. from *Azadirachta indica*.

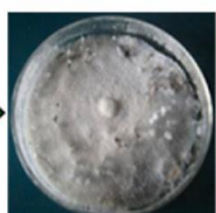
Wang JF, Huang R, Song ZQ, Yang QR, Li XP, Liu SS, Wu SH.

Phytochemistry. 2022 Apr 12;199:113188. PMID: 35421432

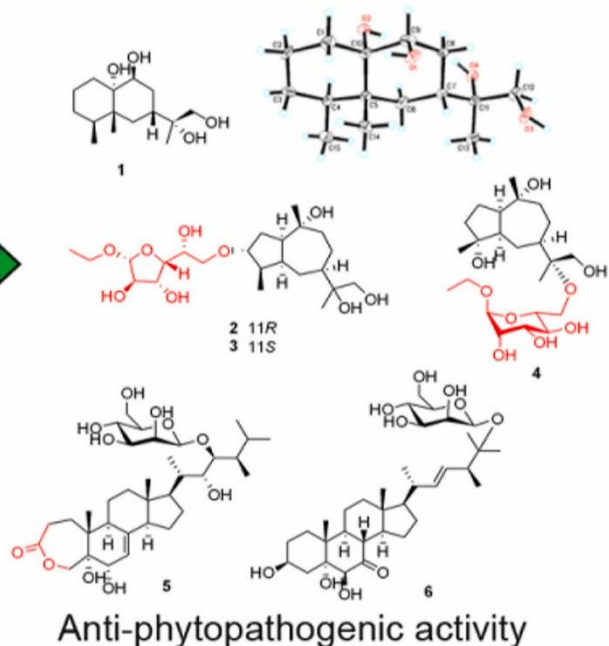
The investigation of the metabolites from the endophytic fungus *Xylaria* sp. YM 311647 in solid fermentation resulted in the isolation of six undescribed compounds, namely xylarioxides A-F, respectively. These included one eremophilane sesquiterpene, three guaiane sesquiterpene glycosides, and two ergostane glycosides. The structures of the compounds were determined by extensive analyses of spectroscopic data, including 1D and 2D NMR, as well as HRESIMS data. The stereochemistry of xylarioxide A was confirmed by X-ray crystallographic analysis. All of the isolated compounds were assayed for their antifungal activities against seven phytopathogenic fungi and two human pathogenic fungi. Among them, xylarioxides A, E and F showed potent activities against the tested phytopathogens. Particularly, xylarioxide E exhibited the highest activity against *Gibberella saubinetii*, *Curvularia lunata*, and *Colletotrichum gloeosporioides* with MIC values of 4, 4, and 8 $\mu\text{g/mL}$, respectively, which were comparable to the positive control of nystatin. Interestingly, guaiane sesquiterpene glycosides have been rarely reported from fungal sources. Additionally, xylarioxide E represented an unusual naturally occurring 3,4-seco-steroidal glycoside with a seven-membered lactone in ring A.



Azadirachta indica



Xylaria sp.
YM 311647



Neem in Aquaculture

Comparative Studies on The Efficiency of Neem Leaves *Azadirachta indica* and Flubendazole Treatment Against *Diplectanum* in Sea Bass *Dicentrarchus labrax*.

Aly SM, Abou El-Gheit SN, Essam El-Din HM.

Acta Parasitol. 2022 Apr 15. PMID: 35426620

Purpose: *Diplectanum* is a life-threatening metazoan infecting the gills of Sea bass *Dicentrarchus labrax* causing a wide-ranging extensive economic loss in the aquaculture sector. This study has focused on verifying the most effective non-toxic dose of the Neem (*Azadirachta indica*) and (flubendazole) bath treatment on infested *D. labrax* fingerlings.

Methods: In the first phase of the experiment, a total of 180 apparently healthy fingerlings were subdivided into six groups for each treatment. The tested concentrations were 0, 50, 100, 150, 200, and 250 mg L⁻¹ for *A. indica* and 0, 10, 20, 30, 40, and 50 mg L⁻¹ for flubendazole. The second phase was conducted for one week in five groups for each treatment. The first group was untreated healthy. The remaining groups were infested and received different concentrations of 0, 50, 100, and 150 mg L⁻¹ & 0, 10, 20, and 30 mg L⁻¹ for *A. indica* and flubendazole, respectively. **Results:** The most toxic dose exhibited high mortality rates at 200 & 250 and 40 & 50 mg L⁻¹ for *A. indica* and flubendazole, respectively. In the second phase of the experiment, the most effective dose was 150 and 30 mg L⁻¹; for *A. indica* and flubendazole, respectively. They demonstrated the lowest mortality rates 20.00 & 20.00 %, prevalence rates 43.33 & 23.33%, and mean parasitic intensities were 2.35 & 2.00 accompanied by the highest therapeutic efficacy value 67.85 & 74.6% for both treatments; respectively. **Conclusion:** The most effective anthelmintic efficacy has been assigned for flubendazole and *A. indica* at 30 and 150 mg L⁻¹.

Neem- Component Analysis

TLC-Based Metabolite Profiling and Bioactivity-Based Scientific Validation for Use of Water Extracts in AYUSH Formulations.

Zahiruddin S, Parveen A, Khan W, Parveen R, Ahmad S.

Evid Based Complement Alternat Med. 2021 Dec 31;2021:2847440. PMID: 35003294

We aimed to develop a chromatographic method for scientific validation of water extract of some important Indian traditional plants used in AYUSH-based formulation as immunomodulator and to evaluate their bioactive potential. Fruits of *Phyllanthus emblica* L. and *Piper nigrum* L., stem of *Tinospora cordifolia* (Willd.) Miers, rhizome of *Curcuma longa* L., leaves of *Ocimum sanctum* L. and *Achillea millefolium* L., roots of *Withania somnifera* L., and stem bark of *Azadirachta indica* A. Juss. were coarsely powdered and extracted in three different solvents (water, ethanol, and hydroethanol). The antioxidant potential was determined through 1, 1-diphenyl-2-picrylhydrazyl and ferric reducing capacity methods. Thin-layer chromatography (TLC) was carried out for the comparative metabolite profiling of the extracts using toluene, ethyl acetate, and formic acid (5 : 4 : 1, v/v/v) as a solvent system. *In vitro* immunomodulatory activity of the extracts has been tested on splenocyte proliferation and pinocytic assay. Hydroethanolic extract (HEE) of most of the plant materials has the highest phenolic and flavonoid contents, followed by water extract (WE) and ethanolic extract (EE), whereas the water extracts of most of the plant material showed better antioxidant activity. Almost all extract exhibited splenocyte proliferation and pinocytic activity in a dose-dependent manner. But water extract showed significantly higher splenocyte proliferation and pinocytic activity as compared to the other two extracts. TLC analysis resulted in detection of totally 63 and 56 metabolites at 254 nm and 366 nm, respectively. Through principal component analysis (PCA), it was observed that metabolite pattern of different extracts from same plant materials may be different or similar. This preliminary result can be used for quality evaluation and to develop a synergy-based polyherbal combination of water extracts of selected plant materials.

Potential Antioxidative Components in *Azadirachta indica* Revealed by Bio-Affinity Ultrafiltration with SOD and XOD.

Fan MX, Chen GL, Guo MQ.

Antioxidants (Basel). 2022 Mar 29;11(4):658. PMID: 35453343

Azadirachta indica (*A. indica*) has been widely used due to its diverse pharmacological activities. However, there are currently few studies on its responsible antioxidant ingredients against superoxide dismutase (SOD) and xanthine oxidase (XOD). In this study, the antioxidant activities of *A. indica* were evaluated by a 2,2'-azinobis-(3-ethyl-benzthiazoline)-6-sulfonic acid) and ferric-ion-reducing antioxidant power method. Meanwhile, total polyphenol and flavonoid content were determined to reveal that they were the highest in ethyl acetate (EA) fraction. Next, compounds with the most antioxidant activity were screened out from EA fraction by bio-affinity ultrafiltration liquid chromatography-mass spectrometry (UF-LC-MS) with SOD and XOD. As a result, gallic acid, protocatechuic acid and (-)-epicatechin were identified as potential SOD ligands with relative binding affinity (RBA) values of 2.15, 1.78 and 1.61, respectively. Additionally, these three ligands could effectively interact with SOD in molecular docking with binding energies (BEs) ranging from -3.84 ± 0.37 to -5.04 ± 0.01 kcal/mol. In addition, carnosic acid exhibited a strong binding affinity to XOD with an RBA value of 2.05 and BE value of -8.24 ± 0.71 kcal/mol. In conclusion, these results indicated that *A. indica* might have good antioxidant activity and antigout potential, and the UF-LC-MS method is suitable and efficient for screening both SOD and XOD ligands from *A. indica*.

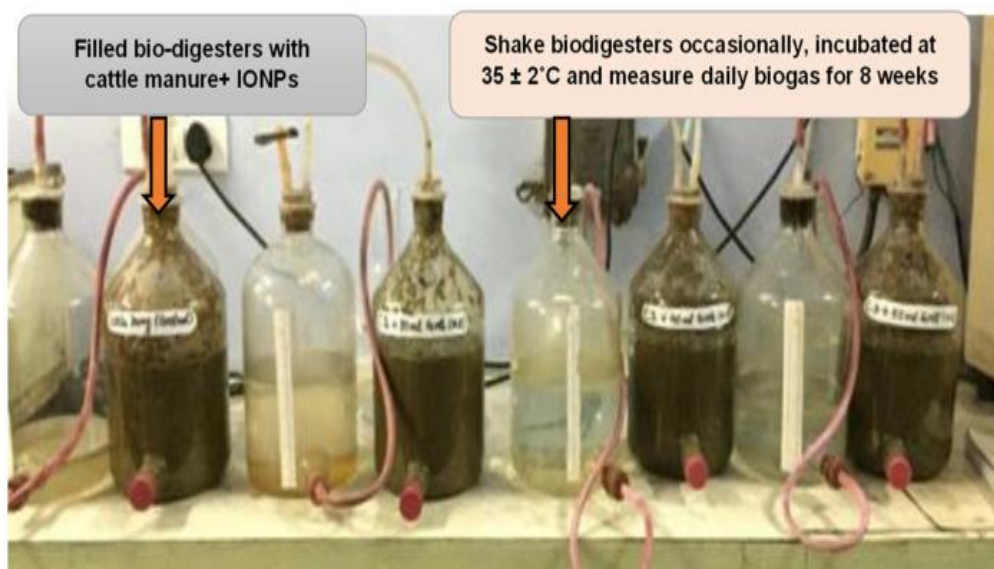
Neem For Sustainable Environment

Biostimulation of Anaerobic Digestion Using Iron Oxide Nanoparticles (IONPs) for Increasing Biogas Production from Cattle Manure.

Singh D, Malik K, Sindhu M, Kumari N, Rani V, Mehta S, Malik K, Ranga P, Sharma K, Dhull N, Malik S, Arya N.

Nanomaterials (Basel). 2022 Jan 31;12(3):497. PMID: 35159841

The effect of synthesised IONPs employing a nontoxic leaf extract of *Azadirachta indica* as a reducing, capping, and stabilizing agent for increasing biogas and methane output from cattle manure during anaerobic digestion (AD) was investigated in this study. Furthermore, the UV-visible spectra examination of the synthesized nanoparticles revealed a high peak at 432 nm. Using a transmission electron microscope, the average particle size of IONPs observed was 30-80 nm, with irregular, ultra-small, semi-spherical shapes that were slightly aggregated and well-distributed. IONPs had a polydisparsity index (PDI) of 219 nm and a zeta potential of -27.0 mV. A set of six bio-digesters were fabricated and tested to see how varying concentrations of IONPs (9, 12, 15, 18, and 21 mg/L) influenced biogas, methane output, and effluent chemical composition from AD at mesophilic temperatures (35 ± 2 °C). With 18 mg/L IONPs, the maximum specific biogas and methane production were 136.74 L/g of volatile solids (VS) and 64.5%, respectively, compared to the control ($p < 0.05$), which provided only 107.09 L/g and 51.4%, respectively. Biogas and methane production increased by 27.6% and 25.4%, respectively using 18 mg/L IONPs as compared to control. In all treatments, the pH of the effluent was increased, while total volatile fatty acids, total solids, volatile solids, organic carbon content, and dehydrogenase activity decreased. Total solid degradation was highest (43.1%) in cattle manure + 18 mg/L IONPs (T5). According to the results, the IONPs enhanced the yield of biogas and methane when compared with controls.



Geo-environmental approach to assess heavy metals around auto-body refinishing shops using bio-monitors.

Zeb J, Tahir H, Othman A, Habeebullah TM, Sayqal A, Assaggaf HM, Ahmed OB, Sultan M, Mohiuddin S, Masood SS, Mirza AZ, Hajira B.

Heliyon. 2022 Jan 21;8(1):e08809. PMID: 35111986

The vehicular industry is looking for continuous challenges to develop the sustainability of its manufacturing, maintenance processes, and vehicle emissions due to marketability, environmental, economic, and policy concerns. The present study focuses on the impact of these processes on the environment. In Pakistan, most of the auto-body refinishing processes are carried out in an open atmosphere. The shades of *Azadirachta indica* (Neem Tree) are generally used for the outdoor practice of scrapping, grinding, and painting in auto-body refinishing shops of Pakistan. *Azadirachta indica* leaves were selected as bio-indicator. For the present work, 26 affected sites and 10 control sites were selected from Karachi city, which is the financial hub and biggest city of Pakistan. Concentrations of different metals (Fe, Co, Cd, Cr, Cu, Mn, Mo, Ni, Pb, and Zn) were determined by atomic absorption spectrophotometer. A geographic information system (GIS) is used to present the variation in concentrations within Karachi city. The only positive correlation was observed in Pb and Mn (0.750). Principal component analysis (PCA) is applied to identify the anthropogenic effect between auto-body refinishing areas and control areas. Almost all analyzed metals show higher concentration at affected sites but Pb (87.14 mg/kg), Mn (46.47 mg/kg) and Fe (146.95 mg/kg) were leading the values, as compared to their concentration at control sites, Pb (48.83 mg/kg), Mn (15.23 mg/kg) and Fe (43.07 mg/kg). All analyzed metals are frequently present in different color pigments, whereas Pb, Mn, and Fe may also come from other sources, like the anti-knocking agent, vehicular exhaust, and scraping of car surface.

Growth of MWCNTs from *Azadirachta indica* oil for optimization of chromium(VI) removal efficiency using machine learning approach.

Uthayakumar H, Radhakrishnan P, Shanmugam K, Kushwaha OS.

Environ Sci Pollut Res Int. 2022 Jan 18. PMID: 35041160

The main objective of the present study is to develop artificial neural networks (ANN) to predict the adsorption efficiency of multi-walled carbon nanotubes (MWCNTs) on Cr(VI) removal. Polydisperse MWCNTs were synthesized at 750 °C on alumina supported Fe-Co-Mo catalyst using CVD (chemical vapor deposition)-assisted spray pyrolysis of *Azadirachta indica* (Neem) oil under inert Argon (Ar) atmosphere. Growth of MWCNTs with inner diameters between 9 and 14 nm was corroborated by scanning electron microscopy (SEM), high resolution transmission electron microscopy (HRTEM), X-ray diffraction analysis (XRD), and Raman spectral evidence assessments. The metal-ion adsorbent capacity (Cr-VI) of the as such prepared MWCNTs was examined for industrial purposes. Different parameters such as adsorption isotherms, kinetics, and thermodynamic parameters were analyzed for the removal of metal ions with MWCNTs. The results of isotherm, kinetic, and thermodynamic study indicated that the process suited well with Langmuir isotherm, pseudo

second-order kinetics, and followed endothermic reaction, respectively. The effects of parameters such as adsorbent dosage, concentration of chromium ion (Cr-VI), pH, and contact time were studied to optimize the maximum removal of Cr(VI). In order to optimize the process conditions using Artificial Neural Networks, Box-Behnken design (BBD) was used to design the batch adsorption experiments, and the resulting datasets were used as the input for ANN. To predict the adsorption efficiency, various ANN architectures were examined using different training algorithms, number of neurons in the hidden layer, and the transfer function for the hidden and output layers. A neural network structure with Levenberg-Marquardt (LM) training algorithm, 14 hidden neurons, and tangent sigmoid transfer function at the hidden layer and logarithmic sigmoid transfer function at the output layer furnished the best level of prediction results. Comparing with experimental data, the optimal model capitulated mean square error (MSE), and correlation coefficient (R^2) of 0.0324 and 0.99512, respectively. The results showed that ANN is well-organized in predicting the adsorption efficiency of MWCNTs for Cr(VI) metal ion removal process.

Remediation of petroleum hydrocarbon contaminated soil using hydrocarbonoclastic rhizobacteria applied through *Azadirachta indica* rhizosphere.

Bhuyan B, Pandey P.

Int J Phytoremediation. 2022 Feb 3:1-11. PMID: 35113751

Crude oil/petroleum hydrocarbons (PHs) are major pollutants worldwide. In the present study, three bacterial isolates -*Pseudomonas aeruginosa* BB-BE3, *P. aeruginosa* BBBJ, and *Gordonia amicalis* BB-DAC were selected for their efficient hydrocarbon degradation and plant growth promotion (PGP) abilities. All three isolates were positive for siderophore production, phosphate solubilization, and IAA production, even in the presence of crude oil. The rhizoremediation ability was validated through pot trials where all three isolates promoted the growth of the *Azadirachta indica* plant in crude oil-contaminated soils. Treatment with the combination of the plant (*A. indica*) and bacteria, i.e., *Pseudomonas aeruginosa* BB-BE3; *P. aeruginosa* BBBJ; *Gordonia amicalis* BB-DAC showed 95.71, 93.28, and 89.88% removal of TPHs respectively, while the treatment with the plant (only) resulted in 13.44% removal of TPHs whereas, in the control (Sterile bulk soil + Crude oil), the hydrocarbon removal percentage was only 5.87%. The plant tissues were analyzed for catalase (CAT) and peroxidase (POX) activities, and the plants augmented with bacterial strains had significantly low CAT and POX activities as compared to uninoculated control. Therefore, the results suggest that the *A. indica* plant, in symbiotic association with these hydrocarbonoclastic rhizobacteria, could be used for bioremediation of crude oil-polluted soil.

Sunlight Photocatalytic Performance of ZnO Nanoparticles Synthesized by Green Chemistry Using Different Botanical Extracts and Zinc Acetate as a Precursor.

López-López J, Tejeda-Ochoa A, López-Beltrán A, Herrera-Ramírez J, Méndez-Herrera P.

Molecules. 2021 Dec 21;27(1):6. PMID: 35011237

In this work, the assessment of *Azadirachta indica*, *Tagetes erecta*, *Chrysanthemum morifolium*, and *Lentinula edodes* extracts as catalysts for the green synthesis of zinc oxide nanoparticles (ZnO NPs) was performed. The photocatalytic properties of ZnO NPs were investigated by the photodegradation of methylene blue (MB) dye under sunlight irradiation. UV-visible (UV-Vis) spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy, Transmission Electron Microscopy (TEM), X-ray Diffraction (XRD), Thermogravimetric (TGA), and Brunauer-Emmett-Teller analysis (BET) were used for the characterization of samples. The XRD results indicate that all synthesized nanoparticles have a hexagonal wurtzite crystalline structure, which was confirmed by TEM. Further, TEM analysis proved the formation of spherical and hemispherical nanoparticles of ZnO with a size in the range of 14-32 nm, which were found in aggregate shape; such a size was well below the size of the particles synthesized with no extract (~43 nm). ZnO NPs produced with *Tagetes erecta* and *Lentinula edodes* showed the best photocatalytic activity, matching with the maximum adsorbed MB molecules (45.41 and 58.73%, respectively). MB was completely degraded in 45 min using *Tagetes erecta* and 120 min using *Lentinula edodes* when subjected to solar irradiation.

Azadirachta indica-wrapped copper oxide nanoparticles as a novel functional material in cardiomyocyte cells: An ecotoxicity assessment on the embryonic development of *Danio rerio*.

Jayakodi S, Shanmugam R, Almutairi BO, Almutairi MH, Mahboob S, Kavipriya MR, Gandusekar R, Nicoletti M, Govindarajan M.

Environ Res. 2022 Mar 24;212(Pt A):113153. PMID: 35341753

This research reports on the production of copper oxide nanoparticles (CuO NPs) through the green synthesis method using *Azadirachta indica* (Ai) flower extract. Synthesized Ai-CuO NPs are characterized by Zeta Potential, TGA, SEM and TEM analysis. The Ai-CuO NPs gave a maximum peak at 270 nm. As per XRD studies, the Ai-CuO NPs obtained were crystalline. FTIR spectrum Ai-CuO NPs showed the presence of functional groups like the O-H group, aromatic group, etc. TEM and SEM assist in investigating the size and morphology of the Ai-CuO NPs, which were spherical and varied in size between 10.11 nm and 17.54 nm. EDAX showed that Ai-CuO NPs were pure with no impurities. The synthesized Ai-CuO NPs were then analyzed for their cytotoxicity at various concentrations (5, 10, 20, 30, 40 and 50 µg/mL) against H9c2 cardiomyocyte cells using MTT assay. DOX-induced H9c2 cell damage of apoptosis and ROS. The nanoparticle formed by Ai-CuO was cured with different concentrations (5, 10 and 20 µg/mL). In zebrafish, 48 hpf and 72 hpf were measured at 75 µM to reduce dysfunction and mortality during organ development. These results can have a beneficial impact on eco-toxicological effects.

Neem- Ethnobotanical Aspects

A Cross-Cultural Analysis of Plant Resources among Five Ethnic Groups in the Western Himalayan Region of Jammu and Kashmir.

Haq SM, Hassan M, Bussmann RW, Calixto ES, Rahman IU, Sakhi S, Ijaz F, Hashem A, Al-Arjani AF, Almutairi KF, Abd Allah EF, Aziz MA, Ali N.

Biology (Basel). 2022 Mar 23;11(4):491. PMID: 35453691

Plant resources have always been valuable in human life, and many plant species are used in medicine, food, and ritual, and resource utilization is closely related to cultural diversity. Our study was conducted from June 2019 to April 2021, during which we aimed to document the local knowledge of plant resources of five ethnic groups, i.e., the Gujjar, Bakarwal, Kashmiri, Pahari, and Dogra communities of the Jammu and Kashmir (J&K) region, Western Himalayas. Through semi-structured interviews (N = 342) and group discussions (N = 38), we collected data on the ethnobotanical uses of plant resources. The data was subjected to hierarchical cluster analysis and ordination techniques (Principal Component Analysis) via, R software of version 4.0.0. Traditional uses were classified into three groups, i.e., single-, double-, and multi-use groups. The study recorded a total of 127 plant species, belonging to 113 genera and distributed among 64 botanical families. The dominant plant families were the Asteraceae, with 8% of all species, followed by Lamiaceae (6%), Polygonaceae (5%) and Ranunculaceae (4%). The recorded plant taxa were frequently used for medicine (51.4% responses), followed by food (14.9%), and fodder (9.5%). Principal component analysis (PCA) separated three groups of provisioning services depending on plant consumption preference levels. Comparative analysis showed remarkable similarities in plant uses (food, medicinal) among the Gujjar and Bakarwal ethnic groups, as both groups share a common culture. Some plants like *Azadirachta indica*, *Brassica campestris*, *Ulmus wallichiana*, *Amaranthus blitum*, and *Celtis australis* were also used for magico-religious purposes. We also recorded some medicinal uses that are new to the ethnobotanical literature of the J&K Himalayas, such as for *Betula utilis*, *Sambucus wightiana*, and *Dolomiaea macrocephala*, in our case for example local medicinal recipe, which is derived from *Dolomiaea macrocephala*, often known as *Nashasta*, used to treat weakness, back pain, and joint pain. Similarly, we also recorded new food uses for *Eremurus himalaicus*. Moreover, we also observed some plants for instance, *Fragaria nubicola*, *Betula utilis* and *Juniperus communis* have spiritual significance (i.e., amulets and scrolls) for this part of the Himalayan region. The present study provides a useful tool for resource management and can help in developing scientifically informed strategies for the conservation of plant resources.

Ethnobotanical and conservation studies of tree flora of Shivalik mountainous range of District Bhimber Azad Jammu and Kashmir, Pakistan.

Khanum H, Ishtiaq M, Bhatti KH, Hussain I, Azeem M, Maqbool M, Hussain T, Mushtaq W, Thind S, Bashir R, Muzamil M, Abdel-Hafez SH, Sayed S.

PLoS

One. 2022 Feb 7;17(2):e0262338. PMID: 35130268

The present study was carried out to explore and document traditional ethnobotanical knowledge of indigenous rural communities of Shivalik mountainous range regarding tree flora of the area, District Bhimber of Azad Jammu and Kashmir, Pakistan. The local people of the area primarily depend on wild flora for life sustenance and cure of different infirmities. In this research, data was collected through visual appraisal and participatory rural approach using questionnaire method by applying semi-structured and structured-interview protocols (S4 Table). To validate and explore novelty of research work, various quantitative ethnobotanical indices like informant consensus factor, use value index, fidelity level, relative frequency of citation, relative importance of plants, rank order of priority, Jaccard index and priority ranking were used. The highest ICF was found for jaundice with 0.91 value followed by ICF values of 0.89, 0.86 and 0.85 for wounds, skin diseases and stomach pains, respectively. Fidelity level predicted that *Azadirachta indica* (Indian lilac tree) ranked first (100%) for its prevalent use in ethnomedicines. Highest use value index (0.61) was found for *Azadirachta indica* while the highest relative frequency of citation (55) was measured for *Melia azedarach*. The relative order of priority index was the highest for *Azadirachta indica* and *Acacia arabica* L. (ROP = 100) depicting their prevalent use in ethnomedicines. For EB study, the highest relative importance (50.8) was found for *Azadirachta indica* with good number of agro-uses and its seeds and bark are sold or marketed in local markets to prepare herbal therapeutics by indigenous practitioners. The Jaccard index depicted that many traditional ethnomedicinal uses of prescribed trees were novel in recipe form or first time reported such as *Senegalia modesta* (Phulai) resin was used to prepare 'Panjoori' a local tonic prepared from cow or buffalo butter mixing with seeds of *Papaver somnifera* (Kashkhash) and *Prunus amygdalus* (Badaam). The wood of many trees like *Pinus roxburghii* (pine), *Dalbergia sissoo* (rosewood), *Senegalia modesta* (Phulai), *Acacia Arabica* (kikar), *Bombax cieba* (cotton tree) is commercially sold as timber in markets (S3 Table). The research proved that population explosion and climate changes have triggered severe biotic and abiotic pressure on tree flora of the study area of Bhimber, AJK. The research describes that plants like *Terminalia belerica* (belerica), *Terminalia arjuna* (arjun tree), *Cassia fistula* (Indian laburnum), *Butea monosperma* (bastard teak), *Phyllanthus emblica* (Indian gooseberry), *Morus laevigata* (Shah toot), *Bauhinia variegata* (orchid tree) and *Flacourtia indica* (Indian plum/ kakoo) are threatened species and their population is highly reduced and if reclamation measures for their conservation are not taken, it may lead towards their complete loss from the area. This research recommends sustainable ethnobotanical use of tree flora, their growth and conservation for green and ecofriendly environment for safe and secure future of human generation.

Ethnobotanical study of Mandi Ahmad Abad, District Okara, Pakistan
Munir M, Sadia S, Khan A, Rahim BZ, Gagosh Nayyar B, Ahmad KS, Khan AM, Fatima I, Qureshi R.

PLoS One. 2022 Apr 7;17(4):e0265125. PMID: 35389994

This study hypothesized that native people have unique traditional knowledge of plant resources in the rural areas and basic objective was the documentation of this valuable inheritance. Ethnobotanical data was collected from a remote rural area of Mandi Ahmad Abad, Union council number NA-144 Tehsil Depalpur District Okara, Pakistan. A total of 94 informants were randomly interviewed to collect data about local names of plant species, mode of administration, recipes and ailments, and ethnobotanical uses through semi-structured questionnaire, interviews and group discussion methods. The collected data was statistically analyzed by calculating use value (UV), frequency of citation (FC), relative frequency of citation (RFC), factor of informant consensus (FIC), family importance value, and relative importance (RI). This study is also compared with ethnobotanical literature by using Jaccard's index (JI) for similarity analysis. A total of 126 species belonging to 52 families were documented. The Poaceae (13spp.), Leguminosae (12spp.), Solanaceae (10spp.) and Cucurbitaceae (10spp.) were dominant families. Highest used value (UV = 0.22) was obtained for *Azadirachta indica*. The minimum used value (UV) was showed by *Alhagi maurorum*, *Eclipta prostrata*, *Hibiscus rosa-sinensis*, *Solanum virginianum* and *Trianthema potulacastrum* (UV = 0.01). Hepatitis, stomach ulcer, bowel disorders, urinary problems, psoriasis, cancer, and leucoderma were the most treated ailments with ICF value of 1, followed by leucorrhea (ICF: 0.89), and vomiting (ICF: 0.86). The highest Jaccard's similarity index value (JI = 0.329) showed that plant species reported in our study was more similar with Arid regions of Northern Punjab, Pakistan. This novel ethnobotanical report concluded that traditional knowledge about use of medicinal plants is decreasing due to allopathic medicines. Immediate steps should be taken for conservation and documentation of traditional knowledge of plants especially those having medicinal properties.

Neem- Ethnopharmacological Aspects

Herbal medicine used for the treatment of diarrhea and cough in Kampala city, Uganda.
Walusansa A, Asiimwe S, Ssenku JE, Anywar G, Namara M, Nakavuma JL, Kakudidi EK.
Trop Med Health. 2022 Jan 7;50(1):5. PMID: 34991719

Background: Globally, diarrheal and respiratory diseases are among the main causes of mortality and morbidity. In Uganda, cities are facing proliferation of trade in herbal medicines (HM), including those for diarrhea and/or cough. Information on the economic, and the ethnopharmacological aspects of these HM is scarce, deterring the sector from achieving optimal capacity to support national development. We profiled the anti-diarrhea and/or anti-cough HM, and the basic economic aspects of HM trade in Kampala city, to support ethnopharmacological knowledge conservation and strategic planning. **Methods:** A cross-sectional survey was conducted on 65 herbalists using semi-structured questionnaires. This was supplemented by an observational survey using a high-resolution digital camera. Data were collected following the guidelines for research on HM, established by Uganda National Drug Authority, and World Health organization. **Results:** Eighty-four plant species from 41 families were documented. Fabaceae and Myricaceae had the highest number of species (9, 10.7% each). Citrus limon (L.) Osbeck was the most commonly cited for cough, with a relative frequency of citation (RFC) of 1.00, and its relative medical importance was not significantly different from the other top 5 species except for Azadirachta indica A.Juss (RFC = 0.87). Entada abyssinica A. Rich (RFC = 0.97) was the most cited for diarrhea. Trees (34, 40.5%) were mostly used, and mainly harvested from wild habitats (55.2%) in 20 districts across Uganda. These HM were mainly sold as powders and concoctions, in markets, shops, pharmacies, and roadside or mobile stalls. The highest prices were Uganda Shillings (UGX) 48,000 (\$13.15)/kg for Allium sativum L, and UGX 16,000 (\$4.38)/kg for C. limon. All participants used HM trade as a sole source of basic needs; majority (60.0%) earned net monthly profit of UGX. 730,000 (\$200) ≤ 1,460,000 (\$400). The main hindrances to HM trade were the; disruptions caused by the COVID-19 pandemic (n = 65, 100%), and the scarcity of medicinal plants (58, 89.2%).

Conclusion: There is a rich diversity of medicinal plant species traded in Kampala to treat diarrhea and cough. The HM trade significantly contributes to the livelihoods of the traders in Kampala, as well as the different actors along the HM value chain throughout the country.



Safety and Efficacy of Medicinal Plants Used to Manufacture Herbal Products with Regulatory Approval in Uganda: A Cross-Sectional Study.

Kaggwa B, Kyeyune H, Munanura EI, Anywar G, Lutoti S, Aber J, Bagoloire LK, Weisheit A, Tolo CU, Kamba PF, Ogwang PE.

Evid Based Complement Alternat Med. 2022 Apr 13;2022:1304839. PMID: 35463071

Introduction: The Uganda National Drug Authority requires phytochemical screening, freedom from microbial contamination, and evidence of safety and efficacy of the constituent plants to register herbal products. Since Uganda has no pharmacopeia, safety, efficacy, and plant processing information are not readily available. We documented the plant materials used to manufacture products in Uganda and established evidence of their safety and efficacy and availability of monographs. **Methods:** The NDA register of herbal products was reviewed, and a product list was extracted. The herbal products were purchased from local pharmacies, and their labels were studied to identify plant ingredients and drug use. Literature was reviewed to document evidence of the safety and efficacy of the plant materials concerning manufacturer's claims. Also, the WHO and available African Pharmacopeia were searched to establish the availability of the plant monographs. **Results:** Of the 84 NDA-registered local products, only 18 were obtained from the market; 82% were indicated for respiratory tract disorders. Thirty-three plant materials were listed with *Eucalyptus globulus* Labill, being the commonest. Several *in vitro* and *in vivo* studies demonstrate efficacy, thus supporting the use of the selected plant species for empirical treatment as stated on the product label. While most plants were safe, some species such as *Albizia coriaria* Oliv. had dose-dependent toxicities that cannot be predicted in combinations. The WHO, African Pharmacopoeia, and West African Herbal Pharmacopoeia had only 16 plant monographs of the 33 plants of interest. Nevertheless, *Aloe vera* (L.) Burm.f., *Azadirachta indica* A.Juss., *Zingiber officinale* Roscoe, and *Allium sativum* L. monographs were published by all three pharmacopoeias. **Conclusions:** Preclinical evidence of safety and efficacy exists in the literature for most of the plants used to manufacture registered herbal products in Uganda. More specific bioassays and clinical trials are required for the products to provide conclusive evidence of safety and toxicity. Monographs are urgently needed for the Ugandan plants.

Neem For Human Health

Antibacterial & Antifungal Effects

Antibacterial activities of plant leaf extracts against multi-antibiotic resistant *Staphylococcus aureus* associated with skin and soft tissue infections.

Akinduti PA, Emoh-Robinson V, Obamoh-Triumphant HF, Obafemi YD, Banjo TT.

BMC Complement Med Ther. 2022 Feb 21;22(1):47. PMID: 35189869

Background: The antibacterial activities of aqueous leaf extracts of *Moringa oleifera*, *Vernonia amygdalina*, *Azadirachta indica* and *Acalypha wilkesiana* against multidrug resistance (MDR) *Staphylococcus aureus* associated with skin and soft tissue infections were investigated. **Methods:** *Staphylococcus aureus* (n = 183) from the skin and soft tissue infections with evidence of purulent pus, effusions from aspirates, wounds, and otorrhea were biotyped, and evaluated for biofilm production. The phenotypic antibiotic resistance and MDR strains susceptibility to plant leaves extract were determined using disc diffusion and micro-broth dilution assays respectively. The correlation of plant extract bioactive components with inhibitory activities was determined. **Results:** High occurrence rate of *S. aureus* were recorded among infant and adult age groups and 13.2% mild biofilm producers from the wound ($p < 0.05$). Of 60.2% MDR strains with overall significant MARI of more than 0.85 ($p < 0.05$), high resistant rates to linezolid (92.7%; 95% CI:7.27-10.52), ofloxacin (94.2%; 95% CI:6.09-8.15), chloramphenicol (91.2%; 95% CI:6.11-8.32), gentamicin (97.3%; 95% CI:6.20-8.22), ciprofloxacin (92.7%; 95% CI: 5.28-7.99) and vancomycin (86.6%; 95% CI:6.81-9.59) were observed. *Vernonia amygdalina* and *Azadirachta indica* showed significant antimicrobial activity at 100 mg/ml and 75 mg/ml, with low susceptibility of less than 10% to 25 mg/ml, 50 mg/ml, and 75 mg/ml *Moringa oleifera*. Alkaloids, saponin and terpenoids were significant in *Moringa oleifera*, *Acalypha wilkesiana*, *Azadirachta indica* and *Vernonia amygdalina* leaves extracts ($p < 0.05$). High inhibitory concentrations at IC₅₀; 3.23, 3.75 and 4.80 mg/ml ($p = 0.02$, CI: - 0.08 - 11.52) and IC₉₀; 12.9, 7.5, and 9.6 mg/ml ($p = 0.028$, CI: 2.72-23.38) were shown by *Acalypha wilkesiana*, *Vernonia amygdalina* and *Moringa oleifera* respectively. Comparative outcome of the plant extracts showed *Acalypha wilkesiana*, *Vernonia amygdalina* and *Moringa oleifera* to exhibit significant inhibition activities ($p < 0.05$) compared to other extracts. Significant median inhibitory concentration (15.3 mg/ml) of *Azadirachta indica* were observed ($p < 0.01$) and strong associations of phytochemical compounds of *Azadirachta indica* ($\eta = 0.527, p = 0.017$), *Vernonia amygdalina* ($\eta = 0.123, p = 0.032$) and *Acalypha wilkesiana* ($\eta = 0.492, p = 0.012$) with their respective inhibitory values. **Conclusion:** Observed high occurrence rate of skin and soft tissue infections caused by biofilm-producing MDR *S. aureus* requires alternative novel herbal formulations with rich bioactive compounds from *Moringa oleifera*, *Acalypha wilkesiana*, *Azadirachta indica* and *Vernonia amygdalina* as skin therapeutic agents.

In vitro efficacy of *Azadirachta indica* leaf extract against methicillin resistant *Staphylococci* isolated from skin infection.

Naeem S, Siddique AB, Zahoor MK, Muzammil S, Nawaz Z, Waseem M, Yasmin A, Asif Zahoor M.

Pak J Pharm Sci. 2021 Nov;34(6(Supplementary)):2303-2308. PMID: 35039267

In this cross-sectional study, the isolation and identification of Methicillin Resistant *Staphylococcus aureus* (MRSA) and Methicillin Resistant *S. epidermidis* (MRSE) was described from skin infections (n=100). Initial isolation was done by conventional procedures followed by amplification/ sequence analysis of 16S rRNA. Methicillin resistance was determined using cefoxitin discs and resistant isolates were screened for *mec-A* gene followed by Minimum Inhibitory Concentrations (MIC) determination of vancomycin. In second phase, we investigated extract of *Azadirachta indica* leaves using Fourier Transformed Infrared Spectroscopy (FTIR-Spectroscopy) and investigated in vitro activity. Initially, total of 28 *Staphylococci* were identified. 16S rRNA gene sequence confirmed *S. aureus* (22), *S. epidermidis* (3) and *S. saprophyticus* (3) isolates. Cefoxitin discs showed (7/22) MRSA, (3/3) (MRSE) and none of the methicillin resistant *S. saprophyticus*. MRSA and MRSE isolates showed presence of *mec-A* gene. However, all isolates were sensitive to vancomycin MIC (0.5-2µg/mL) and sensitive to Linezolid. FTIR-Spectroscopy of *A. indica* indicated the presence of azadirachtin and nimbolinin. The mean zone of inhibition was measured 14.23±1.37 and 13.66±0.70 against MRSA and MRSE isolates, respectively. Altogether, MRSA and MRSE is significant public health concern. However, vancomycin and linezolid were found effective and extract of *A. indica* showed in vitro effects.

Antibiofilm Activity of *Azadirachta indica* and *Catharanthus roseus* and Their Synergistic Effects in Combination with Antimicrobial Agents against Fluconazole-Resistant *Candida albicans* Strains and MRSA.

Neglo D, Adzaho F, Agbo IA, Arthur R, Sedohia D, Tettey CO, Waikhom SD.

Evid Based Complement Alternat Med. 2022 Mar 21;2022:9373524. PMID: 35356250

The rapid emergence and spread of antimicrobial resistance has become a global public health concern that threatens the effective treatment of infectious diseases. One major approach adopted to overcome antimicrobial resistance is the use of plant extracts individually and/or with combination of antibiotics with plant extracts, which may lead to new ways of treating infectious diseases and essentially representing a potential area for further future investigations. In this study, the antifungal activities of *Azadirachta indica* leaf and *Catharanthus roseus* flower extracts against fluconazole-resistant *Candida albicans* strains (isolated from pregnant women with vulvovaginal candidiasis) and anti-methicillin-resistant *Staphylococcus aureus* (MRSA) were evaluated by agar well diffusion, microdilution, and biofilm inhibition assays. Subsequently, the determination of the combined antimicrobial activity of the individual plant extracts with (fluconazole and voriconazole) and (ampicillin, tetracycline, and streptomycin) against *C. albicans* strains and

MRSA, respectively, was evaluated by checkerboard microdilution assay. Results from the study showed that the antimicrobial activity of the two plant extracts determined by time-kill kinetics was fungistatic with their MICs ranging from 0.1 to 4 mg/mL. Interestingly, all extracts were proved as good biofilm inhibitors of resistant *C. albicans* and MRSA from 10.1 to 98.82%. Their combination interaction with fluconazole, voriconazole, ampicillin, tetracycline, and streptomycin ranged from synergy to antagonism as per the parameters used. Overall, these results showed that *A. indica* leaf and *C. roseus* flower extracts have significant antifungal property. Furthermore, *A. indica* leaf and *C. roseus* flower extracts alone or in combination with fluconazole and voriconazole could provide a promising approach to the management of candidiasis caused by drug-resistant strains as well as their interaction with the antibacterial agents to combat the common infections caused by MRSA.

Anticandidal efficacy on Polyimide based denture resin using Photodynamic therapy, chemical and herbal disinfectants and their effect on surface roughness and hardness.

Tulbah HI.

Photodiagnosis Photodyn Ther. 2022 Apr 22:102874. PMID: 35470125

Aim: The present study aimed to evaluate the anticandidal effectiveness of PDT, DL, Triphala, CHX, and NE and their effects on Ra and the hardness of polyamide denture base colonized with *C. Albicans*. **Material and methods:** A total of 50 thermo-injected polyamide denture resins were constructed and inoculated by the American Type Culture Collection (ATCC) of *C. albicans* in an in-vitro setup. The specimens were arbitrarily allocated into five groups, pre-candida count was measured then subjected to the diverse polyamide denture disinfection methodologies: Group 1: PDT, Group 2: Diode Laser, group 3: Triphala, group 4: 0.12% CHX (Control) and group 5 Neem extract. After disinfection protocol, post-candida count (CFU/ml) was assessed. Surface roughness and surface hardness of polyamide dentures were evaluated and statistical differences in the Ra and Vickers hardness was also assessed. Statistical analysis was performed for CFU/mL (log₁₀) for exposed *C. albicans* by two-way ANOVA and Tukey's multiple test ($p > 0.05$). For normality of the data, Kolmogorov Smirnov test was executed. **Results:** The highest anti-microbial efficacy against Candida colonies was displayed by chemical control group 0.12% CHX (11.39 ± 1.8 CFU/ml). This was comparable to herbal NE (12.45 ± 2.9 CFU/ml) ($p > 0.05$). There was no statistical difference found in the surface hardness values among the disinfected groups. Group 2: DL ($1.32 \pm 0.13 \mu\text{m}$) showed the highest Ra value comparable to group 1: PDT ($1.21 \pm 0.22 \mu\text{m}$) **CONCLUSION:** Polyamide denture base colonized with *C. Albicans* and disinfected with 0.12% Chlorhexidine and Neem extract demonstrated the highest antimicrobial efficacy with decreased surface roughness and no alteration in denture hardness.

Antiviral effects

***In silico* study of the inhibition of SARS-COV-2 viral cell entry by neem tree extracts.**
Shadrack DM, Vuai SAH, Sahini MG, Onoka I.

RSC Adv. 2021 Aug 3;11(43):26524-26533. PMID: 35480004

The outbreak of COVID-19, caused by SARS-COV-2, is responsible for higher mortality and morbidity rates across the globe. Until now, there is no specific treatment of the disease and hospitalized patients are treated according to the symptoms they develop. Efforts to identify drugs and/or vaccines are ongoing processes. Natural products have shown great promise in the treatment of many viral related diseases. In this work, using *in silico* methods, bioactive compounds from the neem tree were investigated for their ability to block viral cell entry as spike RBD-ACE2 inhibitors. Azadirachtin H, quentin and margocin were identified as potential compounds that demonstrated viral cell entry inhibition properties. The structural re-orientation of azadirachtin H was observed as the mechanism for viral cell entry inhibition. These compounds possessed good pharmacodynamic properties. The proposed molecules can serve as a starting point towards developing effective anti-SARS-COV-2 drugs targeting the inhibition of viral cell entry upon further *in vitro* and *in vivo* validation.

Anti-COVID-19 potential of *Azadirachta indica* (Neem) leaf extract.

Eze MO, Ejike CECC, Ifeonu P, Udeinya IJ, Udenigwe CC, Uzoegwu PN.

Sci Afr. 2022 Jul;16:e01184. PMID: 35434432

COVID-19 is caused by infection with the "severe acute respiratory syndrome coronavirus-2" (i.e., SARS-CoV-2). This is an enveloped virus having a positive sense, single-stranded RNA genome; like the two earlier viruses SARS-CoV and the Middle East respiratory syndrome (MERS) virus. COVID-19 is unique in that, in the severe case, it has the propensity to affect multiple organs, leading to multiple organ distress syndrome (MODS), and causing high morbidity and mortality in the extreme case. In addition, comorbidities like age, cardiovascular disease, diabetes and its complications, obesity, are risk factors for severe COVID-19. It turns out that a most plausible, simple, single explanation for this propensity for MODS is the pivotal involvement of the vascular endothelium (VE). This is a consequence of the fact that the VE seamlessly connects all the entire vascular bed in the body, thus linking all the target organs (heart, lungs, kidney, liver, brain) and systems. Infection with SARS-CoV-2 leads to hyper-inflammation yielding uncontrolled production of a mixture of cytokines, chemokines, reactive oxygen species, nitric oxide, oxidative stress, acute phase proteins (e.g., C-reactive protein), and other pro-inflammatory substances. In the extreme case, a cytokine storm is created. Displacement of the virus bound to the VE, and/or inhibition of binding of the virus, would constitute an effective strategy for preventing COVID-19. In this regard, the acetone-water extract of the leaf of the Neem (*Azadirachta indica*) plant has been known to prevent the adherence of malaria parasitized red blood cells (pRBCs) to VE; prevent cytoadherence of cancer cells in metastasis; and prevent HIV from invading target T lymphocytes. We therefore hypothesize that this Neem leaf acetone-water extract will prevent the binding of SARS-CoV-2 to the VE, and therefore be an effective therapeutic formulation against COVID-19. It is therefore advocated herein that this extract

be investigated through rigorous clinical trials for this purpose. It has the advantages of being (i) readily available, and renewable in favor of the populations positioned to benefit from it; (ii) simple to prepare; and (iii) devoid of any detectable toxicity.

Neem and Turmeric in the management of Covid Associated Mucormycosis (CAM) derived through network pharmacology.

Datta S, Sarkar I, Sen G, Sen A.

J Biomol Struct Dyn. 2022 Mar 7:1-14. PMID: 35253616

Mucormycosis or 'Black Fungus' has been known to target immunocompromised individuals even before the emergence of COVID-19. Nevertheless, the present circumstances provide the best opening for Covid Associated Mucormycosis (CAM), as the global pandemic is engulfing a large part of human population making them immunocompromised. This drastic increase in Mucormycosis infections has to be addressed as early as possible. There is a growing tendency of relying upon herbal drugs that have minimal side effects and does not compromise our immune system. Recently, the concept of network pharmacology has grabbed the attention of modern science, especially advanced medical sciences. This is a new discipline that can use computational power to systematically catalogue the molecular interactions between botanical formulations and the human body. In this study, Neem and Turmeric was considered as the target plants and an attempt was made to reveal various aspects through which phytocompounds derived from them may effectively manage CAM menace. We have taken a step-by-step approach for identifying the target proteins and ligands associated with Mucormycosis treatment. Functional network analysis and Molecular docking approaches were applied to validate our findings. Quercetin derived from both Neem and Turmeric was found to be one of the main phytocompounds working against Mucormycosis. Along with that, Caffeic acid, Curcumin, Kaempferol, Tetrahydrocurcumin and Myricetin also play a pivotal role in fighting against Black-Fungus. A thorough analysis of our result suggested a triple-front attack on the fungal pathogens and the approaches are necrosis inhibition, iron chelation and immuno-boosting.

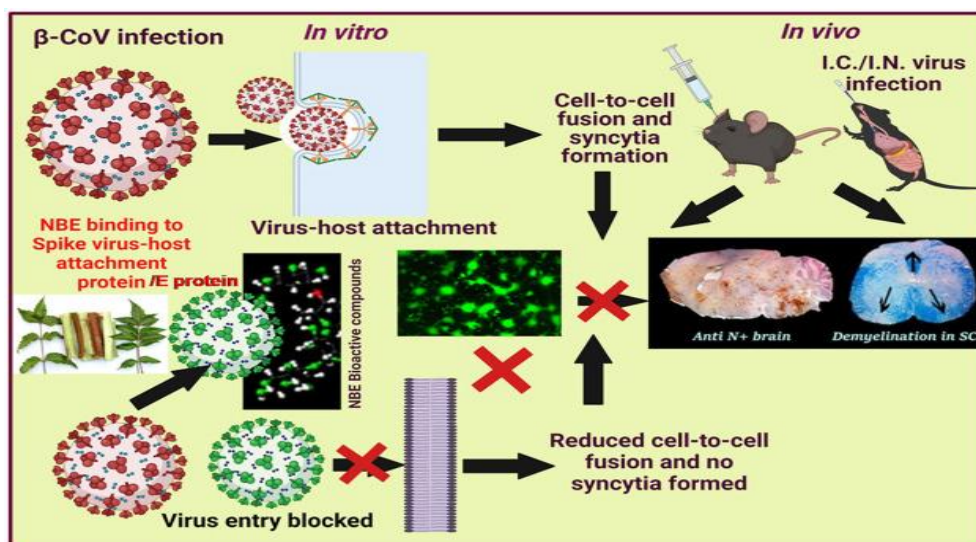
Azadirachta indica A. Juss bark extract and its Nimbin isomers restrict β -coronaviral infection and replication.

Sarkar L, Oko L, Gupta S, Bubak AN, Das B, Gupta P, Safiriyu AA, Singhal C, Neogi U, Bloom D, Banerjee A, Mahalingam R, Cohrs RJ, Koval M, Shindler KS, Pal D, Nagel M, Sarma JD.

Virology. 2022 Feb 15;569:13-28. PMID: 35219218

Emerging mutations in the SARS-CoV-2 genome pose a challenge for vaccine development and antiviral therapy. The antiviral efficacy of Azadirachta indica bark extract (NBE) was assessed against SARS-CoV-2 and m-CoV-RSA59 infection. Effects of in vivo intranasal or oral NBE administration on viral load, inflammatory response, and histopathological changes were assessed in m-CoV-RSA59-infection. NBE administered inhibits SARS-CoV-2 and m-CoV-RSA59 infection and replication in vitro, reducing Envelope and Nucleocapsid gene

expression. NBE ameliorates neuroinflammation and hepatitis in vivo by restricting viral replication and spread. Isolated fractions of NBE enriched in Nimbin isomers shows potent inhibition of m-CoV-RSA59 infection in vitro. In silico studies revealed that NBE could target Spike and RdRp of m-CoV and SARS-CoV-2 with high affinity. NBE has a triterpenoids origin that may allow them to competitively target panoply of viral proteins to inhibit mouse and different strains of human coronavirus infections, suggesting its potential as an antiviral against pan- β -Coronaviruses.



Anti-COVID-19 potential of *Azadirachta indica* (Neem) leaf extract.

Eze MO, Ejike CECC, Ifeonu P, Udeinya IJ, Udenigwe CC, Uzoegwu PN.

Sci Afr. 2022 Jul;16:e01184. PMID: 35434432

COVID-19 is caused by infection with the "severe acute respiratory syndrome coronavirus-2" (i.e., SARS-CoV-2). This is an enveloped virus having a positive sense, single-stranded RNA genome; like the two earlier viruses SARS-CoV and the Middle East respiratory syndrome (MERS) virus. COVID-19 is unique in that, in the severe case, it has the propensity to affect multiple organs, leading to multiple organ distress syndrome (MODS), and causing high morbidity and mortality in the extreme case. In addition, comorbidities like age, cardiovascular disease, diabetes and its complications, obesity, are risk factors for severe COVID-19. It turns out that a most plausible, simple, single explanation for this propensity for MODS is the pivotal involvement of the vascular endothelium (VE). This is a consequence of the fact that the VE seamlessly connects all the entire vascular bed in the body, thus linking all the target organs (heart, lungs, kidney, liver, brain) and systems. Infection with SARS-CoV-2 leads to hyper-inflammation yielding uncontrolled production of a mixture of cytokines, chemokines, reactive oxygen species, nitric oxide, oxidative stress, acute phase proteins (e.g., C-reactive protein), and other pro-inflammatory substances. In the extreme case, a cytokine storm is created. Displacement of the virus bound to the VE, and/or inhibition of binding of the virus, would constitute an effective strategy for preventing COVID-19. In this regard, the acetone-water extract of the leaf of the Neem (*Azadirachta indica*) plant has been known to prevent the adherence of malaria parasitized red blood cells

(pRBCs) to VE; prevent cytoadherence of cancer cells in metastasis; and prevent HIV from invading target T lymphocytes. We therefore hypothesize that this Neem leaf acetone-water extract will prevent the binding of SARS-CoV-2 to the VE, and therefore be an effective therapeutic formulation against COVID-19. It is therefore advocated herein that this extract be investigated through rigorous clinical trials for this purpose. It has the advantages of being (i) readily available, and renewable in favor of the populations positioned to benefit from it; (ii) simple to prepare; and (iii) devoid of any detectable toxicity.

Anti-malarial Effects

Formulation development of Azadirachta indica extract as nanosuppositories improves its intrarectal delivery for the treatment of malaria.

Okeke TC, Umeyor CE, Nzekwe IT, Umeyor IC, Nebolisa NM, Uronnachi EM, Nwakile CD, Ekweogu CA, Aziakpono OM, Attama AA.

Recent Adv Drug Deliv Formul. 2022 Apr 26. PMID: 35473532

Background: Previous folkloric and experimental reports have demonstrated the antimalarial efficacy of Azadirachta indica (AZA) extracts. However, one of the major challenges facing its application for the clinical treatment of malaria is the design of an acceptable dosage form. **Objective:** Consequently, we developed AZA extract-loaded nanostructured lipid carriers (NLC) for the formulation of suppositories, denoted as nanosuppositories, for intrarectal treatment of malaria. **Methods:** Various batches of NLC-bearing AZA extract were formulated based on lipid matrices prepared using graded concentrations of Softisan®154 and Tetracarpidium conophorum or walnut oil. NLC was investigated by size, and differential scanning calorimetry (DSC). Suppository bearing AZA extract-loaded NLC was developed using cocoa butter or theobroma oil, and their physicochemical properties were profiled. In vitro drug release and in vivo antimalarial (using Plasmodium berghei-infected mice) evaluation were investigated. **Results:** NLCs had sizes in nanometer scale ranging from 329.5 - 806.0 nm, and were amorphized as shown by DSC thermograms. Nanosuppositories were torpedo- or bullet- shaped, weighed 138 - 368 mg, softened/liquefied between 4.10 - 6.92 min, and had controlled release behaviour. In vivo antimalarial study revealed excellent antimalarial efficacy of the nanosuppositories comparable with a commercial brand (Plasmotrim®) and better than the placebo (unloaded nanosuppository), and without toxic alterations of hepatic and renal biochemical factors. **Conclusion:** Thus, AZA extract could be rationally loaded in nanostructured lipid carriers (NLC) for further development as nanosuppositories and deployed as an effective alternative with optimum convenience for intrarectal treatment of malaria.

Orodonal Protection

Soft Denture liner and microbial disinfection with contemporary and conventional agents.

AlHamdan EM.

Photodiagnosis Photodyn Ther. 2022 Feb 18;38:102768. PMID: 35183780

Aim: The present study aimed to assess the effect of PDT, herbal and chemical disinfectants on the dental pathogen adherence to soft denture liner colonized with E. coli, C. Albicans, S.aureus, and S. mutans. **Material and methods:** A total of 20 samples of soft-lined removable acrylic complete dentures were fabricated and subjected to the American Type Culture Collection (ATCC) for the inoculation of E.coli, C.albicans, S.mutans and S.aureus in an in-vitro setup. The samples were then randomly divided into four groups and immersed in group 1: 5 µm of Rose Bengal (RB), group 2: Neem extract, group 3: Tea tree oil (TTO), and group 4: 0.12% CHX solutions respectively. Statistical analysis was accomplished by the SPSS 10 statistical software for Windows at a significance level $p < 0.05$. Two-way ANOVA and Tukey's multiple comparison test were used to analyze the data and compare the means and standard deviation values of CFU/mL (log10) for exposed E. coli, C. albicans, S aureus, and S. mutans. **Results:** Intragroup comparison indicated E.coli and C.albicans did not unveil a decrease in reduction CFU/mL (log10) when SDL irradiated with RB 5 µm. Whereas, CFU/mL (log10) values of S.aureus; 2.62 ± 0.68 CFU/mL and S.mutans: 3.41 ± 0.13 CFU/mL plunged significantly ($p < 0.05$). Likewise, S. aureus and S. mutans CFU/mL (log10) strain values display reduction when treated with all four disinfectants while the count of C.albicans unveiled a significant plunge with CHX, TTO and neem extract except RB ($p < 0.05$) **CONCLUSION:** 0.12% CHX and TTO have enhanced antimicrobial efficacy in reducing adhered bacterial colonies of E.coli, C.albicans, S.aureus, and S.mutans (CFU)/mL on a denture soft liner.

Anti-Inflammatory & Anti-Allergic Effects

Natural Anti-Inflammatory and Anti-Allergy Agents: Herbs and Botanical Ingredients.

Saini R, Dhiman NK.

Antiinflamm Antiallergy Agents Med Chem. 2022 Apr 11. PMID: 35410623

Allergies have known to be an abnormally vigorous immune response in which the immune system fights off an allergen or antigen initiating mast cells to release histamine into the blood. Substances which prevent mast cells from releasing histamine are considered as antiallergic agents. The drugs utilized for the treatment of allergy are mast cell stabilizers, steroids, anti-histamine, leukotriene receptor antagonists, and decongestants. Anti-histamine drugs have side effects such as drowsiness, confusion, constipation, difficulty urinating, blurred vision, etc. The use of medicinal plants for effective and safe management of diseases has recently received much attention. Various herbs are utilized for their antiallergic and anti-histaminic properties. Some of the herbs useful in the management of allergic diseases of the respiratory tract like Piper longum, Ocimum tenuiflorum, Solanum

xanthocarpum have been discussed. Ample scientific evidence is available for the anti-histaminic and antiallergic activity of *Azadirachta indica*, *Aloe vera*, *Tinospora cordifolia*, and a number of other such herbs which are safer to use as antiallergic agents have been reported. The review summarizes a wide variety of herbs and botanical ingredients with their common scientific names and distribution for their easy identification and usage as safe antiallergic agents, also discussing their molecular mechanisms involved in combating allergic reactions.

Dermatological Applications

An Insight into the Dermatological Applications of Neem: A Review on Traditional and Modern Aspect.

Singh V, Roy M, Garg N, Kumar A, Arora S, Malik DS.

Recent Adv Antiinfect Drug Discov. 2021;16(2):94-121. **PMID: 34961431**

Background: Dermatological disorders are cutaneous infirmities which are frequently occurring and increasing at an alarming rate. These range from mild itching/redness (dermatitis) to fatal skin cancers and has posed a major health concern. *Azadirachta indica* A. Juss (commonly known as neem), a member of Meliaceae family, is an Indian medicinal plant which has been known for its health promoting effects since ancient times.

Objective: The review highlights the traditional practices, pharmacological aspects, and formulatory approach of neem for the treatment of dermatological disorders. Further, recent patents and novel delivery systems (developed and in pipeline) improving skin delivery and therapeutic profile of neem are discussed. **Results:** Neem is a traditional medicinal plant that has been employed for the prevention and treatment of numerous ailments covering systemic and topical disorders. Scientific studies have validated the traditional claims of neem and attributed these health benefits to the presence of more than 300 structurally diverse and complex compounds. It possesses anti-inflammatory, antibacterial, analgesic, antiviral, antifungal, immunomodulatory and antioxidant activities which substantiate its use as skin therapy. Various novel formulations and associated patents that improved the permeability of neem based products across skin could be found in literature.

Conclusion: Critical appraisal of available literature revealed that neem possesses anti-microbial, anti-inflammatory, antioxidant and antiseptic properties. Thus it has the potential to be developed as a single effective therapy for the management of multimodal skin disorders. Further, pharmaceutical tailoring of neem by implication of novel carriers could enhance its penetrability across skin.



Dermatological aspects of neem.

Neem in Dermatology: Shedding Light on the Traditional Panacea.

Gopinath H, Karthikeyan K.

Indian J Dermatol. 2021 Nov-Dec;66(6):706. PMID: 35283494

The neem tree is commonly used as a traditional panacea for skin ailments in India. For thousands of years, the neem tree has been used as a shield against both agricultural pests and cutaneous infections or infestations. The persistent popularity of neem in skin, hair, and dental care is reflected in the wide range of neem-based personal care products in the Indian market. Indian dermatologists face neem in diverse situations: from neonates placed on a bed of neem leaves to octogenarians who rely on neem chew-sticks for oral hygiene. The traditional uses of neem are being re-explored. Commercial neem formulations are being used as an alternative to synthetic pesticides in several countries. A foreign patent on the antifungal properties of neem sparked India's landmark “biopiracy” battle. We briefly review the relevance of the neem tree in Indian dermatology practice.



Anti-depressant Effects

Anxiolytic and antidepressant-like activities of aqueous extract of *Azadirachta indica* A. Juss. flower in the stressed rats.

Hawiset T, Sriraksa N, Kamsrijai U, Wanchai K, Inkaew P.

Heliyon. 2022 Feb 3;8(2):e08881. PMID: 35198760

The aim of this study was to evaluate whether an aqueous extract of *Azadirachta indica* A. Juss. (*A. indica*) flower had anxiolytic and antidepressant-like effects in the stressed rats. Male Wistar rats were randomly allocated to one of two experimental groups: control or stress. The stress groups were received restraint stress for 3 h. The stressed rats were administered a vehicle, diazepam, fluoxetine, and *A. indica* at doses of 250, 500, and 1000 mg/kg BW for 30 days. The elevated plus-maze test (EPMT), the forced swimming test (FST), and the open field test (OFT) were used to assess anxiolytic and antidepressant-like behaviors. In the EPMT, the percentage of the number of open arm entries and the duration spent in open arms were measured. These measurements were considerably enhanced in the stressed rats treated with diazepam and *A. indica* flower extract at a dose of 500 mg/kg BW. Furthermore, the stressed rats given fluoxetine and *A. indica* flower extract at all doses employed in this study showed a significant reduction in the amount of time the rats were immobilized in the FST. However, there was no significant difference in spontaneous locomotor activity between any of the groups. Additionally, the stressed rats treated with either positive control medications or *A. indica* flower extract exhibited significantly higher brain dopamine (DA) and serotonin (5-HT) levels, but lower blood cortisol levels as compared to the stressed rats treated with vehicle. Moreover, *A. indica* flower extract had no harmful effect on the stressed rats' liver tissue.

Antidiabetic Effects

Fabrication, characterization, anti-inflammatory, and anti-diabetic activity of silver nanoparticles synthesized from *Azadirachta indica* kernel aqueous extract.

Lan Chi NT, Narayanan M, Chinnathambi A, Govindasamy C, Subramani B, Brindhadevi K, Pimpimon T, Pikulkaew S.

Environ Res. 2022 May 15;208:112684. PMID: 34995544

The *Azadirachta indica* is an excellent and pharmaceutically valuable phytochemicals enriched traditional medicinal plant. The purpose of the research was to assess the ability of *A. indica* aqueous kernel extract to synthesize silver nanoparticles as well as their anti-inflammatory and anti-diabetic activity in vitro. The obtained results state that the aqueous kernel extract of *A. indica* can fabricate the silver nanoparticles and be confirmed by standard analytical techniques. Under UV-visible spectrophotometer analysis, the absorbance peak was found at 430 nm was related to the surface plasmon resonance of silver nanoparticles. The FTIR (Fourier-transform infrared spectroscopy) analysis revealed that numbers of functional groups belong to the pharmaceutically valuable phytochemicals,

which act as reducing, capping, and stabilizing agent on silver nanoparticles synthesis. The size and shape of the silver nanoparticles were examined as 19.27-22.15 nm and spherical in shape. Interestingly, this kernel fabricated silver nanoparticles possess a reasonable anti-inflammatory (69.77%) and anti-diabetic (73.5%) activity at 100 $\mu\text{g mL}^{-1}$ and these were partially comparable with standards (anti-inflammatory: 81.15%; anti-diabetic: 87.9%). Thus, the aqueous kernel extract fabricated silver nanoparticles can be considered for further in-vivo study to assess the practical possibility to promote as a pharmaceutical agent.

Anticancer Effects

Nimbolide retards T cell lymphoma progression by altering apoptosis, glucose metabolism, pH regulation, and ROS homeostasis.

Jaiswara PK, Kumar A.

Environ Toxicol. 2022 Feb 24. PMID: 35199915

Nimbolide is reported as one of the potential anticancer candidates of the neem tree (*Azadirachta indica* A. Juss). The cytotoxic action of nimbolide has been well reported against a wide number of malignancies, including breast, prostate, lung, liver, and cervix cancers. Interestingly, only a few in vivo studies conducted on B cell lymphoma, glioblastoma, pancreatic cancer, and buccal pouch carcinoma have shown the in vivo antitumor efficacy of nimbolide. Therefore, it is highly needed to examine the in vivo antineoplastic activity of nimbolide on a wide variety of cancers to establish nimbolide as a promising anticancer drug. In the present study, we investigated the tumor retarding action of nimbolide in a murine model of T cell lymphoma. We noticed significantly augmented apoptosis in nimbolide-administered tumor-bearing mice, possibly due to down-regulated expression of Bcl2 and up-regulated expression of p53, cleaved caspase-3, Cyt c, and ROS. The nimbolide treatment-induced ROS production by suppressing the expression of antioxidant regulatory enzymes, namely superoxide dismutase and catalase. In addition, nimbolide administration impaired glycolysis and pH homeostasis with concomitant inhibition of crucial glycolysis and pH regulatory molecules such as GLUT3, LDHA, MCT1, and V-ATPase, CAIX and NHE1, respectively. Taken together, the present investigation provides novel insights into molecular mechanisms of nimbolide inhibited T cell lymphoma progression and directs the utility of nimbolide as a potential anticancer therapeutic drug for the treatment of T cell lymphoma.

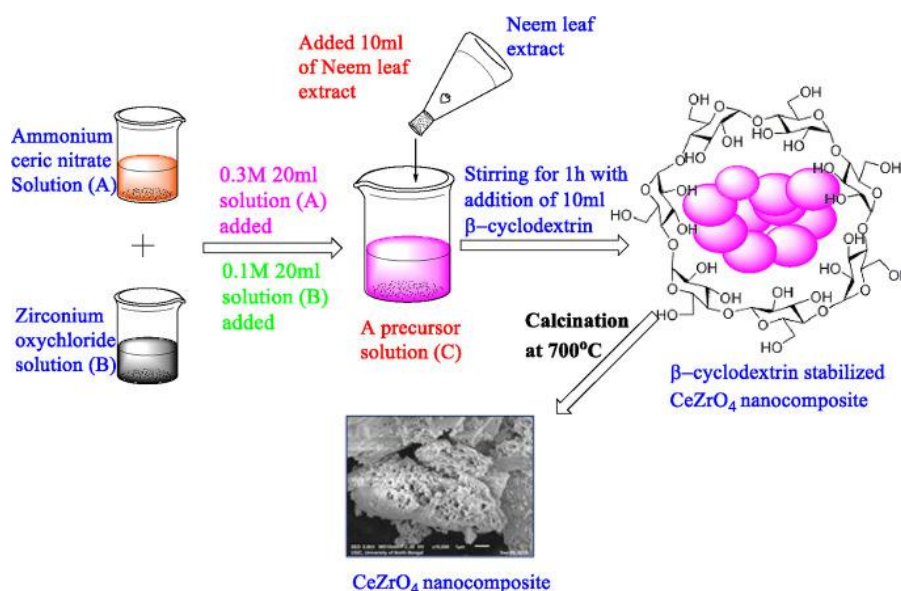
β -Cyclodextrin-Stabilized Biosynthesis Nanozyme for Dual Enzyme Mimicking and Fenton Reaction with a High Potential Anticancer Agent.

Ali S, Sikdar S, Basak S, Rajbanshi B, Mondal M, Roy D, Dutta A, Kumar A, Dakua VK, Chakrabarty R, Roy A, Barman A, Datta A, Roy PK, Chakraborty B, Roy MN.

ACS Omega. 2022 Jan 28;7(5):4457-4470. PMID: 35155938

The myth of inactivity of inorganic materials in a biological system breaks down by the discovery of nanozymes. From this time, the nanozyme has attracted huge attention for its high durability, cost-effective production, and easy storage over the natural enzyme. Moreover, the multienzyme-mimicking activity of nanozymes can regulate the level of reactive oxygen species (ROS) in an intercellular system. ROS can be generated by

peroxidase (POD), oxidase (OD), and Fenton-like catalytic reaction by a nanozyme which kills the cancer cells by oxidative stress; therefore, it is important in CDT (chemo dynamic therapy). Our current study designed to investigate the enzyme mimicking behavior and anticancer ability of cerium-based nanomaterials because the cerium-based materials offer a high redox ability while maintaining nontoxicity and high stability. Our group synthesized CeZrO₄ nanoparticles by a green method using β-cyclodextrin as a stabilizer and neem leaf extract as a reducing agent, exhibiting POD- and OD-like dual enzyme activities. The best enzyme catalytic activity is shown in pH = 4, indicating the high ROS generation in an acidic medium (tumor microenvironment) which is also supported by the Fenton-like behavior of CeZrO₄ nanoparticles. Inspired by the high ROS generation in vitro method, we investigated the disruption of human kidney cells by this nanoparticle, successfully verified by the MTT assay. The harmful effect of ROS in a normal cell is also investigated by the in vitro MTT assay. The results suggested that the appreciable anticancer activity with minimal side effects by this synthesized nanomaterial.



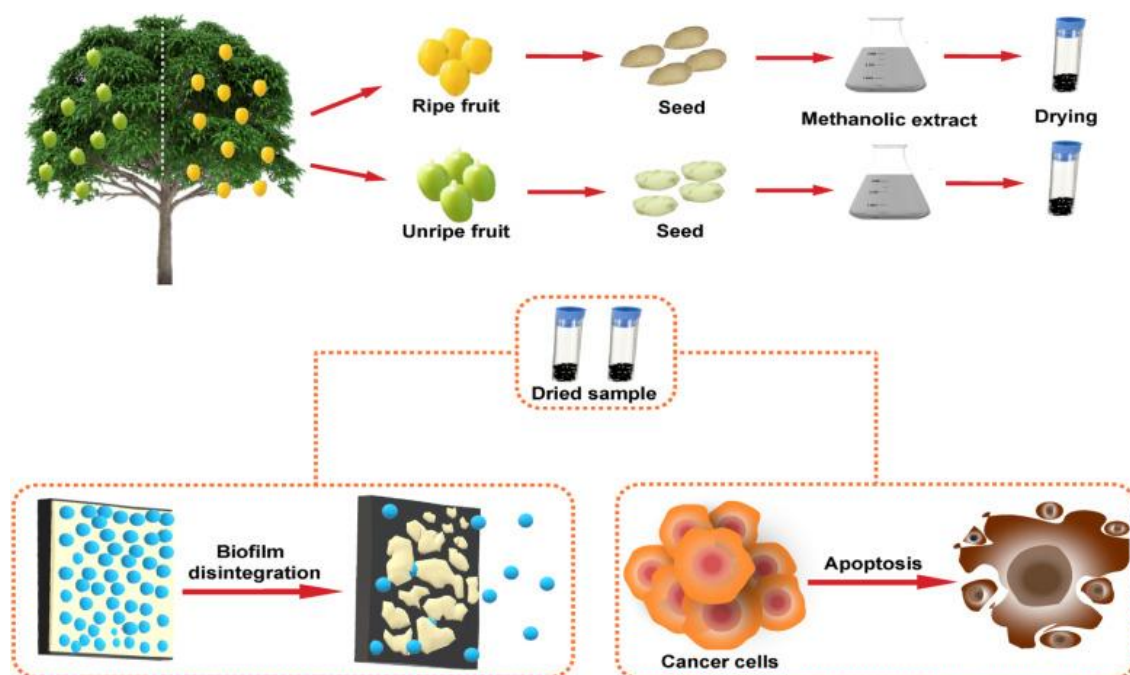
Antibiofilm and anticancer activities of unripe and ripe *Azadirachta indica* (neem) seed extracts.

Guchhait KC, Manna T, Barai M, Karmakar M, Nandi SK, Jana D, Dey A, Panda S, Raul P, Patra A, Bhattacharya R, Chatterjee S, Panda AK, Ghosh C.

BMC Complement Med Ther. 2022 Feb 14;22(1):42. PMID: 35152903

Background: Antibiotic resistances of pathogens and breast cancer warrant the search for new alternative strategies. Phytoextracts can eradicate microbe-borne diseases as well as cancer with lower side effects compared to conventional antibiotics. **Aim:** Unripe and ripe *Azadirachta indica* (neem) seed extracts were explored as potential antibiofilm and anticancer agents in combating multidrug-resistant infectious bacteria as well as anticancer agents against the MDR breast cancer cell lines. **Methods:** Shed-dried neem seeds (both unripe and ripe) were pulverized and extracted using methanol. The chemical components were identified with FTIR and gas chromatography- mass spectrometry. Antibiofilm activity of neem seed extracts were assessed in terms of minimum biofilm inhibitory concentration (MBIC), minimum biofilm eradication concentration (MBEC), and fluorescence microscopic

studies on *Staphylococcus aureus* and *Vibrio cholerae*. Bacterial cells were studied by fluorescence microscopy using acridine orange/ethidium bromide as the staining agents. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values were evaluated to observe the antibacterial activities. Cytotoxicity of the extracts against human blood lymphocytes and the anticancer activity against drug-resistant breast cancer cell lines were assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and fluorescence-activated cell sorting (FACS) studies. **Results:** 4-Ethyl-2-hydroxy-2-cyclopentene-1-one, phthalic acid, and 2-hexyl-tetrahydro thiophane were the major compounds in unripe neem seed, whereas 3,5-dihydroxy-6-methyl-2,3-dihydro-4-H-pyran-4-one and 4-ethylbenzamide were predominant in ripe neem seed. Triazine derivatives were also common for both the extracts. MBIC values of unripe and ripe neem seed extracts for *S. aureus* are 75 and 100 $\mu\text{g/mL}$, respectively, and for *V. cholerae*, they are 100 and 300 $\mu\text{g/mL}$, respectively. MBEC values of unripe and ripe seed extracts are 500 and 300 $\mu\text{g/mL}$, respectively for *S. aureus* and for *V. cholerae* the values are 700 and 500 $\mu\text{g/mL}$, respectively. Fluorescence microscopic studies at 16 and 24 h, after bacterial culture, demonstrate enhanced antibiofilm activity for the ripe seed extract than that of the unripe seeds for both the bacteria. MTT assay reveals lower cytotoxicity of both the extracts towards normal blood lymphocytes, and anticancer activity against breast cancer cell line (MDA-MB-231) with superior activity of ripe seed extract. FACS studies further supported higher anticancer activity for ripe seed extract. **Conclusions:** Methanolic extract of neem seeds could substantially inhibit and eradicate biofilm along with their potent antibacterial and anticancer activities. Both the extracts showed higher antibiofilm and antibacterial activity against *S. aureus* (gram-positive) than *V. cholerae* (gram-negative). Moreover, ripe seed extract showed higher antibiofilm and anticancer activity than unripe extracts.



Nimbolide, a Neem Limonoid, Inhibits Angiogenesis in Breast Cancer by Abrogating Aldose Reductase Mediated IGF-1/PI3K/Akt Signaling.

Nivetha R, Arvindhvv S, Baba AB, Gade DR, Gopal G, K C, Kallamadi KPR, Reddy GB, Nagini S.

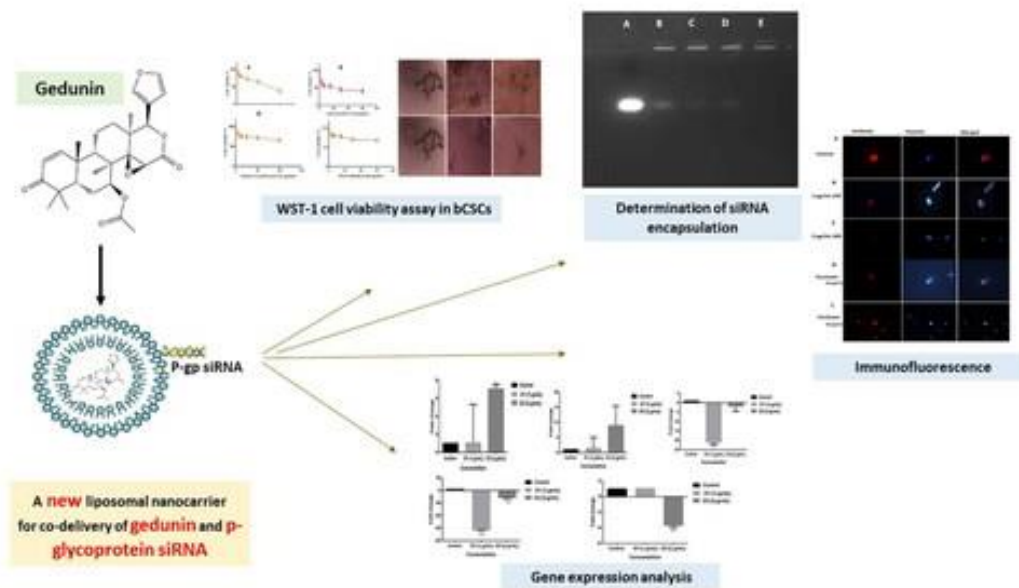
Anticancer Agents Med Chem. 2022 Feb 4. PMID: 35125086

Background & objectives: There is growing evidence to implicate the insulin/IGF-1R/PI3K/Akt signaling cascade in breast cancer development and the central role of aldose reductase (AR) in mediating the crosstalk between this pathway and angiogenesis. The current study was designed to investigate whether nimbolide, a neem limonoid, targets this oncogenic signaling network to prevent angiogenesis in breast cancer. **Methods:** Breast cancer cells (MCF-7, MDA-MB-231), EAhy926 endothelial cells, MDA-MB-231 xenografted nude mice, and tumour tissues from breast cancer patients were used for the study. Expression of AR and key players in IGF-1/PI3K/Akt signaling and angiogenesis was evaluated by qRT-PCR, immunoblotting, and immunohistochemistry. Molecular docking and simulation, overexpression, and knockdown experiments were performed to determine whether nimbolide targets AR and IGF-1R. **Results:** Nimbolide inhibited AR with consequent blockade of the IGF-1/PI3K/Akt and HIF-1 α /VEGF signaling circuit by influencing the phosphorylation and intracellular localisation of key signaling molecules. Downregulation of DNMT-1, HDAC-6, miR-21, HOTAIR, and H19 with upregulation of miR-148a/miR-152 indicated that nimbolide regulates AR and IGF-1/PI3K/Akt signaling via epigenetic modifications. Coadministration of nimbolide with metformin and the chemotherapeutic drugs tamoxifen/cisplatin displayed higher efficacy than single agents in inhibiting IGF-1/PI3K/Akt/AR signaling. Grade-wise increases in IGF-1R and AR expression in breast cancer tissues underscore their value as biomarkers of progression. **Conclusions:** This study provides evidence for the anticancer effects of nimbolide in cellular and mouse models of breast cancer besides providing leads for new drug combinations. It has also opened up avenues for investigating potential molecules such as AR for therapeutic targeting of cancer.

A new liposomal nanocarrier for co-delivery of gedunin and P-glycoprotein siRNA to target breast cancer stem cells.

Rana MS, Ediriweera MK, Rajagopalan U, Karunaratne DN, Tennekoon KH, Samarakoon SR. *Nat Prod Res.* 2022 Jan 27:1-4. PMID: 35084278

Gedunin is a secondary metabolite found in neem tree. Since the first discovery of this compound, its bio-active properties have been continuously evaluated. However, the low hydrophobicity of gedunin decreases its bioavailability and pharmacokinetic profile. In the present investigation, a new liposomal nanocarrier for co-delivery of gedunin and P-glycoprotein (P-gp) siRNA [siRNA coated liposomal gedunin (Lipo-Ged-siRNA)] was developed to improve the anti-proliferative activity of gedunin. Characteristics of prepared Lipo-Ged-siRNA demonstrated promising effects. Lipo-Ged-siRNA showed greater anti-proliferative effects (IC₅₀-8.5 μ g/mL) followed by pure gedunin (IC₅₀- 40.2 μ g/mL) in breast cancer stem cells (bCSCs). Immunofluorescence analysis demonstrated reduced expression of P-gp following exposure to Lipo-Ged-siRNA. Furthermore, Lipo-Ged-siRNA affected the expression of *ABCB1*, *Cyclin D1*, *Bax*, *p53*, and *survivin* genes in bCSCs.



High-throughput, Label-free Proteomics Identifies Salient Proteins and Genes in MDA-MB-231 Cells Treated with Natural Neem-based Electrochemotherapy.

Varadarajan G, Thulasidas JS, Giri P, Camarillo IG, Sundararajan R.

Appl Biochem Biotechnol. 2022 Jan;194(1):148-166. PMID: 34993768

With the absence of the three most common receptor targets, and with high vascularity and higher-grade tumors, triple-negative breast cancer (TNBC) is the most aggressive of all breast cancer subtypes and is in need of additional/alternative/novel treatment strategies. With ~ 15% of the over 2 million new cases each year, there is an unmet need to treat TNBC. MDA-MB-231, human TNBC cells, were treated with neem leaf extract (Neem) and eight, 1200 V/cm, 100 μ s electric pulses (EP), and their viability and proteomic profiles were studied. With EP + Neem, a lower viability of 37% was observed after 24 h, compared to 85% in the neem-only samples, indicating the efficacy of the combinational treatment. The proteomics results indicated significant upregulation of 525 proteins and downregulation of 572 proteins, with a number of different pathways in each case. These include a diverse group of proteins, such as receptors, heat shock proteins, and many others. The upregulated TCA cycle and OXPHOS pathways and the downregulated DNA replication and ubiquitin-mediated proteolytic pathways were associated with effective cell death, demonstrating the potency of this treatment. Viability results reveal the efficacious anticancer effects of the EP + Neem combination, via growth inhibition, on TNBC cells. Proteomics studies could readily identify the effected protein pathways, and their corresponding genes, that are responsible for cell death. This represents a potential therapeutic strategy against TNBC when patients are refractory to standard treatments.

NLGP regulates RGS5-TGF β axis to promote pericyte-dependent vascular normalization during restricted tumor growth.

Dasgupta S, Saha A, Ganguly N, Bhuniya A, Dhar S, Guha I, Ghosh T, Sarkar A, Ghosh S, Roy K, Das T, Banerjee S, Pal C, Baral R, Bose A.

FASEB J. 2022 May;36(5):e22268. PMID: 35363396

Altered RGS5-associated intracellular pericyte signaling and its abnormal crosstalk with endothelial cells (ECs) result chaotic tumor-vasculature, prevent effective drug delivery, promote immune-evasion and many more to ensure ultimate tumor progression. Moreover, the frequency of lethal-RGS5^{high} pericytes within tumor was found to increase with disease progression, which signifies the presence of altered cell death pathway within tumor microenvironment (TME). In this study, we checked whether and how neem leaf glycoprotein (NLGP)-immunotherapy-mediated tumor growth restriction is associated with modification of pericytes' signaling, functions and its interaction with ECs. Analysis of pericytes isolated from tumors of NLGP treated mice suggested that NLGP treatment promotes apoptosis of NG2⁺ RGS5^{high} -functionally altered pericytes by downregulating intra-tumoral TGF β , along with maintenance of more matured RGS5^{neg} pericytes. NLGP-mediated inhibition of TGF β within TME rescues binding of RGS5 with Gai and thereby termination of PI3K-AKT mediated survival signaling by downregulating Bcl2 and initiating pJNK mediated apoptosis. Limited availability of TGF β also prevents complex-formation between RGS5 and Smad2 and rapid RGS5 nuclear translocation to mitigate alternate immunoregulatory functions of RGS5^{high} tumor-pericytes. We also observed binding of Ang1 from pericytes with Tie2 on ECs in NLGP-treated tumor, which support re-association of pericytes with endothelium and subsequent vessel stabilization. Furthermore, NLGP-therapy- associated RGS5 deficiency relieved CD4⁺ and CD8⁺ T cells from anergy by regulating 'alternate-APC-like' immunomodulatory characters of tumor-pericytes. Taken together, present study described the mechanisms of NLGP's effectiveness in normalizing tumor-vasculature by chiefly modulating pericyte-biology and EC-pericyte interactions in tumor-host to further strengthen its translational potential as single modality treatment.

Effects of Supplementation with Dried Neem Leaf Extract on Lipid Peroxidation and Antioxidant Enzyme mRNA Expression in the Pectoralis Major Muscle of Broiler Chickens.

Nakamura K, Shishido M, Shimamoto S, Ogawa G, Khandelwal N, Tatsugawa K, Fujita Y, Ohtsuka A, Ijiri D.

J Poult Sci. 2022 Jan 25;59(1):75-80. PMID: 35125915

This study was conducted to evaluate the effects of dietary supplementation of dried neem (*Azadirachta indica*) leaf extract (DNE) on lipid peroxidation and the expression of genes encoding mRNAs in antioxidant enzymes in the pectoralis major muscle of chickens. A total of 24 male broiler chickens (ROSS308) were divided into three groups ($n=8$) at 21 days of age. The control group of chickens was fed a basal diet, and the remaining two groups of chickens were fed a basal diet supplemented with DNE at a concentration of 0.5% or 2.0% until 35 days of age. Growth performance (body weight, weight gain, feed intake, and feed conversion ratio) and tissue weights did not differ among the three groups. The 2.0% DNE-supplemented diet decreased the muscle malondialdehyde content, a marker of lipid peroxidation, and drip loss compared to the control chickens. In addition, the expression of genes encoding mRNAs of antioxidant enzymes (i.e., Cu/Zn-superoxide dismutase, Mn-superoxide dismutase, glutathione peroxidase 7, and catalase) were higher in the pectoralis major muscle of chickens fed the 2.0% DNE-supplemented diet than in the control chickens. Therefore, DNE supplementation increased the expression of genes encoding mRNAs in antioxidant enzymes and reduced lipid peroxidation and drip loss in the pectoralis major muscle of broiler chickens.

Conservative treatments for feline fibroadenomatous changes of the mammary gland.

Marino G, Pugliese M, Pecchia F, Garufi G, Lupo V, Di Giorgio S, Sfacteria A.

Open Vet J. 2021 Oct-Dec;11(4):680-685.

PMID: 35070864

Background: Fibroadenomatous changes (FAC) of the mammary gland in cats represents a benign, progesterone-associated fibroglandular proliferation of one or more mammary glands that may occur in intact female cats at the time of puberty, during pregnancy or pseudopregnancy, or in female and male cats of any age under progestin treatment. Nowadays, the elective treatment of FAC is based on the progesterone antagonist aglepristone. This study aimed to report the treatment of FAC with a combination of drugs designed to preserve mammary gland integrity, even in pregnant cats. **Case description:** Eight sexually intact female cats with FAC showed mammary glands that were symmetrically enlarged and inflamed in all cases, and the skin was ulcerated in six cats. Four cats were on days 25-32 of pregnancy at presentation. Non-pregnant cats were treated with aglepristone and with a dietary supplement containing maltodextrin and bromelain. The mammary glands were massaged daily with an *Aloe vera* emollient gel. If the gland was inflamed or ulcerated, broad-spectrum antimicrobial, and anti-inflammatory treatments were given, and the ulcers were treated topically with a hypericum and neem-based cream. Two

of the four pregnant cats were treated with the same therapeutic schedule plus cloprostenol to facilitate uterine emptying. Two pregnant cats underwent the same protocol except for aglepristone, which was not administered to safeguard the litter and see if the adjuvant therapy worked independently. At term, they delivered four and three kittens, respectively, that were normally nursed and weaned after 40 days. In all the studied cases, the mammary gland reduced in size 2-3 weeks after the start of the treatment and completely remitted after 4-5 weeks. **Conclusion:** This case series encourages adjuvant therapy in the course of FAC to preserve mammary gland integrity and functionality. Exploiting the natural decline of progesterone at the end of pregnancy (or pseudopregnancy), the therapy may also be used without aglepristone, when its use has other limitations.



The knowns and unknowns of the efficacy of neem oil (*Azadirachta indica*) used as a preventative measure against *Leishmania* sand fly vectors (*Phlebotomus* genus).

Zatelli A, Fondati A, Maroli M; Canine Leishmaniosis Working Group.
Prev Vet Med. 2022 May;202:105618. PMID: 35349879

Since domestic dogs are the main reservoir hosts of *Leishmania infantum* throughout the world, they are the main focus in terms of controlling zoonotic visceral leishmaniosis. To protect dogs from leishmaniosis, chemical repellents of durable efficacy are available in the form of collars, spot-on and sprays. However, the negative effects of chemical pesticides on the environment are well established as they affect animals and plants. This phenomenon has created the need for safer and more environmentally friendly substitutes. Plant extract-based insecticides and/or repellents have therefore been increasingly used by pet owners and veterinarians. Several botanical products have been tested as insecticides and/or repellents against a variety of bloodsucking arthropods that transmit human diseases. Among the products tested against *Leishmania* vectors, neem oil containing azadirachtin is the most studied. This study reviews the scientific literature concerning the efficacy of neem oil

(azadirachtin-based products) against phlebotomine sand fly bites. A questionnaire was also administered to assess Italian veterinarians' attitudes to the use of neem oil. The survey was anonymous and consisted of three closed-ended questions. According to the data reported in the literature, the efficacy of neem oil in reducing the risk of sand fly bites has been tested against *Phlebotomus papatasi*, *Phlebotomus perniciosus*, *Phlebotomus argentipes*, *Phlebotomus orientalis* and *Phlebotomus bergeroti*. The efficacy of the products tested was expressed in percentages, ranging from 74.9% to 100%. The protection time was only available for six out of eight studies, ranging from "only during the first hour" (minimum protection time) to "all night" (expected maximum protection time). As regards the attitude to recommending the use of neem oil, 208 veterinarians participated in the online survey. Of the 126 veterinarians who recommended natural products, 119 (94.44%) reported that they recommended the use of neem oil-based products. Considering the limited data on the duration of protection and the dose of the active ingredient, more studies are required on the efficacy of neem oil-based products in reducing the risk of contracting canine leishmaniasis. These studies should also refer specifically to the concentration of the active ingredient as well as the interval of administration. Until such results are available, the use of azadirachtin-based products as the only topical products for the prevention of leishmaniasis in dogs is not recommended.

Dietary herbaceous mixture supplementation reduced hepatic lipid deposition and improved hepatic health status in post-peak laying hens.

Zhu Y, Zhang X, Du P, Wang Z, Luo P, Huang Y, Liu Z, Zhang H, Chen W. *Poult Sci.* 2022 Mar 24;101(6):101870. PMID: 35472740

Fatty liver hemorrhagic syndrome is characterized by hepatic damage and hemorrhage impairing animal welfare in birds, which was well-known to be moderately relieved through dietary choline chloride supplementation in laying hens. Chinese herb has been proven to exert a positive role on hepatic health in human and rodents. Here, we investigated the effect of herbaceous mixture (HM), which consists of *Andrographis paniculate*, *Silybum marianum*, *Azadirachta Indica*, and *Ocimum basilicum* (2:3.5:1:2), on the hepatic lipid metabolism and health status in laying hens. A total of 240 Hy-line Brown hens (389-day-old) were randomly fed the basal diet with 0 mg/kg choline chloride (negative control, NC), 1,000 mg/kg choline chloride (control, Ctrl), or 300 mg/kg HM for 28 d. Birds fed HM diet exhibited lower serum triglyceride (TG) and low-density lipoprotein cholesterol concentration, and higher high-density lipoprotein cholesterol level than those received NC and Ctrl diets ($P < 0.05$). When compared to control and NC group, the diets with HM decreased the contents of total cholesterol and TG in liver, as well as upregulated the mRNA abundance of hepatic hormone-sensitive lipase and lipoprotein lipase. Meanwhile, the hepatic area and diameter of steatosis vacuoles were also decreased by dietary HM administration ($P < 0.05$), which accompanied by decreased serum alanine aminotransferase activity ($P < 0.05$). Birds fed HM diets enhanced the hepatic antioxidative capacity than those received NC and Ctrl diet. Dietary HM depressed the mRNA level of inflammatory cytokine as compared to NC but not Ctrl group. Collectively, the diet with 300 mg/kg HM has a favorable effect in decreasing the lipid deposition and protecting liver injury by alleviating hepatic oxidant stress and inflammation in post-peak laying hens.