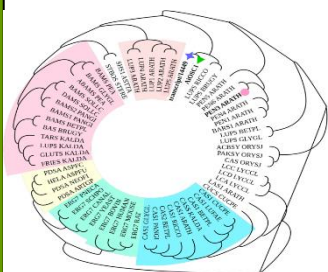


Neem Research Newsletter

Volume 2, Issue 6, 2022



WORLD NEEM ORGANISATION (WNO)



From
The Editor's Desk.....

Neemasthe! This issue of the newsletter features several research and review articles on neem in diverse fields. Scientists have provided the basis for the control of *S. frugiperda* by spinning silk besides revealing the molecular mechanism of *S. frugiperda* larval midgut in response to azadirachtin. Although several papers have underscored the value of neem for orodental protection, tissue dissolution was identified as a severe limitation in harnessing its potent antibacterial activities. Hopefully, in-depth research can circumvent this problem. The neem limonoid gedunin was demonstrated to be a novel microtubule-inhibiting drug candidate for cancer that is able to bypass multidrug resistance mechanisms. A very interesting finding that could positively impact construction industry is the report that neem extract helps to protect steel reinforcement from corrosion thereby increasing the lifetime of steel.

S. Nagini

Core Founding Member, WNO
Chief Scientific Coordinator &

Regional Director, South India



Neem in Agriculture

Biorational Control of *Callosobruchus maculatus* (Coleoptera: Buchidae) in Stored Grains with Botanical Extracts.

Akbar R, Khan IA, Faheem B, Azad R, Zaman M, Altaf R, Usman A, Fawad M, Farid A, Saljoqi AUR, Syed A, Bahkali AH, Elgorban AM, Shah JA, Qayyum A.

Biomed Res Int. 2022 Aug 29;2022:3443578. doi: 10.1155/2022/3443578. eCollection 2022. PMID: 36072466

Globally, around 2000 plant species are used against pest control. The utilization of botanicals is considered the most economic and biodegradable methods for the control of stored grains pests. Therefore, the current study was carried out to investigate the repellency potential of five botanicals against *Callosobruchus maculatus* F. in Haripur, Pakistan. The concentrations of *Azadirachta indica* L., *Nicotiana tabacum* L., *Melia azedarach* L., *Nicotiana rustica* L., and *Thuja orientalis* L. were, i.e., 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0% in four replicates to establish contact effects. The data were recorded after 1, 2, 3, 6, 24, 48, 72, and 96 hours. The repellency effect of these plant species against *C. maculatus* were increased in both the time- and dose-dependent manner, and highest effect was observed at 72 h. In addition, the repellency effect was 91% for *A. indica* (class: V), 86% *M. azedarach*, 82%, *N. tabacum* (class: V), 79% *N. rustica* (class: IV), and 75% *T. orientalis* (class: IV) at 3% concentration against *C. maculatus*. Furthermore, following 96 hours' exposure to treatment the sensitivity response of insects decreases as the time interval increases, i.e., 86% *A. indica* (class: V) was followed by 71% *M. azedarach* (class: IV), 65% *N. tabacum* (class: IV), 61% *N. rustica* (class: IV), and *T. orientalis* 57% (class: III) repellency at highest concentration of 3%. The current study concluded that *A. indica* and *M. azedarach* can be incorporated for the management of *C. maculatus* and these plant species might be helpful in the productions of new biopesticides.

Biosynthesis and chemical composition of nanomaterials in agricultural soil bioremediation: a review.

Rana R, Ferdous J, Rahman M, Rahman F, Huq A, Ali Y, Huda N, Mukhles MB, Rafi MH. *Environ Monit Assess.* 2022 Sep 6;194(10):730. doi: 10.1007/s10661-022-10315-1. PMID: 36066693

Nanomaterials (NMs) are currently being used in agricultural soils as part of a new bioremediation (BR) process. In this study, we reviewed the biosynthesis of NMs, as well as their chemical composition and prospective strategies for helpful and sustainable agricultural soil bioremediation (BR). Different types of NMs, such as nanoparticles, nanocomposites, nanocrystals, nano-powders, and nanotubes, are used in agricultural soil reclamation, and they reflect the toxicity of NMs to microorganisms. Plants (*Sargassum muticum*, *Dodonaea viscosa*, *Aloe Vera*, *Rosemarinus officinalis*, *Azadirachta indica*, *Green tea*, and so on) and microorganisms (*Escherichia coli*, *Shewanella oneidensis*, *Pleurotus sp.*, *Klebsiella oxytoca*, *Aspergillus clavatus*, and so on) are the primary sources for the biosynthesis of NMs. By using the BR process, microorganisms, such as bacteria and plants, can immobilize metals and change both inorganic and organic contaminants in the soil. Combining NMs with bioremediation techniques for agricultural soil remediation will be a valuable long-term solution.

From laboratory to industrial storage - Translating volatile organic compounds into markers for assessing garlic storage quality.

Ludlow RA, Evans G, Graz M, Marti G, Martínez PC, Rogers HJ, Müller CT. *Postharvest Biol Technol.* 2022 Sep;191:111976. doi: 10.1016/j.postharvbio.2022.111976. PMID: 36061628

Garlic (*Allium sativum* L.) has long been grown for its culinary and health-promoting qualities. The seasonal nature of garlic cropping requires that bulbs be stored for many months after harvest to ensure a year-round supply. During this time, quality is known to deteriorate, and efforts have been made to improve the longevity of stored bulbs. Cold temperatures within the stores prolong shelf life, but fine temperature control is needed to avoid freezing damage or cold induced stress. Here, quality traits (alliinase activity, firmness, and water content) are measured in response to a 96 h - 5 °C cold stress, to simulate the effect of non-isothermic temperature control in a - 1.5 °C warehouse. Volatile organic compounds (VOCs) are measured by thermal desorption gas chromatography time of flight mass spectrometry to identify markers of non-isothermic storage in garlic. 129 compounds were putatively identified and four (L-lactic acid, 2,6-dimethylheptadecane, 4-methyldodecane, and methylcyclopentane) showed high predictive accuracy for cold stress. VOCs were also sampled directly from a cold storage facility and the whole profile discriminated between sampling time points. Five VOCs were highly predictive for storage time in the warehouse but were different to VOCs previously shown to discriminate between storage times in a laboratory setting. This indicates the need for realistic warehouse experiments to test quality markers.

Valorization of phyto-biomass with tertiary combination of animal dung for enriched vermicompost production.

Preethee S, Saminathan K, Chandran M, Kathireswari P.

Environ Res. 2022 Sep 23;114365. doi: 10.1016/j.envres.2022.114365. Online ahead of print. PMID: 36162467

A study was conducted for 90 days in two cycles on 45th day (Cycle I), and 90th day (Cycle II) in 144 vermibins with precomposted cow dung (T1), elephant dung (T2), cow dung + elephant dung (T3) in combination with leaf substrates of *Ficus religiosa*, *Azadirachta indica*, *Terminalia catappa*, *Carica papaya*, *Vitex negundo*, *Acalypha indica* and *Borassus flabellifer* to generate nutrient-enriched vermicompost. Different vermibin feedstock materials were retained as experimental setup in other substrates with earthworm (vermicompost) and without earthworm (compost). This method was employed in the current study to decompose environmental leaf debris into the earthworm's mass production and transform it into high-value manure for long-term soil fertility control. The majority of the substrates exhibit pH and electrical conductivity in vermicomposts showed an increment while the total organic carbon and carbon to nitrogen ratio were significantly lowered. A prominent percentage increment of total NPK contents ($P < 0.05$) in vermicompost over initial values (N: 7.09-164.03; P: 4.39-101.09; K: 0.45-84.10). Among the vermibed substrates, *Ficus religiosa* leaf litter mixed with T3 showed stabilized cocoons and juveniles in Cycle I (45 days), while sub-adults and adults growth was favored in Cycle II (90 days). The higher reproductive potential of earthworms

could be due to the composition and palatability of the substrate combination. This study provides a platform for utilizing leaf wastes in combination with animal wastes amended to reproduce earthworms, nutrient enrichment which could benefit soil fertility improvement.

▪
Azadirachtin inhibits the development and metabolism of the silk glands of *Spodoptera frugiperda* and affects spinning behaviour.

Zhao W, Zheng Q, Qin D, Luo P, Ye C, Shen S, Cheng D, Huang S, Liu L, Xu H, Zhang Z.
Pest Manag Sci. 2022 Aug 26. doi: 10.1002/ps.7151. PMID: 36053871

Background: *Spodoptera frugiperda* is a major agricultural pest, and the dispersal of its larvae by spinning silk is one of the causes of crop damage. At present, there are relatively few reports of pest control that affect larvae spinning silk. In this study, the effect of spinning behaviour of the *S. frugiperda* larvae was investigated through a series of experiments. **Results:** The 3rd instar larvae of *S. frugiperda* were exposed to azadirachtin, and the pathological changes in the silk glands of *S. frugiperda* and the differences in their metabolites were analysed by scanning electron microscopy, histological sectioning, transmission electron microscopy and metabolomics. The results showed that azadirachtin could affect the silk gland of *S. frugiperda*. After 48 h of treatment with azadirachtin, the silk gland lumen of *S. frugiperda* appeared vacuolated. KEGG showed that 31 different metabolites were identified, of which 12 were upregulated and 19 were downregulated. These metabolites were enriched in 15 different metabolic pathways, which indicated that the silk gland of *S. frugiperda* was closely related to the formation of fatty acids and energy metabolism for the silk formation process. **Conclusions:** This study provides a preliminary report of the effect of azadirachtin on the spinning behaviour of the *S. frugiperda* larvae. Metabolomic results indicated that histidine, glycine and leucine, which are related to serine protein synthesis, were down-regulated. Azadirachtin can damage the silk glands of *S. frugiperda* and thus affect spinning behaviour. This provides the basis for the control of *S. frugiperda* by spinning silk.

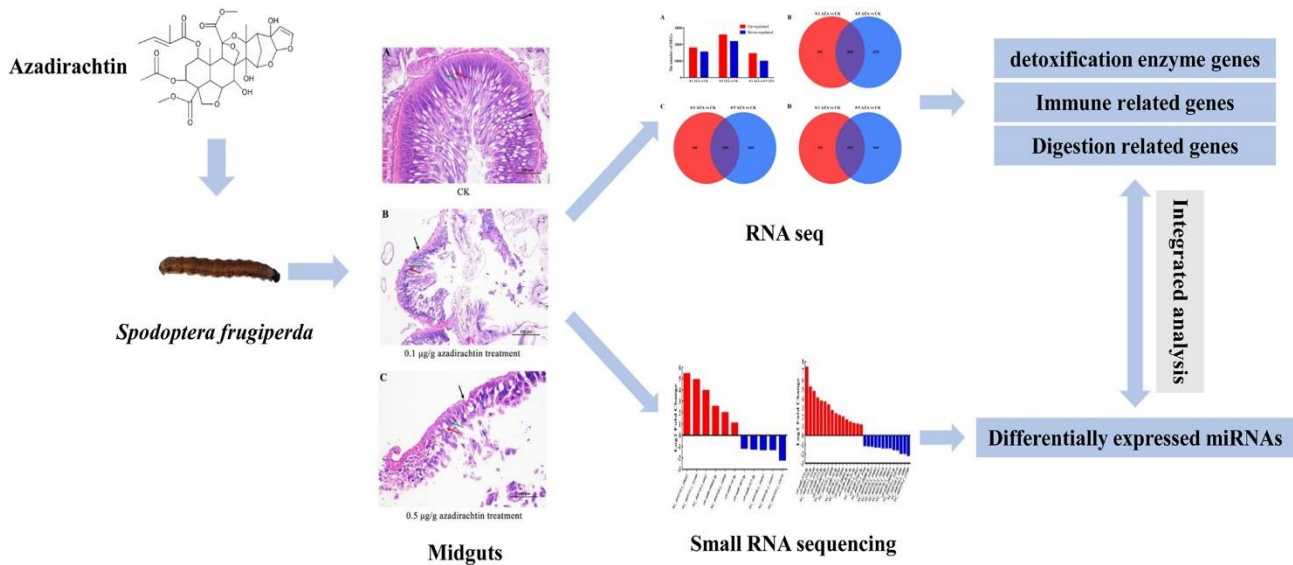
Integrated miRNA and transcriptome profiling to explore the molecular mechanism of *Spodoptera frugiperda* larval midgut in response to azadirachtin exposure.

Shu B, Lin Y, Qian G, Cai X, Liu L, Lin J.

Pestic Biochem Physiol. 2022 Oct;187:105192. doi: 10.1016/j.pestbp.2022.105192. Epub 2022 Aug 2. PMID: 36127051

As a destructive agricultural pest, *Spodoptera frugiperda* has spread worldwide in the past few years. Azadirachtin, an environmentally friendly and most promising compound, showed adverse effects, including mortality and growth inhibition, against *S. frugiperda*. While the effects of azadirachtin on the midgut of this pest remain to be determined. In this study, structural damage was observed in the larval midguts of *S. frugiperda* with azadirachtin exposure. RNA-seq on the larval midguts with different azadirachtin treatments was performed. Compared to the control group, a total of 3344 and 4759 differentially expressed genes (DEGs) were identified in the midguts with 0.1 and 0.5 µg/g azadirachtin exposure, respectively. Among them, the DEGs encoding detoxification enzymes/proteins, immune-related proteins, digestion and absorption-related proteins, and transcript factors were further analyzed. High-throughput sequencing was also used for the identification of

differentially expressed microRNAs in different treatments. A total of 153 conserved miRNAs and 147 novel miRNAs were identified, of which 11 and 29 miRNAs were affected by 0.1 and 0.5 $\mu\text{g/g}$ azadirachtin treatments, respectively. The integrated analysis found that 13 and 178 miRNA versus mRNA pairs were acquired in the samples with 0.1 and 0.5 $\mu\text{g/g}$ azadirachtin treatments, respectively. The results of high-throughput sequencing were confirmed by real-time quantitative polymerase chain reaction (RT-qPCR). These results provide useful information for revealing the molecular mechanism of *S. frugiperda* larval midgut in response to azadirachtin.



Neem-Based Insecticides Deter Oviposition Activity in Spotted Wing Drosophila (Diptera: Drosophilidae) in Sweet Cherries.

Gyawaly S, Rijal J.

J Econ Entomol. 2022 Aug 10;115(4):1310-1313. doi: 10.1093/jee/toac074.

PMID: 35604376

Spotted wing drosophila, *Drosophila suzukii* (Matsumura), is an important invasive insect pest in the United States. Spotted wing drosophila females have serrated ovipositor that enables them to deposit eggs inside healthy, ripening softskinned fruits. In many berry and fruit crops, spotted wing drosophila is primarily controlled using synthetic insecticides in the organophosphate, pyrethroid, and spinosyn groups. However, alternative, reduced-risk insecticides with short residuals are necessary to minimize the possibilities of insecticide resistance and address other negative effects of synthetic insecticides. Neem-based products as alternative plant-based insecticides have antifeedant, repellent, antiovipository, and molting interference effects on insects and have been shown to affect the oviposition activities of some tephritid flies. We conducted laboratory studies to evaluate the oviposition deterrent activities of selected neem-based insecticides that contain either azadirachtin, or CHENO, or a combination of both, on spotted wing drosophila using sweet cherry in California. The results of the study showed that some of the neem oil products could reduce the degree of spotted wing drosophila oviposition by half compared with the control.

Neem for Aquaculture

Isolation and Characterization of *Aeromonas jandaei* from Nile Tilapia in Lake Volta, Ghana, and Its Response to Antibiotics and Herbal Extracts.

Abarike ED, Atuna RA, Agyekum S, Akongyuure DN, Alhassan EH.

J Aquat Anim Health. 2022 Sep;34(3):140-148. doi: 10.1002/aah.10165.PMID: 36165569

Production of Nile Tilapia *Oreochromis niloticus* contributes to economic growth in many countries. However, there has been a decline in its production over the years due to the influx of bacterial infections, with *Aeromonas jandaei* as an emerging threat. In this study, we identified and characterized *A. jandaei* from cage-cultured Nile Tilapia in Akosombo Stratum II of Lake Volta in Ghana and evaluated its response to commonly used antibiotics using the disc diffusion and agar well diffusion methods for herbal extracts at various concentrations (10, 30, 50, 70, and 100 mg/mL). The herbs considered included guava *Psidium guajava* leaf, bitter leaf *Vernonia amygdalina*, neem *Azadirachta indica* leaf, and their cocktail (GBNL in the ratio of 1:1:1). The bacterium was isolated from swab samples from the head kidneys of 27 moribund Nile Tilapia collected from nine fish farms. Samples were screened for *A. jandaei* by culturing and identification using morphological and molecular techniques. The bacterium isolate from fish in the study, identified as *A. jandaei* GH-AS II, had 92-93% identity to *A. jandaei* reference strains. Infection of healthy Nile Tilapia ($n = 210$) with the bacterium isolate showed that 1.0×10^5 CFU/mL was the lethal dose causing 50% mortality. Antibiotic susceptibility testing showed that *A. jandaei* GH-AS II was resistant to tetracycline and ampicillin. Herbal extracts at the various concentrations inhibited the growth of the bacterium isolate, with a significant increment in the zones of inhibition with increasing concentrations of leaf extracts. However, GBNL showed prominence compared to the other extracts only at 100 mg/mL. Management of *A. jandaei* GH-AS II by using herbal extracts at Nile Tilapia farms in Lake Volta may be recommended since the use of antibiotics, such as tetracycline and ampicillin, may not yield the needed result.

Neem For Human Health

Antiviral Effects

Potential of *Azadirachta indica* as a Capping Agent for Antiviral Nanoparticles against SARS-CoV-2.

Foka FET, Manamela N, Mufamadi SM, Mufhandu HT.

Biomed Res Int. 2022 Sep 15;2022:5714035. doi: 10.1155/2022/5714035. eCollection 2022. PMID: 36158879

A rare type of pneumonia later on referred to as COVID-19 was reported in China in December 2019. Investigations revealed that this disease is caused by a coronavirus previously identified as SARS-CoV-2, and since then, it has become a global pandemic with new strains emerging rapidly as a result of genetic mutations. Various therapeutic options are being explored in order to eradicate this pandemic even though approved vaccine candidates are being currently rolled out globally. Most medicinal plant extracts have astonishing properties, and they can therefore be used in the biosynthesis of effective antiviral nanoparticles. In this systematic review, we aimed to highlight the specific attributes that make *Azadirachta indica* (neem plant) a suitable candidate for the biosynthesis of anti-SARS-CoV-2 nanoparticles. A systematic investigation was therefore carried out in PubMed, Scopus, Web of Science, and AJOL databases with the keywords "Nanoparticles," "Biosynthesis," "Antivirals," "SARS-CoV-2," and "*Azadirachta indica*." 1216 articles were retrieved by the 21st of February 2022, but we screened studies that reported data on biomedical and antimicrobial assessment of *Azadirachta indica* extracts. We also screened studies that were reporting nanoparticles possessing antiviral properties against SARS-CoV-2, narrowing our results to 98 reports. Herein, the SARS-CoV-2 viral structure is briefly discussed with nanoparticles of biomedical importance in the design of SARS-CoV-2 antivirals. Most importantly, we focused on the biomedical and antiviral properties of *Azadirachta indica* extracts that could be of importance in the design of potential anti-SARS-CoV-2 nanoformulations.

Anti-inflammatory Effects

Antiosteoporosis and bone protective effect of nimbolide in steroid-induced osteoporosis rats.

Huo J, Ding Y, Wei X, Chen Q, Zhao B.

J Biochem Mol Toxicol. 2022 Sep 9:e23209. doi: 10.1002/jbt.23209. Online ahead of print. PMID: 36086868

Background: Osteoporosis is a metabolic, hereditary, progressive disease characterized by unusual bone production across the skeleton and a loss the bone tissue microstructure and mass. In this experimental study, we scrutinized the antiosteoporosis effect of nimbolide against glucocorticoid (GCs) induced osteoporosis in rats. **Methods:** Swiss albino female rats were employed for the current experiment study and the rats were divided into different

groups. Dexamethasone (0.1 mg/kg/day) was used for induction the osteoporosis and the rats were received the different doses of nimbolide (2.5, 5, and 7.5 mg/kg) for the estimation of bone protective effects. The body weight was estimated (initially and finally). Hormones, bone metabolic markers, bone turnover markers, bone structure, biomechanical, histomorphometric dynamic, biochemical markers, and histomorphometric static parameters were analyzed. **Results:** The body weight of GCs group rats considerably suppressed and nimbolide treatment remarkably improved the body weight. Nimbolide treated group exhibited the enhancement of bone metabolic, bone structure markers, and histomorphometric dynamic markers, which was suppressed during the GCs-induced osteoporosis. GCs-induced osteoporosis rats exhibited the enhancement of procollagen type 1 C-terminal propeptide (P1CP), carboxy-terminal crosslinked telopeptide of type 1 collagen (CTX-1), Dickkopf-1 (DKK1), tartrate-resistant acid phosphatase 5b (TRACP 5b), and suppressed the level of bone alkaline phosphatase (BAP), which was reversed by the nimbolide treatment. Nimbolide treatment remarkably improved the level of estradiol and suppressed the level of parathyroid hormone (PTH), which was altered during the osteoporosis. Nimbolide treatment significantly ($p < 0.001$) improved the level of calcium, magnesium, and phosphorus in the serum and bone tissue. Nimbolide treatment also altered the level of bone metabolic markers and suppressed the level of inflammatory cytokines. **Conclusion:** Based on the findings, we may conclude that nimbolide has antiosteoporosis properties via balancing the bone mass and improving vitamin and hormone levels.

Dermatological Effects

***Cassia alata*, *Coriandrum sativum*, *Curcuma longa* and *Azadirachta indica*: Food Ingredients as Complementary and Alternative Therapies for Atopic Dermatitis-A Comprehensive Review.**

Chew YL, Khor MA, Xu Z, Lee SK, Keng JW, Sang SH, Akowuah GA, Goh KW, Liew KB, Ming LC.

Molecules. 2022 Aug 26;27(17):5475. doi: 10.3390/molecules27175475.PMID: 36080243

Traditional medicine is critical in disease treatment and management. Herbs are gaining popularity for disease management and treatment. Therefore, they can be utilised as complementary and alternative treatment (CAT) ingredients. Atopic dermatitis (AD) is one of the common non-communicable diseases. It is characterised by chronic inflammatory skin disease with intense pruritus and eczematous lesions. AD is associated with oxidative stress, microbial infection, and upregulation of inflammatory cytokines. Both children and adults could be affected by this skin disorder. The prevalence of AD is increasing along with the country's level of development. This review revisited the literature on four medicinal herbs widely used as complementary medicine to manage AD. These therapeutic herbs are commonly eaten as food and used as spices in Asian cuisine. The four food herbs reviewed are *Cassia alata*, *Coriandrum sativum*, *Curcuma longa* Linn, and *Azadirachta indica*. Their traditional uses and phytochemical content will be covered. Four relevant pharmacological and biological activities of the plants crucial in AD management have been reviewed and discussed, including anti-inflammatory, anti-microbial, antioxidant, and wound recovery.

Hepatoprotective Effects

The *Azadirachta indica* (Neem) Seed Oil Reduced Chronic Redox-Homeostasis Imbalance in a Mice Experimental Model on Ochratoxine A-Induced Hepatotoxicity.

Nikolova G, Ananiev J, Ivanov V, Petkova-Parlapanska K, Georgieva E, Karamalakova Y. *Antioxidants (Basel)*. 2022 Aug 28;11(9):1678. doi: 10.3390/antiox11091678. PMID: 36139752

Liver damage severity depends on both the dose and the exposure duration. Oxidative stress may increase the Ochratoxine-A (OTA) hepatotoxicity and many antioxidants may counteract toxic liver function. The present study aims to investigate the hepatoprotective potential of *Azadirachta indica* (*A. indica*; neem oil) seed oil to reduce acute oxidative disorders and residual OTA toxicity in a 28-day experimental model. The activity of antioxidant and hepatic enzymes, cytokines and the levels of oxidative stress biomarkers –MDA, GSPx, Hydroxiprolin, GST, PCC, AGEs, PGC-1, and STIR-1 were analyzed by ELISA. The free radicals ROS and RNS levels were measured by EPR. The protective effects were studied in BALB/C mice treated with *A. indica* seed oil (170 mg/kg), alone and in combination with OTA (1.25 mg/kg), by gavage daily for 28 days. At the end of the experiment, mice treated with OTA showed changes in liver and antioxidant enzymes, and oxidative stress parameters in the liver and blood. *A. indica* oil significantly reduced oxidative stress and lipid peroxidation compared to the OTA group. In addition, the hepatic histological evaluation showed significant adipose tissue accumulation in OTA-treated tissues, while treatment with 170 mg/kg *A. indica* oil showed moderate adipose tissue accumulation.

Orodonal Protection

Medicinal Plants Used as an Alternative to Treat Gingivitis and Periodontitis

Neeraj Rani, Rajeev K. Singla, Sonia Narwal, Tanushree, Nitish Kumar, Md. Mominur Rahman

Evidence-Based Complementary and Alternative Medicine, vol. 2022, Article ID 2327641, 14 pages, 2022. <https://doi.org/10.1155/2022/2327641>

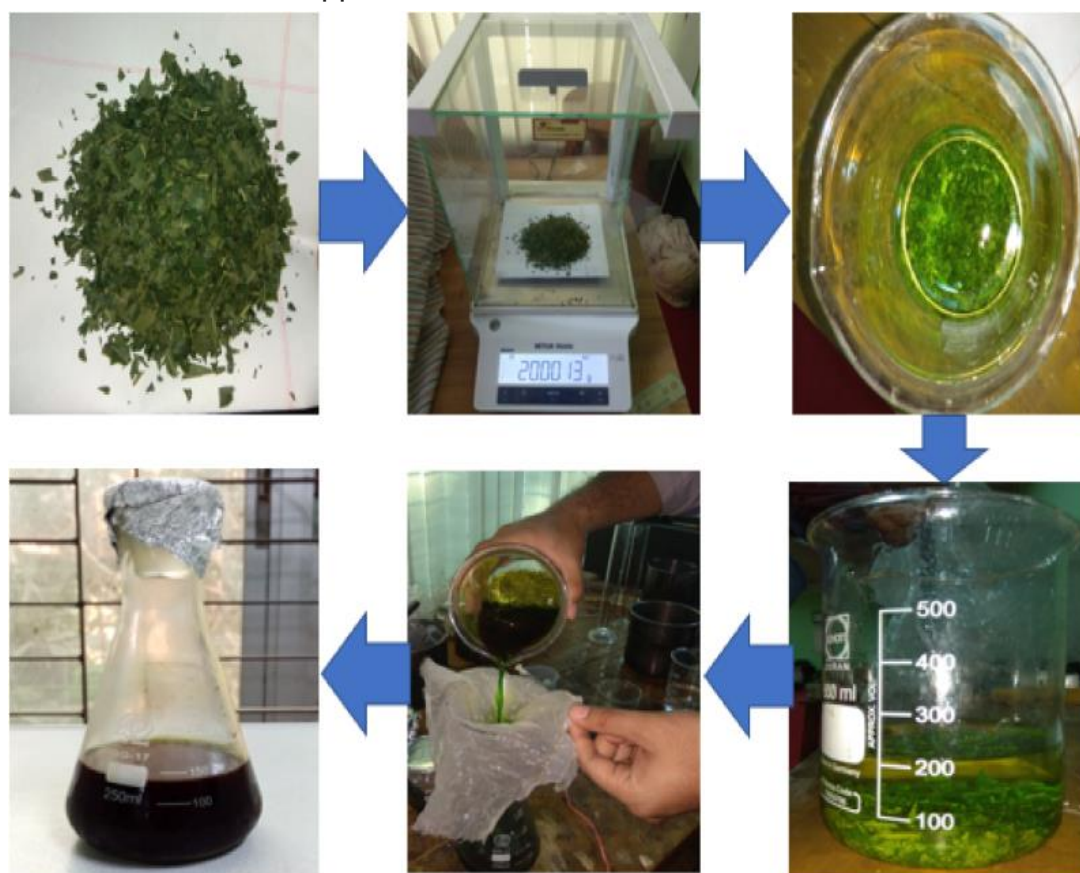
For various ailments, natural remedies have been traditionally used. To defend against common disorders, medicinal plants are progressively used as nutritional supplements. Gingivitis and periodontitis are widespread and can affect most of the world's population. Gingivitis is a very common, nondestructive inflammatory disease of gums that causes redness and irritation of the gingiva (gums), but periodontitis causes permanent damage to teeth' subsidiary structures. Herbal medicines are getting popular for the treatment of such types of disorders due to being economical with their medicinal effectiveness, compatibility, and nontoxicity. Traditional chemical therapies can cause cell toxicity along with their disease-curing effects. In this article, we discussed the medicinal plants that can be used as an alternative for the treatment of gingivitis (early-stage gum disease) and periodontitis (chronic-stage gum disease).

Development of SiC-TiO₂-Graphene neem extracted antimicrobial nano membrane for enhancement of multiphysical properties and future prospect in dental implant applications.

Chowdhury MA, Hossain N, Shahid MA, Alam MJ, Hossain SM, Uddin MI, Rana MM.

Heliyon. 2022 Sep 14;8(9):e10603. doi: 10.1016/j.heliyon.2022.e10603. eCollection 2022 Sep. PMID: 36158080

This paper presents the coating technology on Nano membrane using SiC-TiO₂-Graphene with varying percentages of *Azadirachta indica* (Neem) extract with an objective to develop new coating materials. The nanomembranes have been synthesized by electrospinning machine over aluminum foil paper using the raw materials PVA grain, SiC, TiO₂, Graphene, and neem. The nanomembranes have been characterized by SEM, XRD, FTIR, Surface Roughness, antibacterial, and Cytotoxicity test. FTIR analysis established the presence of PVA and neem indicating the formation of different organic compounds. It also confirmed that no chemical reaction occurred during the synthesis process. The membrane's roughness analysis obtained average roughness values from 1.15 to 3.84. The formation of homogeneous and smooth membranes with the formation of micropores was confirmed by SEM analysis. Miller Indices identified different types of crystal structures in XRD analysis. Antibacterial activity increased with the increase of the percentage of neem confirmed by the antibacterial test. No toxic effects were observed from the membrane during the cytotoxicity test. The obtained data confirmed that the synthesized nanomembrane could be used in different biomedical applications.

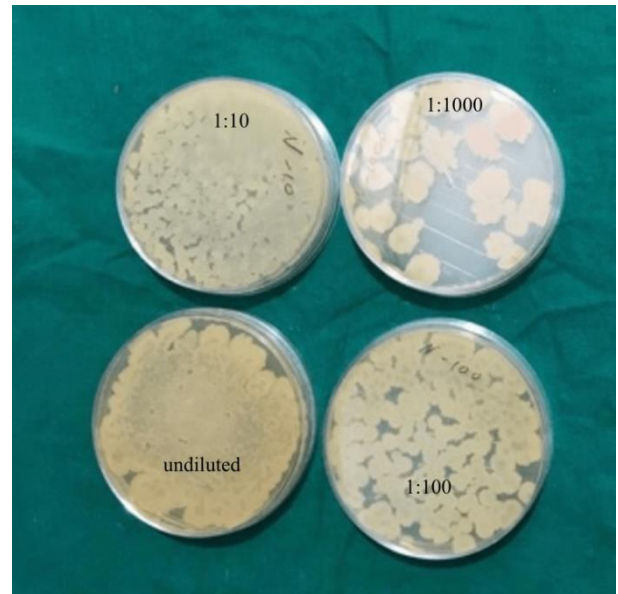


Individual and Synergistic Effects of Tea Tree Oil and Neem Extract on *Candida albicans* Adhesion to Denture Soft Liner.

Singhania A, Sathe S, Ranka R, Godbole S.

Cureus. 2022 Aug 10;14(8):e27869. doi: 10.7759/cureus.27869. eCollection 2022 Aug. PMID: 36110485

Background Inflammation is present in denture stomatitis. Denture stomatitis's etiology is complex, but there is evidence that it is brought on by *Candida albicans* growing in biofilms on its surface. **Objective** This study aimed to examine and assess the effectiveness of several herbal products, such as tea tree oil and neem extracts, on *Candida albicans* adhesion to denture soft liners. **Method** Each wall of the tissue culture plate was filled with 30 acrylic blocks lined with soft liners, followed by the addition of 0.1 ml of the standardized *Candida albicans* suspension, which was then left to incubate for 48 hours. Each specimen was placed in a disinfectant solution for 10 minutes. A colony was counted after 0.1ml of the solution was plated on a sabouraud dextrose agar (SDA) plate and cultured for 72 hours. Data were compared using a one-way analysis of variance (ANOVA) test. **Results** The mean colony forming units (CFU) per ml for combined tea tree oil and neem extract was least (0.40), followed by tea tree oil (2.30), followed by neem extract (30.33). The treated blocks were effective in reducing the growth of *Candida albicans*. **Conclusion** Combining tea tree oil and neem extract significantly reduced the growth of *Candida albicans*, suggesting a new form of intraoral effective antifungal treatment.



Effect of Herbal and Chemical Solution in Tissue Dissolution by using Conventional Irrigation and Sonic Irrigation System.

Bhavsar BA, Selvi T, Paliwal A, Ansari F, Beohar DS, Joseph T.

J Pharm Bioallied Sci. 2022 Jul;14(Suppl 1):S863-S867. doi: 10.4103/jpbs.jpbs_28_22. Epub 2022 Jul 13. PMID: 36110684

Introduction: An essential part of root canal cleaning is irrigating the system to remove any remaining debris and tissue remains. The purpose of root canal therapy is to shape and clean the endodontic space, reduce the bacterial burden, and remove the pulp tissue. In this research, several irrigation solutions with and without sonic irrigation were evaluated to see how quickly tissue dissolves. **Method:** A tissue sample was taken from a cow (68 ± 3 mg) with no statistically significant difference between groups. All five test tubes in each group were immersed in irrigant, and each group contained one subgroup with five test tubes. Separate weights were recorded for every irrigant that had been passed through the filter paper. Thus, the quantity of pulp dissolved by different irrigating solutions was quantified using a filtering technique. **Results:** It was revealed that there was a substantial difference between the groups. Sonic and non-sonic irrigation of sodium hypochlorite resulted in a substantial difference in tissue disintegration. **Conclusion:** This research found that sodium

hypochlorite was more effective than EDTA and saline in dissolving pulp. Despite several studies claiming that neem has potent antibacterial activities, tissue dissolution has not been shown in experiments using this herb.

Antibacterial Efficacy of Herbal Solutions in Disinfecting Gutta Percha Cones Against *Enterococcus Faecalis*.

Vanapatla A, Nanda N, Satyarth S, Kawle S, Gawande HP, Gupte JM.

J Pharm Bioallied Sci. 2022 Jul;14(Suppl 1):S748-S752. doi: 10.4103/jpbs.jpbs_111_22. Epub 2022 Jul 13. PMID: 36110725

Objective: Even when endodontic procedures are performed with the utmost care, reinfection can occur. One possible explanation is that contaminated gutta-percha cones were used in the root canals. The study's goal is to assess the antimicrobial activity and efficacy of Neem, Turmeric, and Calotropis Procera in disinfecting gutta percha cones prior to obturation. **Materials and methods:** 80 Gutta percha (GP) cones were contaminated with 0.5ml Ferlands standard *Enterococcus Faecalis* for 10 minutes. 20 cones each were treated up to 5 minutes in their prepared neem, turmeric, calotropis and sodium hypochlorite solutions. Then, they placed in BHI broth and vortex is done for 1 minute. One loopful of broth was stroked on 20 blood agar plates of 5 each neem, turmeric, calotropis and sodium hypochlorite. The plates were then incubated for 24 hours aerobically at 37°C and the colony forming units were counted with a digital colony counter. **Results:** Mean colony forming units were compared in all the groups and there was a statistically significant difference present among the groups ($p < 0.01$). Sodium Hypochlorite (NaOCL) as control group showed average of 5 colony forming units (CFU). Turmeric had the least average of 55 CFU and was the most effective of the experimental groups. Neem and Calotropis had the higher average of 130 CFU and 200 CFU respectively, making it the least effective of the solutions tested. **Conclusion:** All of the herbal solutions tested positive for disinfection of GP points. When compared to Neem and Calotropis, Turmeric was the most effective and has superior antibacterial activity.

The Effectiveness of Herbal Versus Non-Herbal Mouthwash for Periodontal Health: A Literature Review.

Tidke S, Chhabra GK, Madhu PP, Reche A, Wazurkar S, Singi SR.

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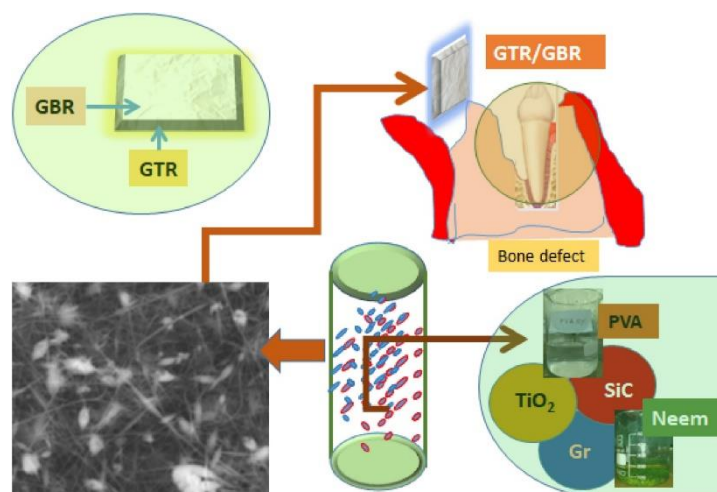
Dental plaque is a biofilm of microorganisms that present naturally on the exposed tooth surface; it is the main etiological factor for many periodontal conditions and other oral health issues and its regular removal from the oral cavity can prevent many periodontal problems. Despite several experiments using herbal oral care products to reduce dental plaque or gingivitis, the findings remain inconclusive. We performed a systematic literature search on PubMed and Cochrane Library for randomized controlled trials (RCTs) dating from 2001 up to and including the year 2021. The keywords and Medical Subject Headings (MeSH) terms comprised combinations of the following: herbal, clove oil, peppermint oil, ginger, basil, ajwain, betel leaf extract, neem, lavender, non-herbal, chlorhexidine, fluorides, hydrogen fluoride, hydrogen fluoride, stannous fluoride, and mouthwashes. Each of the titles that the search elicited was screened and duplicates were removed from the gathered results. The full-text versions of the remaining articles were downloaded and examined by title and

abstract. Handsearching was not carried out. We initially identified 21 studies; 14 studies, which did not fulfill the selection criteria, were excluded. All the included studies reported a reduction in plaque index (PI) and gingival index (GI) scores in both herbal and non-herbal groups. Two studies reported the superiority of the non-herbal mouthwash over the herbal one while five of the studies showed no significant difference in PI and GI scores between herbal and non-herbal mouthwash, implying equal efficacy of both, i.e., Triphala, aloe vera, tea tree, and polyherbal groups like *Zingiber officinale*, *Rosmarinus officinalis*, and *Calendula officinalis*, and chlorhexidine. Current research suggests that herbal mouthwashes are as effective as non-herbal mouthwashes for reducing dental plaque in the short term; however, the evidence is based on low-quality trials.

Development of SiC–TiO₂-Graphene neem extracted antimicrobial nano membrane for enhancement of multiphysical properties and future prospect in dental implant
MA Chowdhury, N Hossain, MA Shahid, MJ Alam

Heliyon, 2022 <https://doi.org/10.1016/j.heliyon.2022.e10603>

This paper presents the coating technology on Nano membrane using SiC–TiO₂-Graphene with varying percentages of *Azadirachta indica* (Neem) extract with an objective to develop new coating materials. The nanomembranes have been synthesized by electrospinning machine over aluminum foil paper using the raw materials PVA grain, SiC, TiO₂, Graphene, and neem. The nanomembranes have been characterized by SEM, XRD, FTIR, Surface Roughness, antibacterial, and Cytotoxicity test. FTIR analysis established the presence of PVA and neem indicating the formation of different organic compounds. It also confirmed that no chemical reaction occurred during the synthesis process. The membrane's roughness analysis obtained average roughness values from 1.15 to 3.84. The formation of homogeneous and smooth membranes with the formation of micropores was confirmed by SEM analysis. Miller Indices identified different types of crystal structures in XRD analysis. Antibacterial activity increased with the increase of the percentage of neem confirmed by the antibacterial test. No toxic effects were observed from the membrane during the cytotoxicity test. The obtained data confirmed that the synthesized nanomembrane could be used in different biomedical applications.



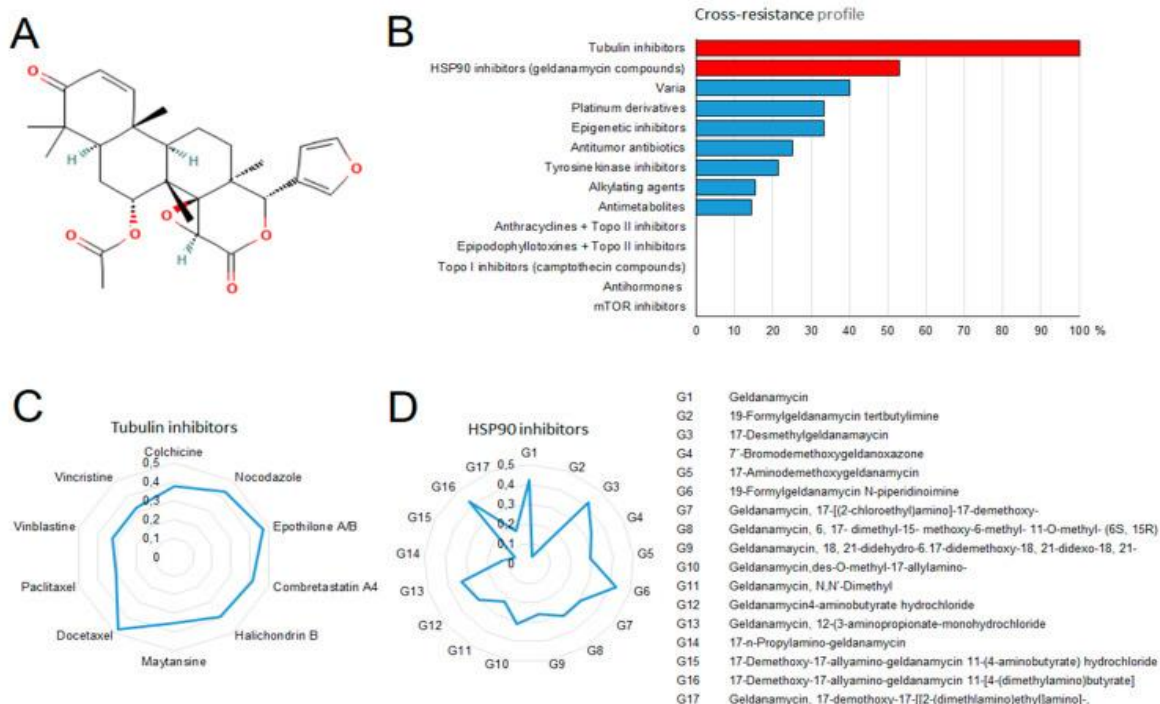
Anticancer Effects

Identification of Gedunin from a Phytochemical Depository as a Novel Multidrug Resistance-Bypassing Tubulin Inhibitor of Cancer Cells.

Khalid SA, Dawood M, Boulos JC, Wasfi M, Drif A, Bahramimehr F, Shahhamzehei N, Shan L, Efferth T.

Molecules. 2022 Sep 9;27(18):5858. doi: 10.3390/molecules27185858.PMID: 36144591

The chemotherapy of tumors is frequently limited by the development of resistance and severe side effects. Phytochemicals may offer promising candidates to meet the urgent requirement for new anticancer drugs. We screened 69 phytochemicals, and focused on gedunin to analyze its molecular modes of action. Pearson test-base correlation analyses of the $\log_{10}IC_{50}$ values of 55 tumor cell lines of the National Cancer Institute (NCI), USA, for gedunin with those of 91 standard anticancer agents revealed statistically significant relationships to all 10 tested microtubule inhibitors. Thus, we hypothesized that gedunin may be a novel microtubule inhibitor. Confocal microscopy, cell cycle measurements, and molecular docking in silico substantiated our assumption. Agglomerative cluster analyses and the heat map generation of proteomic data revealed a subset of 40 out of 3171 proteins, the expression of which significantly correlated with sensitivity or resistance for the NCI cell line panel to gedunin. This indicates the complexity of gedunin's activity against cancer cells, underscoring the value of network pharmacological techniques for the investigation of the molecular modes of drug action. Finally, we correlated the transcriptome-wide mRNA expression of known drug resistance mechanism (ABC transporter, oncogenes, tumor suppressors) $\log_{10}IC_{50}$ values for gedunin. We did not find significant correlations, indicating that gedunin's anticancer activity might not be hampered by classical drug resistance mechanisms. In conclusion, gedunin is a novel microtubule-inhibiting drug candidate which is not involved in multidrug resistance mechanisms such as other clinically established mitotic spindle poisons.



Methanolic neem (*Azadirachta indica*) stem bark extract induces cell cycle arrest, apoptosis and inhibits the migration of cervical cancer cells in vitro.

Kumar S, Mulchandani V, Das Sarma J.

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Background: Cervical cancer remains one of the significant causes of mortality in women due to the limitations of current treatment strategies and their associated side effects. Investigation of alternative medicine, including phytomedicine, has shown effective anti-cancer potential with fewer side effects. *Azadirachta indica* (commonly known as neem) is known for its medicinal properties. The present study investigated the anti-cancer potential of methanolic neem stem bark extract (MNBE) against cervical cancer using HeLa, SiHa, and ME-180 cell lines. **Methods:** Cytotoxic effect of MNBE on cultured cell lines was evaluated by MTT and clonogenic assay. The growth-inhibiting effect of MNBE was further confirmed by performing cell cycle analysis and apoptosis assay using flow cytometry. The anti-migratory effect of MNBE was evaluated by using wound healing and Boyden chamber assay. Real-time PCR was used to determine the mRNA expression, and western blot and flow cytometry was used to determine the protein levels of growth and migration-related genes. **Results:** MNBE significantly suppressed the growth and survival of cervical cancer cells in a dose-dependent manner by inducing cell cycle arrest and apoptosis. In addition, the growth inhibitory effect of MNBE was specific to cervical cancer cells than normal cells. Cell cycle arrest was correlated to transcriptional downregulation of cyclin dependent kinase 1 (CDK1), cyclin A, and cyclin B. Additionally, MNBE treatment resulted in the upregulation of active caspase-3 protein and downregulation of pro-survival genes, Bcl2, and survivin at mRNA level and NFkB-p65 at the protein level. Furthermore, MNBE inhibited the migration of cervical cancer cells accompanied by modulation of migration-related genes, including zona occludens-1 (ZO-1), matrix metalloproteinase 2 (MMP2), focal adhesion kinase (FAK), N-cadherin, snail, and E-cadherin. **Conclusion:** In summary, the present study provides the first evidence of MNBE in restricting cervical cancer cell growth and migration, which warrants further investigation for developing novel anti-cancer drugs.

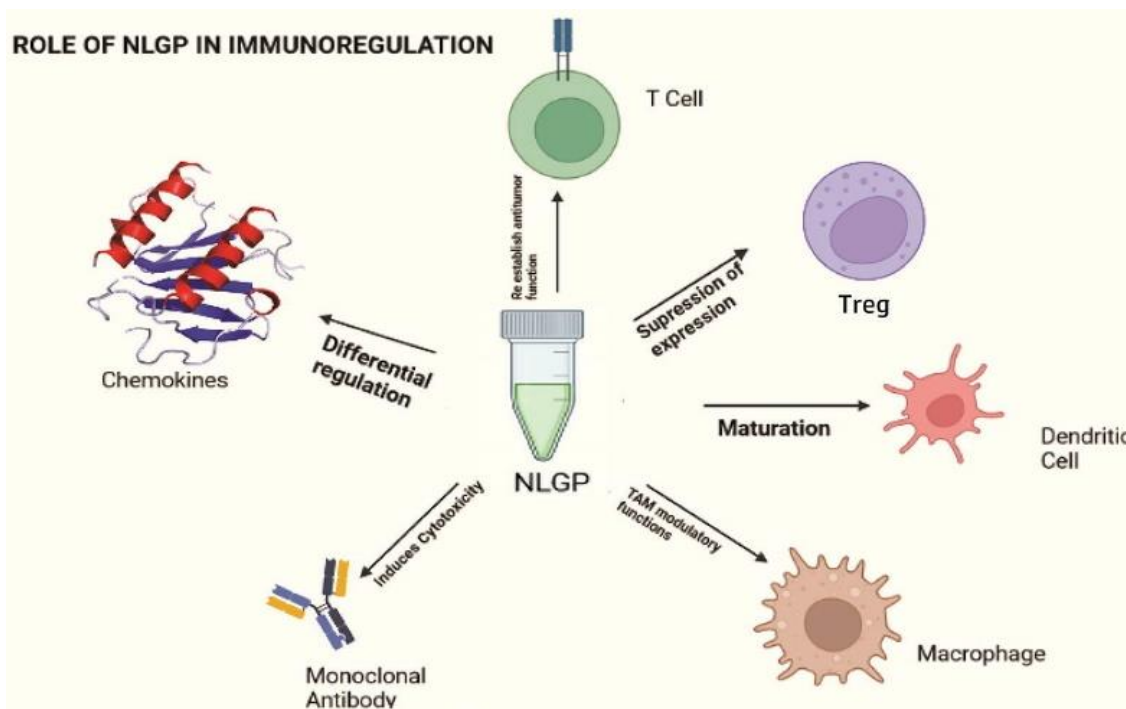
Neem Leaf Glycoprotein in immunoregulation of cancer.

Singh A, Chatterjee A, Rakshit S, Shanmugam G, Mohanty LM, Sarkar K.

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Cancer is a disease having global consequences. Though several new strategies and treatments have been developed so far, they often come with malicious side effects and this paved ways for demand of naturally extracted/driven product as potent anti-cancer agent owing to their reduced toxicity and side effects. One such common Indian household plant Neem (*Azadirachta Indica*) and its extract have variegated immunomodulatory effects as anti-cancer agent. Neem Leaf Glycoprotein (NLGP) modifies immune cells present in the tumor surroundings as well as in the peripheral system, rather than directly attacking the cancer cells. NLGP acts as a natural immunomodulator showing several functions like sustained tumor growth regulation by stimulating central and effector memory cells as a

vaccination adjuvant, normalization of angiogenic activities, controls hypoxia, improves immune evasion techniques as well as suppresses the activity of several immunological cells (Tregs, myeloid-derived suppressor cells, and tumor-associated macrophages) which promote tumor growth and metastasis in the tumor microenvironment (TME). NLGP prioritises type1 immune-microenvironment which consists of T-bet⁺IFN- γ -producing group 1 innate lymphoid cell (ILC) (ILC1 and natural killer cells), CD8⁺ cytotoxic T cells (TC1), and CD4⁺ T helper1 (Th1) cells. In this review we aim to summarize detailed activity of NLGP in cancer immunoregulation.



Neem for Animal Health

Structural modification of octadecanoic acid-3,4-tetrahydrofuran diester and the acaricidal activity and mechanism of its derivatives against *Sarcoptes scabiei* var. *Cuniculi*.

Li L, Zhang Y, Liu T, Xing R, Peng S, Song X, Zou Y, Zhao X, Jia R, Wan H, Yin L, Ye G, Shi F, Zhang Y, Yue G, Yin Z.

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Octadecanoic acid-3,4-tetrahydrofuran diester is a compound with acaricidal activity isolated and extracted from neem oil. In this study, a series of derivatives were obtained by structural modification of octadecanoic acid-3,4-tetrahydrofuran diester. The acaricidal activity of these derivatives indicated that introduction of benzyloxy substitution at the 2-position of the furan ring and the formation of a benzoate at the 3,4-position of the furan ring (benzoic acid-2-benzyloxy-3,4-tetrahydrofuran diester) could enhance the acaricidal activity. At

concentration of 20, 10, and 5 mg/ml, the median lethal time (LT₅₀) values of benzoic acid-2-benzyloxy-3,4-tetrahydrofuran diester were 16.138, 47.274, and 108.122 min, respectively. The LC₅₀ value of benzoic acid-2-benzyloxy-3,4-tetrahydrofuran diester at 60 min was 5.342 mg/ml. Transmission electron microscopy showed that after treatment with benzoic acid-2-benzyloxy-3,4-tetrahydrofuran diester, the body structure of mites was destroyed; dermal organelles were dissolved; nuclear chromatin was ablated. Further, transcriptome sequencing analysis was used to get insight into the acaricidal mechanism of benzoic acid-2-benzyloxy-3,4-tetrahydrofuran diester. The results showed that its acaricidal mechanism is related to interfering "energy metabolism" in *S. scabiei*, including processes such as citric acid cycle, oxidative phosphorylation pathway and fatty acid metabolism. Additionally, through the activity detection of the mitochondrial complexes of *S. scabiei*, it was further verified that the acaricidal mechanism of benzoic acid-2-benzyloxy-3,4-tetrahydrofuran diester was related to the energy metabolism system of *S. scabiei*.

Neem- Industrial Applications

Effect of organic inhibitor on the corrosion behaviour of reinforced cement concrete **R Dharmaraj, K Narayanan, M Ramalingam P Vinodhini Pon Rajalinggam**

Materials Today Proceedings <https://doi.org/10.1016/j.matpr.2022.09.353>

Neem tree production results in a significant amount of garbage produced each year. Reduced environmental pollution as a result of neem seed coverage is necessary. In order to partially replace cement in concrete, the usage of Neem extract was researched. In this research work, the corrosion inhibitor impact that has been added to concrete of steel reinforcement by means of NEEM extract. NEEM extract helps to protect the steel reinforcement from corrosion and increase the lifetime of steel. The neem extract (organic) as an accelerator in concrete behalf of using the chemical admixture. In this research articles M25 grade of concrete was used. In the neem extract can add in concrete at various ratios percentage as 0 % (M0), 0.5 % (M1), 1 % (M2), 1.5 (M3)%, and 2 % (M4). The mechanical test like compression test split tensile and flexural behavior test were performed at different curing days for various substitution of extract in concrete mix. The durability test like water absorption test was conducted after 28 days of curing. The strength properties shows good resistant against bending stress, it improves upto 14.08 % and 9.71 % for compressive and split tensile strength for 1 % substitution of extract, compared to control concrete mix. From the finding, substitution of NEEM extract shows good protection for concrete elements.