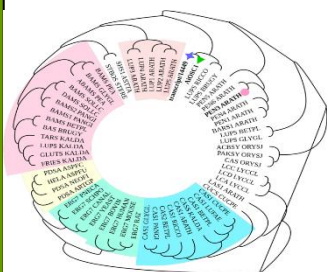


# Neem Research Newsletter

## Volume 2, Issue 3, 2022



**WORLD NEEM ORGANISATION (WNO)**



From  
The Editor's Desk.....

Neem Greetings! It is heartening to note publication of a benchmark study on economic impact of neem coated urea (NCU) on Indian agriculture. The researchers demonstrate the positive contribution of NCU in enhancing the yield and net returns of almost all reference crops by minimizing the use of urea as well as other fertilizers and pesticides. Phytochemical identification and characterization of neem components continue to interest researchers. On the health front, some novel findings include the protective effects of neem against osteoporosis and identification of molecular targets of neem limonoids. The pace of neem research seems to be increasing and hopefully, this newsletter may soon become a fortnightly or even weekly publication.

S. Nagini

Core Founding Member, WNO  
Chief Scientific Coordinator &

Regional Director, South India



## Neem in Agriculture

### **Fumigant toxicity of essential oils against *Frankliniella occidentalis* and *F. insularis* (Thysanoptera: Thripidae) as affected by polymer release and adjuvants.**

**Gharbi K, Tay JW.**

*Insects*. 2022 May 24;13(6):493. doi: 10.3390/insects13060493. PMID: 35735830

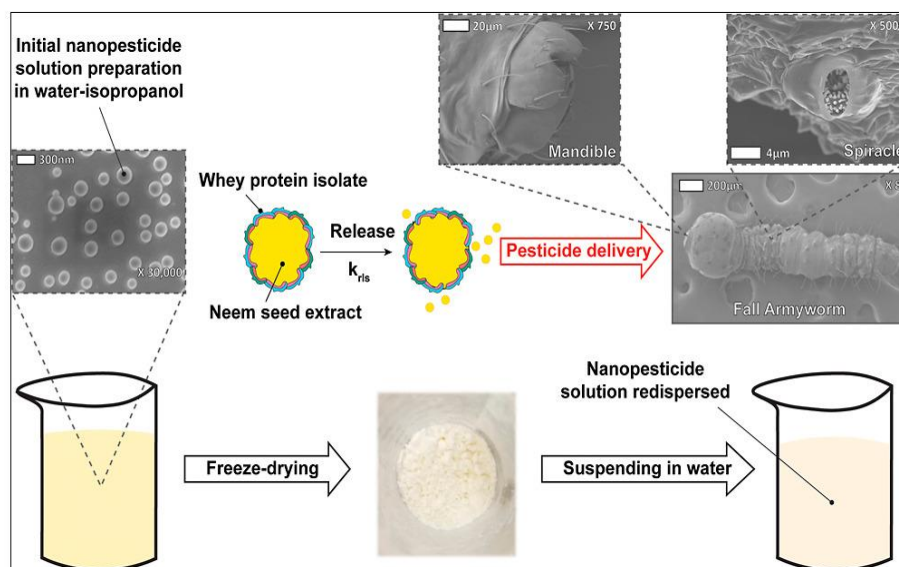
*Frankliniella occidentalis* is among the most economically significant pests of greenhouse crops, whose resistance to conventional insecticides has created demand for biopesticides such as essential oils. We assessed the fumigant toxicity of linalool against *F. occidentalis*, *F. insularis*, and *Solanum lycopersicum*. Thrips were fumigated with polyacrylamide hydrogels containing either (*R*)-linalool, (*S*)-linalool, racemic linalool, or a binary mixture of (*R*)-linalool with one of twelve adjuvants (i.e., peppermint, cedarwood, neem, clove, coconut, jojoba, soybean, olive,  $\alpha$ -terpineol, 1,8-cineole, trans-anethole, or (*R*)-pulegone). *Solanum lycopersicum* seedlings were exposed to (*R*)-linalool or a mixture of (*R*)-linalool and peppermint oil via conditioned hydrogels or foliar spray. For *F. insularis*, (*R*)-linalool was more toxic than (*S*)-linalool, with LC<sub>50</sub> values of 11.7 mg/L air and 16.7 mg/L air, respectively. Similarly for *F. occidentalis*, (*R*)-linalool was more toxic than (*S*)-linalool, with LC<sub>50</sub> values of 29.0 mg/L air and 34.9 mg/L air, respectively. Peppermint oil and  $\alpha$ -terpineol were the only synergists, while the other adjuvants exhibited varying degrees of antagonism. All seedling treatments demonstrated phytotoxicity, but symptoms were most severe for foliar sprays and mixtures containing peppermint oil. While hydrogels conditioned in linalool may be a favorable substitute to conventional insecticides, the cross-resistance demonstrated herein indicates that expectations should be metered.

### **Novel Biopesticides Based on Nanoencapsulation of Azadirachtin with Whey Protein to Control Fall Armyworm.**

**Bae M, Lewis A, Liu S, Arcot Y, Lin YT, Bernal JS, Cisneros-Zevallos L, Akbulut M.**

*J Agric Food Chem*. 2022 Jun 21. doi: 10.1021/acs.jafc.2c01558. Online ahead of print. PMID: 35727694

Biopesticides have become a global trend in order to minimize the hazards derived from synthetic chemical pesticides and improve the safety, efficacy, and environmental friendliness of agricultural pest management. Herein, we report a novel biopesticide composite encapsulating azadirachtin with the size of  $260.9 \pm 6.8$  nm and its effects on the insect pest *Spodoptera frugiperda* (fall armyworm). The nanocomposite biopesticide was produced via nano emulsification and freeze-drying process using whey protein isolate as a nanocarrier matrix to encapsulate azadirachtin, a natural insect-killing compound obtained from neem seed. We found that the nanocomposite biopesticide acted quicker and with greater efficacy than bulk azadirachtin treatment with corresponding LC<sub>50</sub> values within 11 days of *S. frugiperda* larvae survival. Through confocal microscopy, we found the enhanced biodistribution of the nanocomposite to all parts of the insect body. Photodegradation assays revealed an enhanced UV stability facilitated by light-scattering stemming from the intrinsic nanostructure and UV scavenging vitamin-E component.



**Ecdysone modulates both ultrastructural arrangement of hindgut and attachment of *Trypanosoma cruzi* DM 28c to the rectum cuticle of *Rhodnius prolixus* fifth-instar nymph.**

**Mendonça Lopes D D, Provençano AF AF, Mello CB CB, Feder D D, Albuquerque Cunha JMA JM, Sant'Anna NF NF, Curty Lechuga G GC, Cabral Bourguignon S SC, de Souza W W, de Souza Garcia E ES, Folly E EC, Azambuja P P, Gonzalez MS MS.**

*Exp Parasitol.* 2022 May-Jun;236-237:108247. doi: 10.1016/j.exppara.2022.108247. Epub 2022 Mar 17. PMID: 35307367

Studies on the effects of azadirachtin treatment, ecdysone supplementation and ecdysone therapy on both the ultrastructural organization of the rectum in 5th-instar nymph of *Rhodnius prolixus* and the ex vivo attachment behavior of *Trypanosoma cruzi* under these experimental conditions were carried out. Control insects had a typical and significant organization of the rectum cuticle consisted of four main layers (procuticle, inner epicuticle, outer epicuticle, and wax layer) during the entire period of the experiment. Both azadirachtin treatment and ecdysone supplementation avoid the development of both outer epicuticle and wax layer. Oral therapy with ecdysone partially reversed the altered organization and induce the development of the four main rectal cuticle layers. In the same way, the ex vivo attachment of *T. cruzi* to rectal cuticle was blocked by azadirachtin treatment but ecdysone therapy also partially recovered the parasite adhesion rates to almost those detected in control insects. These results point out that ecdysone may be a factor responsible - directly or indirectly - by the modulation of rectum ultrastructural arrangement providing a superficial wax layer to the attachment followed by metacyclogenesis of *T. cruzi* in the rectum of its invertebrate hosts.



## **Effects of azadirachtin on detoxification-related gene expression in the fat bodies of the fall armyworm, *Spodoptera frugiperda*.**

**Yu H, Yang X, Dai J, Li Y, Veeran S, Lin J, Shu B.**

*Environ Sci Pollut Res Int.* 2022 Mar 16. doi: 10.1007/s11356-022-19661-6. Online ahead of print. PMID: 35294689

The fall armyworm, *Spodoptera frugiperda*, has become a worldwide pest and threatens world food production. A previous study indicated that azadirachtin, the most effective botanical insecticide for *S. frugiperda*, inhibits larval growth of the insect. The effect of azadirachtin on the tissues of the larvae, however, remains to be determined. In this study, the effects of azadirachtin on the structure of fat bodies were analyzed. Comparative transcriptomic analysis was conducted between controls and samples treated with 0.1 µg/g azadirachtin for 7 days to explore potential relevant mechanisms. The expression of 5356 genes was significantly affected after azadirachtin treatment, with 3020 up-regulated and 2336 down-regulated. Among them, 137 encode detoxification enzymes, including 53 P450s, 20 GSTs, 27 CarEs, 16 UGTs, and 12 ABC transporters. Our results indicated that azadirachtin could destroy fat body structure and change the mRNA levels of detoxification-related genes. The up-regulated genes encoding detoxification enzymes might be related to detoxifying azadirachtin. Our results elucidate a preliminary mechanism of azadirachtin detoxification in the fat bodies of *S. frugiperda* larvae.

## **Purification and Characterization of Gum-Derived Polysaccharides of *Moringa oleifera* and *Azadirachta indica* and Their Applications as Plant Stimulants and Bio-Pesticidal Agents.**

**Shobana N, Prakash P, Samrot AV, Jane Cypriyana PJ, Kajal P, Sathiyasree M, Saigeetha S, Stalin Dhas T, Alex Anand D, Sabesan GS, Muthuvenkatachalam BS, Mohanty BK, Visvanathan S.**

*Molecules.* 2022 Jun 9;27(12):3720. doi: 10.3390/molecules27123720. PMID: 35744846

Plant gums are bio-organic substances that are derived from the barks of trees. They are biodegradable and non-adverse complex polysaccharides that have been gaining usage in recent years due to a number of advantages they contribute to various applications. In this study, gum was collected from *Moringa oleifera* and *Azadirachta indica* trees, then dried and powdered. Characterizations of gum polysaccharides were performed using TLC, GC-MS, NMR, etc., and sugar molecules such as glucose and xylose were found to be present. Effects of the gums on *Abelmoschus esculentus* growth were observed through root growth, shoot growth, and biomass content. The exposure of the seeds to the plant gums led to bio stimulation in the growth of the plants. Poor quality soil was exposed to the gum polysaccharide, where the polysaccharide was found to improve soil quality, which was observed through soil analysis and SEM analysis of soil porosity and structure. Furthermore, the plant gums were also found to have bio-pesticidal activity against mealybugs, which showed certain interstitial damage evident through histopathological analysis.

## **A benchmark study on economic impact of Neem Coated Urea on Indian agriculture.**

**Ramappa KB, Jadhav V, Manjunatha AV.**

*Sci Rep.* 2022 May 31;12(1):9082. doi: 10.1038/s41598-022-12708-1.PMID: 35641568

The policy of mandatory production and distribution of Neem Coated Urea (NCU) was implemented by the Government of India since 2015. In this article, authors have made an attempt to explore the benefits of NCU recognized by the producers of six major crops such as paddy, maize, sugarcane, tur, jute and soybean across six major states viz., Karnataka, Maharashtra, Madhya Pradesh, Bihar, Punjab and Assam. The results reveal that NCU use has contributed positively in terms of increasing the yield levels of main product and by-products, as well as net returns with regard to almost all reference crops however; the extent varies from crop to crop. Moreover, NCU has helped reduce the cost of production by minimizing the cost of urea as well as other fertilizers and pesticides usage. Interestingly, the diversion of urea has stopped completely, post the production and distribution of NCU. Hence, it is concluded that the application of NCU has a positive impact on Indian agriculture, by way of increasing yield levels & returns for the farming community. These results are in line with the PM's vision of doubling farmers' income by 2022 and Sustainable Development Goals of the Country.

## **Harnessing endophytic fungi for biosynthesis of selenium nanoparticles and exploring their bioactivities.**

**Hussein HG, El-Sayed ER, Younis NA, Hamdy AEHA, Easa SM.**

*AMB Express.* 2022 Jun 8;12(1):68. doi: 10.1186/s13568-022-01408-8.PMID: 35674975

In the light of the fast growing several applications of selenium nanoparticles (SeNPs) in different industrial and agricultural sectors, this paper was conducted to explore the suitability of endophytic fungi as nano-factories for SeNPs. Thus, 75 fungal isolates were recovered from plant tissues and tested for their efficacy to biosynthesize SeNPs. Four promising strains were found able to synthesis SeNPs with different characteristics and identified. These strains were *Aspergillus quadrilineatus* isolated from the twigs of *Ricinus communis*, *Aspergillus ochraceus* isolated from the leaves of *Ricinus communis*, *Aspergillus terreus* isolated from the twigs of *Azadirachta indica*, and *Fusarium equiseti* isolated from the twigs of *Hibiscus rose-sinensis*. The synthesized SeNPs were characterized by several techniques viz., UV-Vis, X-ray diffraction, Dynamic light scattering analyses, High resolution transmission electron microscopy, and Fourier transform infrared spectroscopy, to study their crystalline structure, particle sized distribution, and morphology. Furthermore, the in vitro antimicrobial and antioxidant activities were evaluated. SeNPs synthesized by the four strains showed potent antifungal and antibacterial potentials against different human and phyto- pathogens. Moreover, SeNPs synthesized by the respective strains showed promising antioxidant power with IC<sub>50</sub> values of 198.32, 151.23, 100.31, and 91.52 µg mL<sup>-1</sup>. To the best of our knowledge, this is the first study on the use of endophytic fungi for SeNPs' biosynthesis. The presented research recommends the use of endophytic fungi as facile one-pot production bio-factories of SeNPs with promising characteristics.

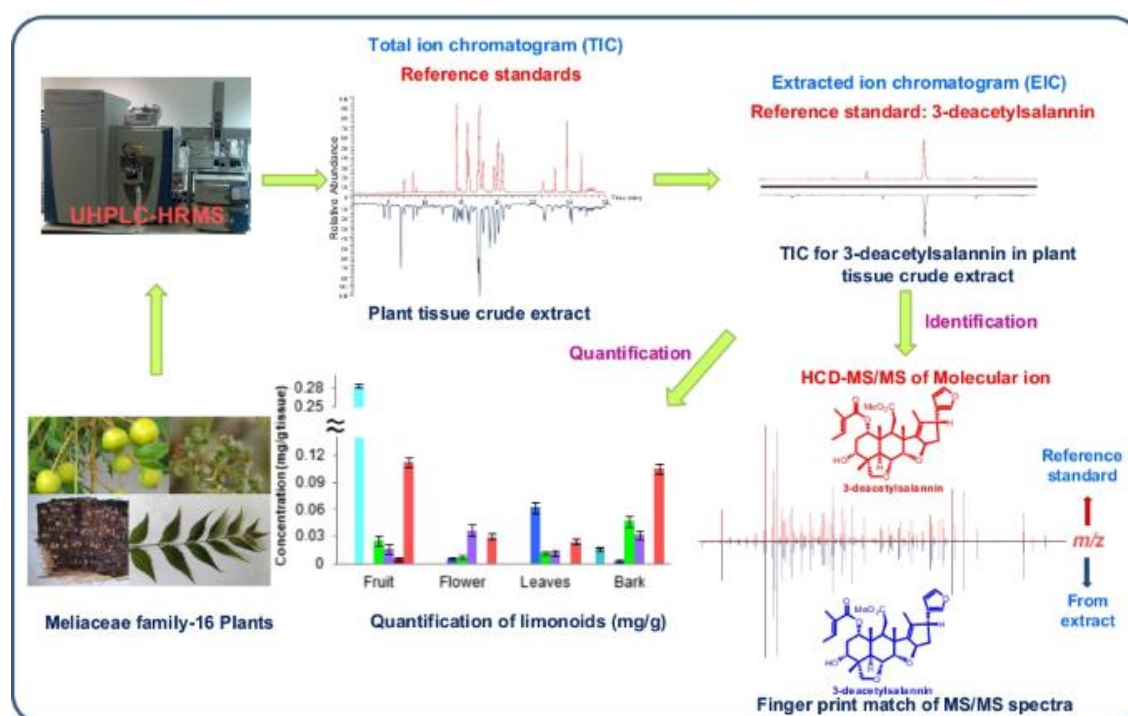
# Neem- Component Characterization

Ultra-high performance liquid chromatography Q-Orbitrap MS/MS-based profiling and quantification of limonoids in Meliaceae plants.

Mulani FA, Nandikol SS, Kajjihundi JS, Pathappa N, Puttappa S, Thulasiram HV.

*Anal Bioanal Chem.* 2022 Jun 21. doi: 10.1007/s00216-022-04169-2. Online ahead of print. PMID: 35727329

Meliaceae plants have been extensively used in agriculture, folklore, and traditional medicine. They are the major storehouses for structurally diverse limonoids (meliacins) possessing various bioactivities like antifeedant, insecticidal, antimicrobial, etc. However accurate detection of these tetranortriterpenes from the vast pool of metabolites in plant tissue extracts or biological sample is a crucial challenge. Though the mass spectrum (MS) provides the molecular mass and the corresponding elemental composition, it cannot be relied precisely. The exact identification of a specific metabolite demands the MS/MS spectrum containing the signature product ions. In the present study, we have developed the UHPLC Q-Orbitrap-based method for identification, quantification, and characterization of limonoids in different plant tissue extracts requiring minimum plant material. Using this method, we carried out the limonoid profiling in different tissue extracts of sixteen Meliaceae plants and the identification of limonoids was performed by comparing the retention time (RT), ESI-(+)-MS spectrum, and HCD-MS/MS of the purified fifteen limonoids used as reference standards. Our results revealed that early intermediates of the limonoid biosynthetic pathway such as azadiradione, epoxyazadiradione, and gedunin occurred more commonly in Meliaceae plants. The MS/MS spectrum library of the fifteen limonoids generated in this study can be utilized for identification of these limonoids in other plant tissue extracts, botanical fertilizers, agrochemical formulations, and biopesticides.



# Neem- Genomic Analysis

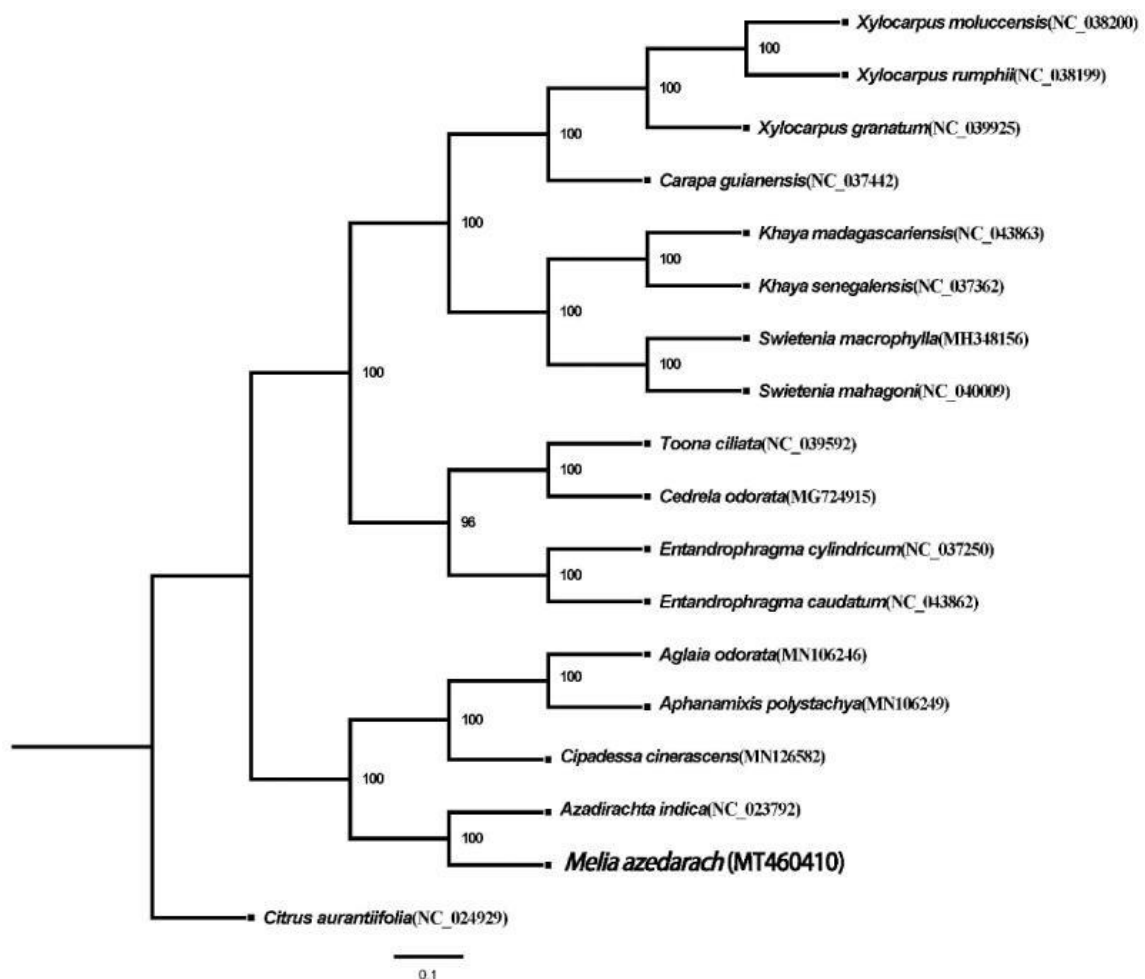
The complete genome sequence of *Melia azedarach* Linn. (Meliaceae): a multi-purpose pesticide species.

Liao B, Tan J, Zhou W, Wang Y, Li Y, Chen X.

*Mitochondrial DNA B Resour.* 2022 Jun 20;7(6):1103-1105. doi:

10.1080/23802359.2020.1815600. eCollection 2022. PMID: 35756442

The plant genus *Melia* has two or four species in the modern world, and is a natural source of a traditional pesticide, Toosendanin. In this study, we report the complete chloroplast genome of *Melia azedarach*, assembled from whole-genome high-throughput sequencing data, as a resource for future studies on the taxonomy and evolution of *Melia*. The chloroplast genome was 160,393 bp in length, with a large single-copy region of 87,598 bp, a small single-copy region of 18,709 bp, separated by two inverted repeat regions of 27,043 bp each. It was predicted to contain a total of 133 genes, with an overall GC content of 37.37%. Phylogenetic analysis placed *M. azedarach* closest to *Azadirachta* sp. in Meliaceae.





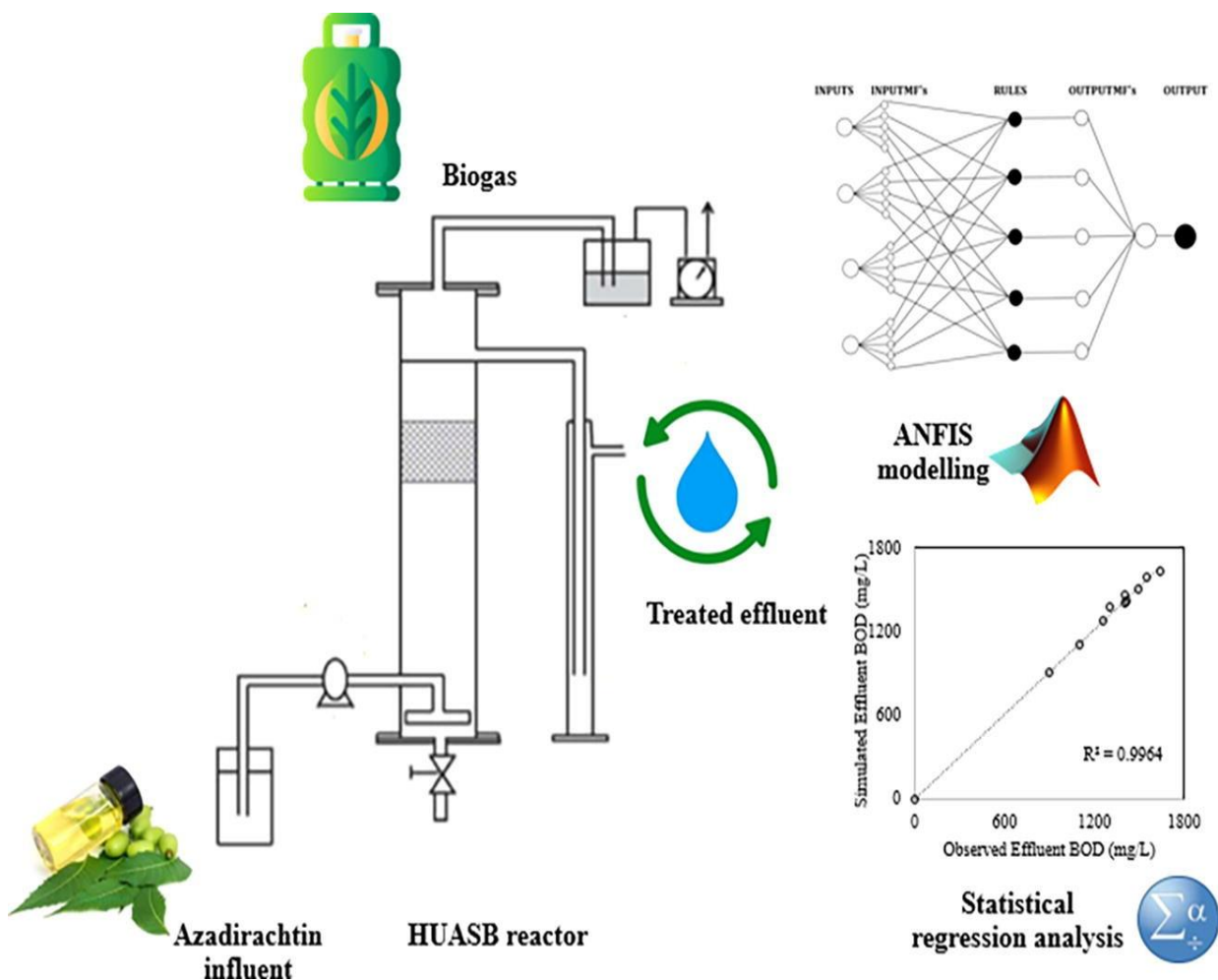
# Neem For Sustainable Environment

Experiments and adaptive-network-based fuzzy inference system modelling in a hybrid up-flow anaerobic sludge blanket reactor to assess industrial azadirachtin effluent quality.

Mullai P, Vishali S, Sobiya E.

*Bioresour Technol.* 2022 May 27;358:127395. doi: 10.1016/j.biortech.2022.127395. Online ahead of print. PMID: 35636676

Experimental investigations were carried out for the treatment of industrial azadirachtin effluent in a hybrid up-flow anaerobic sludge blanket (HUASB) reactor continuously for 115 days in three stages at mesophilic temperature (30 - 35°C). An adaptive-network-based fuzzy inference system (ANFIS) modelling and statistical regression analysis were applied with the raw data. In the ANFIS modelling as well as in the statistical regression analysis, the operating parameters such as initial pH, influent COD, effluent COD and biogas generation ( $X_1$ ,  $X_2$ ,  $X_3$  and  $X_4$ ) were taken as variables and effluent BOD values as a response ( $Y$ ). The average percentage error (APE) values of ANFIS modelling were 2.18, 12.29, and 0.01%, for stage-I, II and III respectively. These values indicated that ANFIS modelling performed well in all the three stages and provided more accurate results.



# Neem For Human Health

## Osteoporotic Remedy

**Biochemometry-Based Discovery of Phenylpropanoids from *Azadirachta indica* Fruits as Inhibitors of In Vitro Osteoclast Formation.**

**Tahir A, Kampleitner C, Wirglauer T, Grienke U, Hoffmann O, Rollinger JM.**

*Molecules*. 2022 Jun 4;27(11):3611. doi: 10.3390/molecules27113611.PMID: 35684547

(1) Background: Inhibition of osteoclast differentiation is the key approach in treating osteoporosis. However, using state-of-the-art treatments such as bisphosphonates and estrogen-based therapy is usually accompanied by many side effects. As opposed to this, the use of natural products as an osteoporotic remedy delivers promising outcomes with minimal side effects. (2) Methods: In the present study, we implemented a biochemometric workflow comprising (i) chemometric approaches using NMR and mass spectrometry and (ii) cell biological approaches using an osteoclast cytochemical marker (TRAP). The workflow serves as a screening tool to pursue potential in vitro osteoclast inhibitors. (3) Results: The workflow allowed for the selective isolation of two phenylpropanoids (coniferyl alcohol and sinapyl alcohol) from the fruits of neem tree (*Azadirachta indica*). These two isolated phenylpropanoids showed a very promising dose-dependent inhibition of osteoclast differentiation with negligible effects in terms of cell viability. (4) Conclusion: The presented workflow is an effective tool in the discovery of potential candidates for osteoclast inhibition from complex extracts. The used biochemometric approach saves time, effort and costs while delivering precise hints to selectively isolate bioactive constituents.

## Wound Healing

**Hypericum and neem oil for dehiscenced post-surgical wounds: a randomised, controlled, single-blinded phase III study.**

Arena R, Strazzeri MG, Bianchi T, Peghetti A, Merli Y, Abbenante D, Olivari D.

*J Wound Care*. 2022 Jun 2;31(6):492-500. doi: 10.12968/jowc.2022.31.6.492.

PMID: 35678785

**Objective:** To evaluate the clinical efficacy of a hypericum and neem oil dressing, Primary Wound Dressing [ONE] (1PWD) (Kerecis AG, Switzerland), in a patient population with dehiscence of surgical wounds with critical colonisation/infection. Efficacy was defined as resolution of inflammatory/infective symptoms. **Method:** A randomised, controlled, single-blinded, parallel-arms phase III study was conducted comparing the experimental medication to silver-based dressings. All patients were evaluated at enrolment, on days 7, 14, 21 and 28. Improvement of inflammatory/infective symptoms was measured by detecting seven items of the Bates-Jensen Wound Assessment Tool (BWAT). Pain was assessed using the Numeric Rating Scale (NRS). **Results:** The study enrolled 99 patients. Follow-up was completed in 49 patients in the experimental group and 48 patients in the control group.

Overall BWAT evaluation demonstrated similar outcomes between the groups:  $t=0.23$ ,  $p$ -value= $0.81$ , 95% confidence interval (CI):  $-13.3-10.8$ . Furthermore, when evaluating the seven items of the BWAT relating to inflammatory signs, there was not a significant difference between the groups:  $t=0.38$ ,  $p=0.35$ , 95% CI:  $-2.8-2.7$ . However, when an analysis using the NRS pain scale was performed, a statistically significant pain reduction was demonstrated in favour of the experimental group:  $t=7.8$ ,  $p<0.0001$ , 95% CI:  $2.918-4.8819$ . **Conclusion:** This randomised controlled trial confirmed the efficacy of 1PWD, an investigational product, in the management of surgical dehiscence with critical colonisation or infection, with the added benefit of significant pain reduction when compared with a silver-based dressing.

## Orodonal Protection

### Evaluation of *In Vitro* Antiprotease Activity of Selected Traditional Medicinal Herbs in Dentistry and Its *In Silico* PASS Prediction.

Assiry AA, Bhavikatti SK, Althobaiti FA, Mohamed RN, Karobari MI.

*Biomed Res Int.* 2022 Jun 6;2022:5870443. doi: 10.1155/2022/5870443. eCollection 2022. PMID: 35707383

**Background:** Dental/oral diseases are one of the significant public health problems globally. Herbal medicines for managing oral diseases are considered an effective alternative to synthetic compounds due to their lower side effect. *Azadirachta indica*, *Terminalia chebula*, *Camellia sinensis*, and *Piper nigrum* are used to control and prevent oral inflammations in dentistry. In this study, we have evaluated the protease inhibition activity of these plant extracts, and further, the binding mode of the active ingredient of these plants with trypsin was studied using molecular docking. **Methods:** In this study, protease inhibition activity was carried out using aqueous extracts of the plant parts such as *Azadirachta indica* (neem) twig, *Terminalia chebula* (Haritaki) fruit, *Camellia sinensis* (green tea) powder, and *Piper nigrum* (kali miri) seed. Next, to explore the binding mode of active ingredients azadirachtin, chebuligenic acid, catechin, and piperine with trypsin, we employed a molecular docking study using AutoDock4.2. **Results:** The results revealed that the *Azadirachta indica* plant extract showed an IC<sub>50</sub> value of  $96.19 \mu\text{g mL}^{-1}$ , *Camellia sinensis* IC<sub>50</sub> value of  $188.50 \mu\text{g mL}^{-1}$ , *Piper nigrum* IC<sub>50</sub> value of  $371.20 \mu\text{g mL}^{-1}$ , and *Terminalia chebula* IC<sub>50</sub> value of  $639.48 \mu\text{g mL}^{-1}$ , when compared with standard drug diclofenac sodium, had IC<sub>50</sub> value  $93.00 \mu\text{g mL}^{-1}$ . Further, the docking result reveals that all the main active ingredients of these plants have significant binding affinity and prefer the same binding pocket of trypsin. **Conclusion:** Hence, our results show the importance of traditional plants *Azadirachta indica*, *Terminalia chebula*, green tea, and *Piper nigrum* to control oral disease conditions. As they show significant protease inhibition activity, hence, the active ingredient could act as a potential anti-inflammatory agent and further help to prevent or control oral disease conditions such as gingivitis and periodontitis.

## **Comparative Evaluation of *Azadirachta indica* (Neem) Chip and Soft Tissue Diode Lasers as a Supplement to Phase I Periodontal Therapy in Localized Chronic Moderate Periodontitis: A Randomized Controlled Clinical Trial.**

**Ganvir MN, Parwani SR, Chaudhary DS, Parwani R, Dadlani H, Vikey AK, Kawadkar KP, Jaju NS, Armogida NG, Spagnuolo G.**

*Int J Dent.* 2022 Jun 15;2022:6109040. doi: 10.1155/2022/6109040. eCollection 2022. PMID: 35756957

**Introduction:** The current trial aimed to assess and compare the efficacy of neem chip and diode laser as a local drug delivery (LDD) agent as a supplement to phase I periodontal therapy in treatment of localized chronic moderate periodontitis. *Materials and Methodology.* Fourteen systemically healthy participants with 4-6 mm deep periodontal pockets at least in three quadrants (with no alveolar bony defect amenable to respective or regenerative osseous surgery, as seen in orthopantomograph) were selected for the trial. One week after phase I therapy, 10% absorbable chip of neem (commercially prepared by staff of a pharmacy college, Sheriguda, India) was placed in the periodontal pocket on one site, and soft tissue diode laser pocket sterilization was performed on the other site of the arch. Remaining one site was considered as a control. Parameters recorded clinically were plaque index (PI), papillary bleeding index (PBI), probing pocket depth (PPD), and relative attachment level (RAL) measured at baseline, 21<sup>st</sup> day, and one month postoperatively.

**Results:** Statistically significant improvements were observed in all clinical parameters at one month as compared to baseline for both treatment groups. **Conclusion:** Neem chip supplemented with phase I therapy showed best improvement in clinical parameters followed by laser supplemented with phase I therapy in comparison to phase I therapy alone at one month follow-up. *Clinical Significance.* Neem chips are nature's products, affordable without side effects, with a potential to be used as a local drug delivery agent in treating moderate chronic periodontitis.

## **Comparative Evaluation of Antimicrobial Efficacy of Zinc Oxide Eugenol with Zinc Oxide Mixed with Three Herbal Products to be Used as Root Canal Filling Material: An *In Vitro* Study.**

**Wasnik MB, Mittal R, Sajjanar A, Gahlod N, Khekade S, Shukla H.**

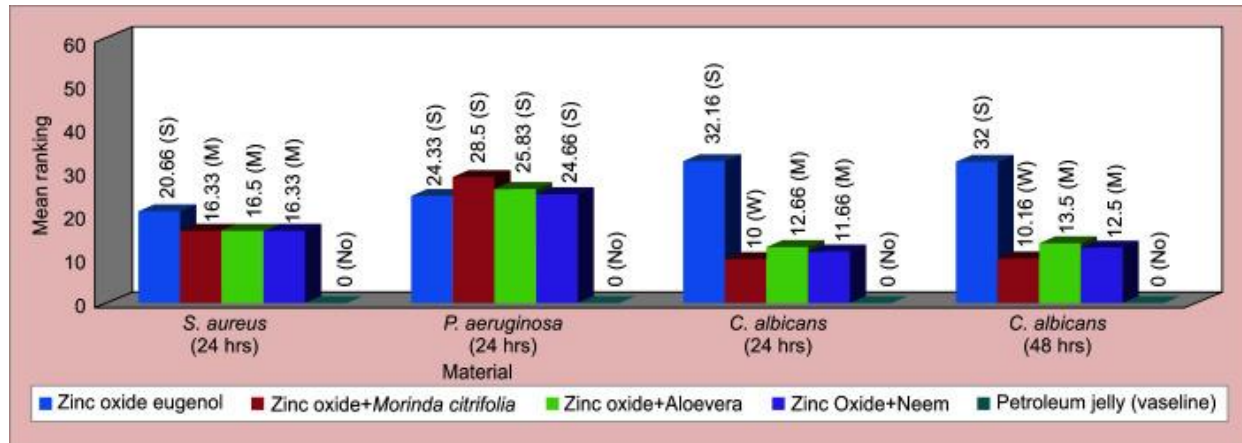
*Int J Clin Pediatr Dent.* 2022;15(Suppl 1):S40-S46. doi: 10.5005/jp-journals-10005-2129. PMID: 35645491

**Introduction:** Primary teeth with pulpal involvement and those having periapical issues should be retained until their normal exfoliation because their premature loss may lead to adverse aberrations in the future dentition. Root canals harbor different types of microorganisms and root canal infections generally are polymicrobial in nature. One of the most common and preferred root canal filling material which is commonly used for primary teeth is zinc oxide eugenol (ZOE) cement. **Aims and objectives:** To evaluate and compare the antimicrobial efficacy of ZOE with zinc oxide powder mixed with *Morinda citrifolia* extract, *Aloe vera* extract, and neem extract against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*. **Materials and methods:** The material used in the study were zinc oxide powder, eugenol liquid, *M. citrifolia* extract, *A. vera* extract, neem extract, petroleum jelly (Vaseline). The zinc oxide powder was mixed with minimum inhibitory concentration (MIC) percentage value of herbal extract.



**Result:** Zinc oxide eugenol showed strong inhibitory effect against *S. aureus* and *C. albicans*. For *P. aeruginosa*, zinc oxide+*M. citrifolia* showed strong inhibitory. Petroleum jelly (Vaseline) was used as control agent which showed no inhibitory effect.

**Conclusion:** The test root canal filling materials, i.e., ZOE, zinc oxide powder mixed with *M. citrifolia* extract, *A. vera* extract, and neem extract, respectively showed varied antimicrobial activity against the microorganisms tested, i.e., *S. aureus*, *P. aeruginosa*, and *C. albicans*.



### Herbal Agents versus Ethylene Diamine Tetra Acetic Acid on Removal of the Smear Layer-A Systematic Review of In Vitro Studies.

Teja KV, Janani K, Alqahtani AA, Robaian A, Alhalabi F, Merdad KA, Alam MK, Shrivastava D, Jose J, Srivastava KC.

*Int J Environ Res Public Health.* 2022 Jun 3;19(11):6870. doi: 10.3390/ijerph19116870. PMID: 35682452

This systematic review aimed to compare the efficacy of herbal agents with ethylene diamine tetraacetic acid (EDTA) in removing the smear layer during root canal instrumentation. The research question in the present study was to assess: "Is there a significant difference in reducing smear layer comparing EDTA and herbal agents?" Electronic databases (PubMed, Scopus, and Web of Science) were searched from their start dates to April 2022 using strict inclusion and exclusion criteria, and reviewed following PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) 2020 guidelines. Only in vitro studies comparing herbal agents with EDTA were included in the current systematic review. Two reviewers independently assessed the included articles. A total of 625 articles were obtained from an electronic database. Eighteen papers were included for review of the full text, out of which, ten papers were excluded because they did not meet the inclusion criteria. Finally, eight articles were included in the systematic review. The present systematic review considered only in vitro studies; hence, the result cannot be completely translated to strict clinical conditions. The results of the present systematic review have shown that *quixabeira*, *morindacitrifolia*, *oregano* extract, and neem show better smear layer removal compared to other herbal agents, whereas they showed reduced smear layer removal when compared with EDTA. Although, it was seen that most of the included studies did not report a high quality of evidence. Hence, the present systematic review concludes that herbal agents have reported to show inferior smear layer removal when compared to EDTA. Thus, as far as herbal based alternatives are concerned, there is no highest level of evidence to state its real benefit when used as a chelating root canal irrigant.

## Antidiabetic Effects

**Use of haematological signatures in conjunction with conventional biomarkers to assess Reno-protective effects of Gedunin in diabetic nephropathy of Streptozotocin induced diabetic rats.**

**Mazumdar S, Marar T, Patki J.**

*J Diabetes Metab Disord.* 2022 Jan 20;21(1):323-332. doi: 10.1007/s40200-022-00977-6. eCollection 2022 Jun. PMID: 35673464

**Purpose:** The present study was aimed at analysing the reno-protective potential of Gedunin (a limonoid from Neem leaves) through haematological and serological assays and renal histopathology studies in streptozotocin induced diabetic rats. **Method:** Four weeks old Wister albino rats were chosen for the study. The rats were divided into four groups of 8 rats each. Group 1 (normal control rats); Group 2 (diabetic control rats); Group 3 (Drug control rats: normal rats treated with 1 mg/kg body weight of Gedunin thrice a week through oral gavage for 2 weeks); Group 4 (diabetic rats treated with Gedunin similar to Group 3). Diabetes was induced using a single intraperitoneal injection of streptozotocin (50 mg/kg) in the pre-determined groups. After completion of treatment, the rats were sacrificed and haematological and serological parameters including functional indices of blood cells and renal markers were evaluated through spectrophotometric methods. Renal histology studies were carried out and images were captured on Olympus SC 100, 10 Mega Pixel camera. **Results:** Streptozotocin induced diabetes lead to marked reduction in the RBC count, haemoglobin, PCV, WBC count, lymphocyte, elevated levels of fasting blood glucose, kidney function markers such as urea, creatinine, uric acid in rats. Administration of Gedunin significantly ( $P < 0.05$ ) ameliorated the elevated levels of fasting glucose, RDW%, MCHC%, NLR% and platelet count. A significant reversal of renal tissue damage was also observed. **Conclusion:** The restoration of renal serological markers and reversal of renal tissue injuries are all indicative of promising reno-protective potential of Gedunin. Remarkable improvement of haematological parameters supports their use as reno-protection markers.

**Assessment of Antioxidative and Alpha-Amylase Potential of Polyherbal Extract.**

**Patwekar M, Patwekar F, Mezni A, Sanaullah S, Fatema SR, Almas U, Ahmad I, Tirth V, Mallick J.**

*Evid Based Complement Alternat Med.* 2022 May 31;2022:7153526. doi: 10.1155/2022/7153526. eCollection 2022. PMID: 35685725

The present study aims to prepare a polyherbal formulation (PHF) of *Azadirachta indica* (Neem), *Aloe barbadensis* (Aloe vera), *Allium sativum* (garlic), *Acacia arabica* (Babul), and *Aegle marmelos* (Bel) and evaluation of antidiabetic and antioxidant activity utilizing the in vitro model. Air-dried powder of 5 medicinal plants, which are divided into equal portions, and PHF, is prepared by the soxhlet technique using polar and nonpolar solvents. The PHF is screened for the phytochemical screening, and then the antidiabetic activity is determined by alpha-amylase inhibition. The extracts thus obtained are also subjected to the inhibition assay by the use of (DNS) dinitro salicylic acid. The antioxidant

activity was determined by the DPPH radical scavenging assay, H<sub>2</sub>O<sub>2</sub> scavenging assay, and TBARS assay. In in vitro study, the result revealed polyherbal formulation in which hot water extract has the topmost inhibitory effect on alpha-amylase activity, ranging from 20.4% to 79.5% with an IC<sub>50</sub> value of 48.98 ± 0.31 µg/ml. This extract clearly showed the effective lowering of postprandial hypertriglyceridemia (PPHG). In the antioxidant activity carried out by using the (DPPH) radical scavenging assay, the highest result was obtained by the concentration of 250 µg/ml, which was around 77.2 ± 0.05 with statistical significance compared with control (a:  $p < 0.01$ ; b:  $p < 0.001$ ), while in the GTA method, the highest result was obtained by the concentration of 250 µg/ml, which was around 78.2 ± 0.05, and in the case of the TBARS assay, the concentration of 250 µg/ml gave around 76.2 ± 0.03 antioxidant value. In conclusion, the study shows that polyherbal formulation has superior antidiabetic activity and antioxidant properties.

## Anticancer Effects

### Anti-Cancer Activity of Gedunin by Induction of Apoptosis in Human Gastric Cancer AGS Cells.

Zhou H, Li F, Li Y.

*Appl Biochem Biotechnol.* 2022 Jun 27. doi: 10.1007/s12010-022-04001-8. Online ahead of print. PMID: 35759172

Currently, gastric cancer is considered one of the major causes of high mortality and morbidity worldwide. Recent advances in therapeutics, clinical treatment, staging procedures, and imaging techniques are high, yet the prevalence of gastric cancer has not been reduced. Usage of the synthetic drug has many side effects that can lead to other ailments. Gedunin, a phytochemical derived from *Azadirachta indica* (neem tree), exhibits several pharmacological activities including antitumor, anti-inflammatory, antiulcer, antipyretics, antibacterial, antifungal, anti-diabetic, and antimalarial properties. In the current investigation, the effect of gedunin on the cell viability; reactive oxygen species (ROS) generation by DCFH-DA staining; mitochondrial membrane potential (MMP) by Rh-123 staining; apoptosis by AO/EtBr staining; cell migration and wound healing ability by wound scratch assay; and Bcl-2, Bax, caspase-3, and caspase-9 by ELISA techniques were analyzed in the AGS cells. The treatment with gedunin effectively inhibited the cell viability with IC<sub>50</sub> = 20µM, increased the ROS generation, and triggered the apoptosis in AGS cells. The gedunin-treated AGS cells also demonstrated a decreased MMP status. The increment in the ROS generation leads to oxidative stress which in turn induce the apoptosis. The activity of Bax gene was upregulated and the activity of Bcl-2 gene was down-regulated in the AGS cells after the treatment with gedunin. In the AGS cells treated with gedunin, the caspase-3 and caspase-9 activities were increased. In overall, these findings suggested that gedunin can be used as a potent chemotherapeutic agent in the future to treat gastric cancer.

## **Molecular Targets of Nimbolide for Anti-Cancer Therapy: An Updated Review.**

**Elumalai P, Ezhilarasan D, Raghunandhakumar S.J**

*Environ Pathol Toxicol Oncol.* 2022;41(2):69-88. doi: 10.1615/J  
*EnvironPatholToxicolOncol.2021040263. PMID: 35695653*

Cancer is a major cause of death worldwide with an increasing incidence rate and is considered a major public health problem. Distance metastasis to other tissues, high toxicity, and drug resistance of cancer cells to chemotherapy demand novel therapeutic approaches to treat cancer. Natural compounds from medicinal plants have been studied for therapeutic use in various malignancies. Nimbolide is an active principal compound from *Azadirachta indica*, which is an Asian traditional medicinal plant utilized historically as a remedy for a variety of diseases due to its antioxidant, anti-inflammatory, anti-cancer, and antimicrobial properties. It is a limonoid triterpene possessing potent anti-cancer effects in various types of cancers. It has been reported to induce multiple cytotoxic effects in tumor cells by modulating the cell proliferation, cell cycle, apoptosis, and metastasis by altering the various molecular signaling pathways. In the present review, we summarized all the in vitro and in vivo studies reporting the molecular targets of nimbolide for the therapeutic approaches in different types of cancer cells. We analyzed research publications up to September 2021 on the effect of nimbolide in various malignancies and the molecular mechanism of action. Nimbolide targets different signaling pathways including epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), insulin like growth factor (IGF), Wingless and INT-1 (Wnt)/ $\beta$ -catenin, mitogen-activated protein kinases (MAPK)/c-Jun N-terminal kinases (JNK), phosphoinositide 3-kinase (PI3K)/AKT, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ )/nuclear factor kappa B (NF- $\kappa$ B), and death receptor 5 (DR5) in several cancer cells. Nimbolide's widespread availability and absence of side effects, as well as understanding the molecular mechanism of nimbolide's action, will be useful to develop a therapeutic agent against cancer.

## **Design, synthesis and cytotoxic activity studies of alkyne linked analogues of Nimbolide.**

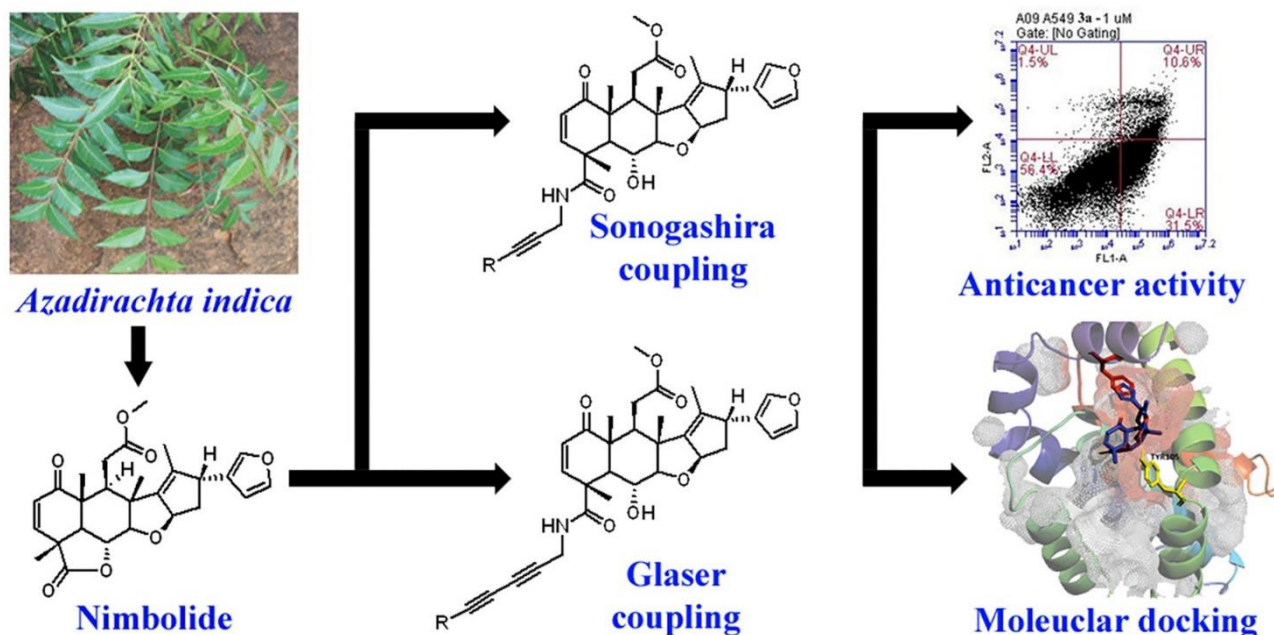
**Manga B, VenkateswaraRao B, Sudeshnakopparapu, Balaji AS, Jadav SS, Ramalingam V, Babu KS.**

*Fitoterapia.* 2022 Jun 24:105246. doi: 10.1016/j.fitote.2022.105246. Online ahead of print. PMID: 35760229

A series of novel nimbolide derivatives bearing various substitutions on 28th position was designed and synthesized using Sonogashira (2a-2p) and Glaser coupling (3a-3e) reactions. The synthesized derivatives were assessed for in vitro cytotoxic activity against four different human cancer cell lines (A549 cells, MCF-7 cells, MDA-MB-231 cells, and HCT15 cells) and normal cell line (HEK cells) using MTT assay. Among the screened derivatives, the compound 3a showed potent activity against A549 cells with IC<sub>50</sub> value of 0.23  $\mu$ M as comparing with parent molecule 1 (1.48  $\mu$ M) and the standard drug doxorubicin (0.82  $\mu$ M). As well, the flow cytometry analysis confirmed that the compounds 1 and 3a arrest the cell cycle progress at S phase and induce the early apoptosis in the lung cancer. The qRT-PCR analysis revealed that the compounds 1 and 3a downregulate the Bcl2 expression and upregulates the Bax gene expression level in A549 cells. The strong binding



affinity of the compounds 1 and 3a with Bcl2 was also confirmed using molecular docking analysis. Together, the results suggested that the compound 3a is a promising anticancer agent against lung cancer is deserved for further investigation.



**Antiproliferative efficacy of the antioxidant bioactive compounds of defatted seeds of *Azadirachta indica* and *Momordica charantia* against the regulatory function of tumor suppressor gene inducing oral carcinoma.**

**Sharma Y, Kaur A, Mishra R, Kulkarni S, Bharadwaj M, Bala K.**

*J Biomol Struct Dyn.* 2022 Jun 8:1-15. doi: 10.1080/07391102.2022.2083015. Online ahead of print. PMID: 35674735

The present study focuses on the antiproliferative activity of polyphenolic flavonoids found in defatted seeds of *Azadirachta indica* and *Momordica charantia* with the regulatory function of tumor suppressor genes inducing Oral Squamous Cell Carcinoma. Polyphenolic flavonoid in extracts was characterized using chromatographic analysis and has confirmed the presence of quercetin, rutin and tannic acid in the extracts of *A. indica* and *M. charantia*. According to DPPH assay and reducing power assays, free radical scavenging was found to be high in ethanolic extract of defatted seeds. Antiproliferative efficacies of defatted seed extracts against KB cell line (mouth) were studied by MTT assay and revealed that aqueous extract of defatted seeds of *M. charantia* has exhibited maximum antiproliferative activity against KB cells. Antioxidant activity of defatted seed extracts were observed on treated KB cells by determining enzymatic activity (SOD, Cat, and GST) and nonenzyme content (GSH and MDA Content). Using the AutoDock tool, quercetin, rutin and tannin acid revealed that mutant p53, TWIST related protein, TGF- $\beta$  and Snail I have the best binding energy results. MD simulation was observed on best docking results between the molecule and identified flavonoid by Desmond V 5.9 package. This leads to the conclusion that bioactive extracts with antiproliferative activity, antioxidant capacity and polyphenols with binding efficacy against tumor suppressor gene regulatory function could be used as a herbal remedy.

## Drug Targeting

### **Molecular docking study of GSK-3 $\beta$ interaction with nomilin, kihadanin B, and related limonoids and triterpenes with a furyl- $\delta$ -lactone core.**

**Vergoten G, Bailly C.**

*J Biochem Mol Toxicol.* 2022 Jun 10:e23130. doi: 10.1002/jbt.23130. Online ahead of print. PMID: 35686814

Glycogen synthase kinase-3 $\beta$  (GSK-3 $\beta$ ) is a target enzyme considered for the treatment of multiple human diseases, from neurodegenerative pathologies to viral infections and cancers. Numerous inhibitors of GSK-3 $\beta$  have been discovered but thus far only a few have reached clinical trials and only one drug, tideglusib (1), has been registered. Natural products targeting GSK-3 $\beta$  have been identified, including the two anticancer limonoids obacunone (5) and gedunin (4), both presenting a furyl- $\delta$ -lactone core. To help identifying novel GSK-3 $\beta$  ligands, we have performed a molecular docking study with 15 complementary natural products bearing a furyl- $\delta$ -lactone unit (such as limonin (6) and kihadanins A (8) and B (9)) or a closely related structure (such as cedrelone (10) and nimbolide (11)). The formation of GSK-3 $\beta$ -binding complexes for those natural products was compared to reference GSK-3 $\beta$  ATP-competitive inhibitors LY2090314 (3) and AR-A014418 (2). Our in silico analysis led to the identification of two new GSK-3 $\beta$ -binding natural products: kihadanin B (9) and nomilin (7). The latter surpassed the reference compounds in terms of calculated empirical energy of interaction ( $\Delta E$ ). Nomilin (7) can possibly bind to the active site of GSK-3 $\beta$ , notably via the furyl- $\delta$ -lactone core and its 1-acetyl group, implicated in the protein interaction. Compound structure-binding relationships are discussed. The study should help the discovery of novel natural products targeting GSK-3 $\beta$ .

## Neem in Veterinary Science

### **A survey on ethnoveterinary medicines used by the tribal migratory shepherds of Northwestern Himalaya.**

**Radha, Prakash S, Sharma N, Kumar A, Kumari N, Puri S, Pundir A, Kumar V, Sharma AK, Rais N, Dey A, Lorenzo JM, Mekhemar M, Kumar M.**

*J Ethnopharmacol.* 2022 Jun 20:115467. doi: 10.1016/j.jep.2022.115467. Online ahead of print. PMID: 35738470

**Ethnopharmacological relevance:** Tribal migratory shepherds (Gaddi) living in the Northwestern Himalayan region are well known for their nomadic lifestyle in which tribal migratory shepherds along with their livestock which mainly include sheep and goat migrate seasonally in the Northwestern Himalayan region from high hills to low hills for the survival of their livestock from cold winters and hot summer. Due to harsh environmental conditions, heavy snowfall, heavy rainfall, wild animal attacks, no medical facilities, no transportation, and no electricity facilities tribal migratory shepherds mostly rely on plant species mentioned for ethnoveterinary use to treat livestock disease. **Aim of study:** The aim of our study is to conduct ethnoveterinary survey for the first time to document the contemporary oral

ethnoveterinary knowledge of Gaddi shepherds living in Northwestern Himalayan region. **Methodology:** The documentation of plant species mentioned for ethnoveterinary use was executed through extensive field surveys from 2017 to 2019. Data were collected through direct interviews by administering questionnaire among tribal migratory shepherds. **Results:** A total of 181 plant species mentioned for ethnoveterinary use belonging to the same or different families were documented during the seasonal migration of shepherds from Northwestern Himalayan region. Most frequently occurring family of plant species mentioned for ethnoveterinary was Poaceae, leaves and roots were reported to be the most frequently used plant parts. Most frequently documented plant species for ethnoveterinary use were *Cynodon dactylon*, *Chenopodium album*, *Zanthoxylum armatum*, *Aloe vera*, *Azadirachta indica* and *Cannabis sativa*. *Chrysopogon serrulatus*, *Cynodon dactylon*, and *Vitex negundo* were reported with high use reports. Some of the endemic species of Northwestern Himalayan region such as *Elymus himalayanus* and *Euphorbia pilosa* and one endangered species *Picrorhiza kurroa* were reported in current study. **Conclusion:** It is observed that there is a need to raise awareness among the tribal migratory shepherds about the sustainable use and conservation of some of the plant species mentioned for ethnoveterinary use. This study provided an inventory of plant species mentioned for ethnoveterinary use having significant ethnoveterinary potential, however there is need of scientific evaluations by in vitro, in vivo and clinical studies.

#### **Influence of *Azadirachta indica* and *Cnidioscolus angustidens* dietary extracts on equine fecal greenhouse gas emissions.**

**Alvarado TD, Elghandour MMY, Ekanem NJ, Alcalá-Canto Y, Velázquez AE, Pacheco EBF, Purba RAP, Salem AZM.**

*J Equine Vet Sci.* 2022 Jun 15:104049. doi: 10.1016/j.jevs.2022.104049. Online ahead of print. PMID: 35716836

The present study was conducted to investigate the flavonoid extracts of *Azadirachta indica* (AZN), *Cnidioscolus angustidens* (CNA), and their combination at dosages (MIX) of 0, 0.6, 1.2, and 1.8 mL for their ability to reduce greenhouse gases and fermentation profiles in an in vitro study using horse feces and a nutrient-dense diet (as substrate). The quantity of greenhouse gas and fermentation profiles and were determined in in vitro incubation for 48 h. Extracts of AZN, CNA, and MIX reduced total gas production in the incubated and degraded substrates in a dose-dependent and time-dependent manner. Production of CH<sub>4</sub> was reduced (P <0.05) by 4.41-54.54% in the incubated substrates and by 1.16 - 61.82% in the degraded substrates. However, AZN and MIX reduced (P <0.05) CO by 4.43 - 12.85% in the incubated substrates and by 0.70 - 16.78% in the degraded substrates. In like manner, the plant extracts and combination reduced (P <0.05) H<sub>2</sub>S gas emission in a dose-dependent and time-dependent manner by 18.37-67.35% in the incubated substrates and by 8.51-67.23% in the degraded substrates. Extracts maintained pH within the normal range (6.05-7.05), reduced dry matter digestibility and metabolizable energy, and improved (P <0.05) concentration of short chain fatty acids. Overall, extracts of AZN and CNA and their combinations had a positive effect on reducing the greenhouse gas production with no deleterious effect on cecal horses' fermentation activities.