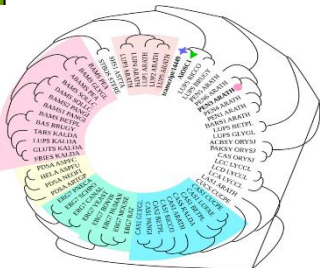




Neem Research Newsletter

Volume 2, Issue 9, 2022



WORLD NEEM ORGANISATION (WNO)



From
The Editor's Desk.....

A very Happy Neem Year to All! This is the last issue of the newsletter for 2022 and hopefully, there will be exciting discoveries and developments in neem research in 2023. In this issue abstracts of several papers showcasing the multifaceted attributes of neem are included. Neem-derived products and growth-regulating insecticides of synthetic origin are suggested as offer alternatives for insecticide resistance management programs. Azadirachtin in combination with an NPK fertilizer proved to have a greater lethal impact on the larvae of *Aedes albopictus*. The whole genome sequence of *Melia azedarach* comprising 237.16 Mb with a contig N50 of 8.07 Mb, and an improved genome sequence of *Azadirachta indica* comprising 223.66 Mb with a contig N50 of 8.91 Mb are unravelled. Moreover, genome skimming data, transcriptomes, and other published genomes were comprehensively analyzed to determine the genes and proteins that produce superior wood and valuable limonoids. The nimbin analog N2 was shown to alleviate oxidative stress and apoptosis and alter the expression of polycystic ovarian syndrome key genes. Neem gum polysaccharide (NGP) in conjugation with Polyvinyl alcohol (PVA) in the form of nanofibers exhibits antimicrobial properties and accelerates wound healing suggesting the tissue engineering potential of the device. Nimbidiol attenuated inflammation protecting kidneys from fibrosis and dysfunction in type-1 diabetes. A bionanocomposite made up of neem leaf powder (NLP), zinc oxide (ZnO), and amino acid (l-cysteine)-functionalized polyaniline (PANI) was found to remove anionic and cationic dyes from synthetic and real water samples. A simple two-step method was developed for efficient separation of oil/water mixtures using superhydrophobic *Azadirachta indica* leaves.

S. Nagini

Core Founding Member, WNO
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Neem in Agriculture

Sublethal effects of growth-regulating insecticides of synthetic and botanical origins on the biological parameters of *Spodoptera frugiperda* (Lepidoptera: Noctuidae).

Martins LN, Geisler FCS, Rakes M, Araújo MB, Amandio DTT, da Rosa APSA, Ribeiro LP, Bernardi D.

Bull Entomol Res. 2022 Dec 21:1-9. doi: 10.1017/S000748532200058X. PMID: 36539283

The objective of this study was to evaluate the effects of growth-regulating insecticides of synthetic (e.g., Certero 480 SC, Intrepid 240 SC, Match EC and Mimic 240 SC) and botanical origins (e.g., Azamax 1.2 EC, Agroneem 850 EC, Azact 2.4 EC and Fitoneem 850 EC) on the biological parameters and fertility life table of *Spodoptera frugiperda* (J.E. Smith) under laboratory conditions. Larvae were fed insecticides that were incorporated into artificial diets. To develop the fertility life table, the following biological parameters were evaluated: survival at 7 days after infestation (d.a.i) and survivorship at adult eclosion, duration of the neonate-to-adult eclosion period, larval and pupal weights and total fecundity (number of total eggs per female). The results indicated that *S. frugiperda* neonates surviving LC₂₅ or LC₅₀ concentrations of the evaluated insecticides showed longer larval and egg-to-adult periods, lower larval and pupal weights and reduced fecundity, when compared to the control treatment. Larvae exposed to Azamax at LC₂₅ or LC₅₀ concentrations showed the greatest increase in generation duration (75 d). In addition, *S. frugiperda* adults emerged from pupae when larvae reared on an artificial diet containing growth regulating insecticides of synthetic and botanical origins produced fewer females per female per generation (R_0). As well as, lower rates of natural population increase per day (r_m) compared to insects fed the control diet. Our findings indicated that, neem-derived products and growth-regulating insecticides of synthetic origin may be employed within integrated management strategies that aim to keep populations of *S. frugiperda* below levels that cause economic damage. Similarly, they offer alternatives for insecticide resistance management programs.

Evaluation of selected botanicals for the management of maize weevil (*Sitophilus zeamais*) on maize (*Zea mays* L.) grain under laboratory condition in Gabilay District, Somaliland.

Barre J, Jenber AJ.

Heliyon. 2022 Nov 30;8(12):e11859. doi: 10.1016/j.heliyon.2022.e11859. PMID: 36478808

One of the most reliable crops in the research region is maize. However, a variety of post-harvest insect pests like *Sitophilus zeamais* and Angoumois grain moths pose a hazard to the cultivation and storage of maize. Hence, a laboratory experiment was conducted in Gabilay District Somaliland in 2021 to assess selected botanicals effectiveness against maize weevil. The treatments consisted of six botanicals namely neem seed, neem leaves, garlic, lantana, ginger, and pepper tree leaves at 50 g/kg of each were evaluated. Malathion 5% dust at 0.05 g/kg as standard check and the control/untreated check/were included for comparison. The Experiment was designed in a Completely Randomized Design and replicated three times, All the botanical powders were more effective than the control by causing high insect mortality, by lowering grain damage, weight loss, and emergence of F1 progeny. Neem seed and Garlic showed 100% adult mortality, which had

a similar effect to Malathion 5% dust. Neem seeds produced the lowest F1 offspring, followed by garlic. Neem seeds and garlic had the least amount of seed damage among the botanicals (0%) and (4%), respectively. The control/untreated check/resulted in the most seed damage (45%). Similarly, the highest weight loss among the botanicals was recorded on the control check, Ginger and Pepper tree 11%, 5%, and 4% respectively. The ability of seeds to germinate was unaffected by the botanicals. A conclusion, neem seed and garlic leaves are the most effective treatment against maize weevil.

Performance of a hermetic device and neem (*Azadirachta indica*) in storing wheat seed: Evidence from participatory household trials in central Bangladesh.

Krupnik TJ, Hossain K, Timsina J, Uddin MM, Baksh ME, Hasan MZ, Gathala MK.

J Stored Prod Res. 2022 Dec;99:102024. doi: 10.1016/j.jspr.2022.102024.PMID: 36466545

Smallholder farmers in Bangladesh often use low-density polyethylene (LDPE) bags contained within woven polypropylene bags to store wheat seed during the summer monsoon that precedes winter season planting. High humidity and temperature during this period can encourage increased seed moisture and pests, thereby lowering seed quality. Following a farm household survey conducted to inform trial design, eighty farmers were engaged in an action research process in which they participated in designing and conducting trials comparing traditional and alternative seed storage methods over 30 weeks. Factorial treatments included comparison of hermetic SuperGrainbags® (Premium RZ) against LDPE bags, both with and without the addition of dried neem tree leaves (*Azadirachta indica*). SuperGrainbags® were more effective in maintaining seed moisture at acceptable levels close to pre-storage conditions than LDPE bags. Both seed germination and seedling coleoptile length were significantly greater in hermetic than LDPE bags. Neem had no effect on seed moisture, germination, or coleoptile length. SuperGrainbags® were also more effective in abating seed damage during storage, although inclusion of neem within LDPE bags also had significant damage. Quantification of seed predating insects and diseases suggested that SuperGrainbags® also suppressed Coleopteran pests and blackspot, the latter indicative of *Fusarium graminearum*. Conversely, where farmers used LDPE bags, neem also had an additional though limited pest suppressive effect. Post-storage treatment scoring by farmers revealed a strong preference for SuperGrainbags® and no preference differences for or against neem. This study demonstrates a process by which farmers can be involved in the participatory co-design and testing of alternative wheat storage options, and stresses the need to develop SuperGrainbag® supply chains so hermetic storage can be made widely available.

Integrated Management of *Meloidogyne Incognita* and Soilborne Fungi Infecting Cucumber Under Protected Cultivation.

Patil JA, Yadav S, Ram S, Kumar A, Kumar S.

J Nematol. 2022 Nov 20;54(1):20220042. doi: 10.2478/jofnem-2022-0042. PMID: 36457368

Relative efficacy of various approaches for management of *Meloidogyne incognita* and the soilborne fungus *Fusarium oxysporum* f. sp. *cucumerinum* has been tested in cucumber under protected cultivation conditions for two seasons. Management practices, namely, chemicals (fumigant, nonfumigant, and fungicide), organic amendments (neem cake,

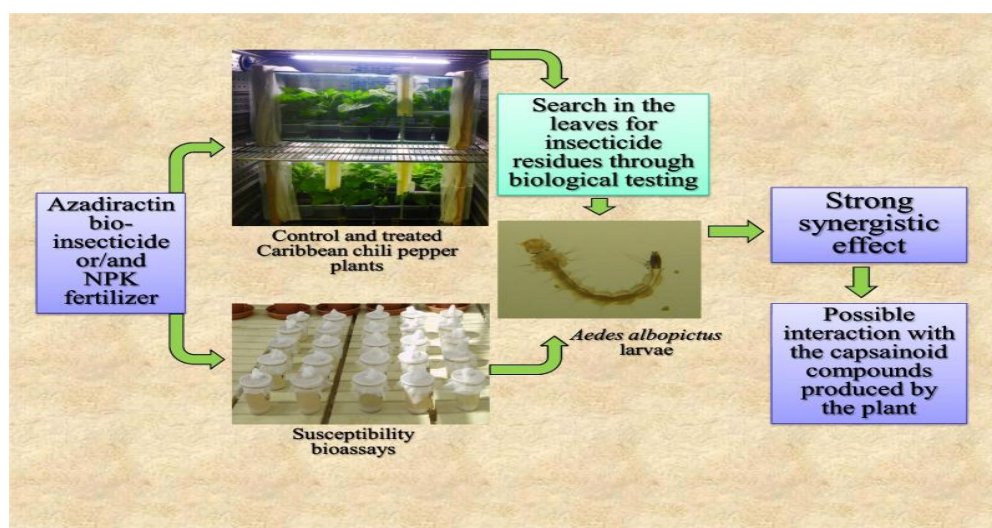
leaves, and oil opted as soil and seed treatment), and biocontrol agents (egg-parasitic fungus and *Purpureocillium lilacinum*), were combined for the management of the disease complex in a randomized block design. Two significant parameters were measured: plant growth parameters (shoot length, dry shoot weight, dry root weight, and yield) and disease parameters (galls per plant, final nematode population, egg masses per plant, and fungal incidence). All treatments significantly improved plant growth parameters and reduced nematode reproduction as compared to untreated check. The integration of formalin and neem oil seed treatment favors the low root galling index compared to all other treatments in both the seasons. Formalin and neem oil seed treatment reduced the nematode population and fungal incidence, and increased the yield of cucumber during both the seasons.

The bio-interactions between plants, insecticides and fertilizers: an innovative approach for the research of xenobiotic substances.

Darriet F.

Nat Prod Bioprospect. 2022 Dec 1;12(1):41. doi: 10.1007/s13659-022-00360-1. PMID: 36450969

In this experiment carried out on Caribbean chili pepper plants (*Capsicum chinensis*), the bio-insecticide azadirachtin in combination with an NPK fertilizer proved to have a greater lethal impact on the larvae of *Aedes albopictus* than each substance on its own. This synergistic effect is noticeably important when both inputs are sprayed directly on the leaves of the plant (foliar application). While the plants treated with azadirachtin or NPK alone cause a 33.6% and 36.4% mortality respectively of the *Ae. albopictus* larvae, the combination of the two inputs induces a 74.4% mortality on the mosquito larvae. To account for this synergistic effect phenomenon inside the plant, the azadirachtin + NPK combination most likely interacts with the capsaicinoid compounds naturally produced by the plant. Not only does this study carried out on azadirachtin reveal major results but the methodology itself offers a most interesting approach on how to boost the agricultural inputs within the plants. As a matter of fact, this research axis demands developing since the control of pests harmful to men has been dramatically lacking insecticide molecules acting on new targets over the past three decades.



Azadirachtin-based insecticide impairs testis morphology and spermatogenesis of the southern armyworm *Spodoptera eridania* (Lepidoptera: Noctuidae).

Scudeler EL, Daquila BV, Carvalho SF, Conte H, Padovani CR, Santos DCD.

Pest Manag Sci. 2022 Dec 24. doi: 10.1002/ps.7338. PMID: 36565161

Background: In the search for alternative tools for integrated pest management, azadirachtin, a botanical insecticide, has been used with the most promising activity against *Spodoptera* spp., but the mechanism of cytotoxicity on reproductive organs remains unclear. *Spodoptera eridania* (Stoll, 1782) is a polyphagous pest with great economic importance that has become an important target to elucidate the action of azadirachtin on the reproductive organs of insect pests, helping to understand the deleterious effects caused by its exposure. This study evaluated the effects of chronic exposure to azadirachtin on the morphology and ultrastructure of *S. eridania* larval testes as well as larval development. **Results:** Azadirachtin exposure (6 or 18 mg a.i. L⁻¹) caused a progressive increase in cumulative mortality and reduced gain in body mass after five days. Testicular structure indicated a reduction in their size with internal morphological changes such as spermatogonia, spermatogonial, spermatocytes and spermatid cysts in degeneration. The occurrence of cell death in germ and somatic cells was evidenced by the TUNEL technique. Electron microscopy revealed changes in cystic cells, such as cytoplasmic membrane rupture and cytoplasmic vacuolization. Chromatin compaction, changes in the rough endoplasmic reticulum and Golgi complex cisternae were observed in germ cells. Apoptotic bodies occurred between germ cell cysts. **Conclusion:** Azadirachtin damaged the testes of *S. eridania* larvae, and these changes compromised spermatogenesis and consequently the development of the reproductive potential of this specimen, making azadirachtin a promising botanical insecticide for application in integrated pest management programs.

Meliaceae genomes provide insights into wood development and limonoids biosynthesis.

Cui G, Li Y, Yi X, Wang J, Lin P, Lu C, Zhang Q, Gao L, Zhong G.
Plant Biotechnol J. 2022 Dec 1. doi: 10.1111/pbi.13973. PMID: 36453987

Meliaceae is a useful plant family owing to its high-quality timber and its many limonoids that have pharmacological and biological activities. Although some genomes of Meliaceae species have been reported, many questions regarding their unique family features, namely wood quality and natural products, have not been answered. In this study, we provide the whole genome sequence of *Melia azedarach* comprising 237.16 Mb with a contig N50 of 8.07 Mb, and an improved genome sequence of *Azadirachta indica* comprising 223.66 Mb with a contig N50 of 8.91 Mb. Moreover, genome skimming data, transcriptomes, and other published genomes were comprehensively analyzed to determine the genes and proteins that produce superior wood and valuable limonoids. Phylogenetic analysis of chloroplast genomes, single-copy gene families, and single nucleotide polymorphisms revealed that Meliaceae should be classified into two subfamilies: Cedreloideae and Melioideae. Although the Meliaceae species did not undergo additional whole-genome duplication events, the secondary wall biosynthetic genes of the woody Cedreloideae species, *Toona sinensis*, expanded significantly compared to those of *A. indica* and *M. azedarach*, especially in downstream transcription factors and cellulose/hemicellulose biosynthesis-related genes. Moreover, expanded special oxidosqualene cyclase catalogs can help diversify Sapindales skeletons, and the clustered genes that regulate terpene chain elongation, cyclization, and modification would support their roles in limonoid biosynthesis. The expanded clans of terpene synthase, O-methyltransferase, and cytochrome P450, which are mainly derived from tandem duplication, are responsible for the different limonoid classes among the species. These results are beneficial for further investigations of wood development and limonoid biosynthesis.

Neem for Human Health

Nimbin analog N2 alleviates high testosterone induced oxidative stress in CHO cells and alters the expression of Tox3 and Dennd1a signal transduction pathway involved in the PCOS zebrafish.

Sudhakaran G, Rajesh R, Murugan R, Velayutham M, Guru A, Boopathi S, Muthupandian S, Gopinath P, Arockiaraj J.

Phytother Res. 2022 Nov 30. doi: 10.1002/ptr.7685. PMID: 36450691

Polycystic ovarian syndrome (PCOS) is a hormonal disorder that causes enlargement of ovaries and follicular maturation arrest, which lacks efficient treatment. N2, a semi-natural triterpenoid from the neem family, was already reported to have antioxidant and anti-inflammatory properties in our previous report. This study investigated the anti-androgenic property of N2 on testosterone-induced oxidative stress in Chinese Hamster Ovarian cells (CHO) and PCOS zebrafish model. The testosterone exposure disrupted the antioxidant enzymes and ROS level and enhanced the apoptosis in both CHO cells and PCOS zebrafish. However, N2 significantly protected the CHO cells from ROS and apoptosis. N2 improved the Gonado somatic index (GSI) and upregulated the expression of the SOD enzyme in zebrafish ovaries. Moreover, the testosterone-induced follicular maturation arrest was normalized by N2 treatment in histopathology studies. In addition, the gene expression studies of Tox3 and Dennd1a in zebrafish demonstrated that N2 could impair PCOS condition. Furthermore, to confirm the N2 activity, the in-silico studies were performed against PCOS susceptible genes Tox3 and Dennd1a using molecular docking and molecular dynamic simulations. The results suggested that N2 alleviated the oxidative stress and apoptosis in-vitro and in-vivo and altered the expression of PCOS key genes.

Neem Oil or Almond Oil Nanoemulsions for Vitamin E Delivery: From Structural Evaluation to in vivo Assessment of Antioxidant and Anti-Inflammatory Activity.

Rinaldi F, Hanieh PN, Maurizi L, Longhi C, Uccelletti D, Schifano E, Del Favero E, Cantù L, Ricci C, Ammendolia MG, Paolino D, Froiio F, Marianecchi C, Carafa M.

Int J Nanomedicine. 2022 Dec 20;17:6447-6465. doi: 10.2147/IJN.S376750. PMID: 36573206

Purpose: Vitamin E (VitE) may be classified in "the first line of defense" against the formation of reactive oxygen species. Its inclusion in nanoemulsions (NEs) is a promising alternative to increase its bioavailability. The aim of this study was to compare O/W NEs including VitE based on Almond or Neem oil, showing themselves antioxidant properties. The potential synergy of the antioxidant activities of oils and vitamin E, co-formulated in NEs, was explored. **Patients and methods:** NEs have been prepared by sonication and deeply characterized evaluating size, ζ -potential, morphology (TEM and SAXS analyses), oil nanodroplet feature, and stability. Antioxidant activity has been evaluated in vitro, in non-tumorigenic HaCaT keratinocytes, and in vivo through fluorescence analysis of *C. elegans* transgenic strain. Moreover, on healthy human volunteers, skin tolerability and anti-inflammatory activity were evaluated by measuring the reduction of the skin erythema induced by the application of a skin chemical irritant (methyl-nicotinate). **Results:** Results

confirm that Vitamin E can be formulated in highly stable NEs showing good antioxidant activity on keratinocyte and on *C. elegans*. Interestingly, only Neem oil NEs showed some anti-inflammatory activity on healthy volunteers. **Conclusion:** From the obtained results, Neem over Almond oil is a more appropriate candidate for further studies on this application.

Appraisal of selected ethnomedicinal plants as alternative therapies against onychomycosis: Evaluation of synergy and time-kill kinetics.

Mohsin SA, Shaukat S, Nawaz M, Ur-Rehman T, Irshad N, Majid M, Hassan SSU, Bungau S, Fatima H.

Front Pharmacol. 2022 Nov 24;13:1067697. doi: 10.3389/fphar.2022.1067697. eCollection 2022.PMID: 36506532

Introduction: This study aims at the biological profiling of *Allium sativum*, *Zingiber officinale*, *Nigella sativa*, *Curcuma longa*, *Mentha piperita*, *Withania somnifera*, *Azadirachta indica*, and *Lawsonia inermis* as alternatives against onychomycosis to combat the treatment challenges. **Methods:** An extract library of aqueous (DW), ethyl acetate (EA), and methanol (M) extracts was subjected to phytochemical and antioxidant colorimetric assays to gauge the ameliorating role of extracts against oxidative stress. RP-HPLC quantified therapeutically significant polyphenols. Antifungal potential (disc diffusion and broth dilution) against filamentous (dermatophytes and non-dermatophytes) and non-filamentous fungi (yeasts; *Candida albicans*), synergistic interactions (checkerboard method) with terbinafine and amphotericin-B against resistant clinical isolates of dermatophytes (*Trichophyton rubrum* and *Trichophyton tonsurans*) and non-dermatophytes (*Aspergillus* spp., *Fusarium dimerum*, and *Rhizopus arrhizus*), time-kill kinetics, and protein estimation (Bradford method) were performed to evaluate the potential of extracts against onychomycosis. **Results:** The highest total phenolic and flavonoid content along with noteworthy antioxidant capacity, reducing power, and a substantial radical scavenging activity was recorded for the extracts of *Z. officinale*. Significant polyphenolics quantified by RP-HPLC included rutin ($35.71 \pm 0.23 \mu\text{g}/\text{mgE}$), gallic acid ($50.17 \pm 0.22 \mu\text{g}/\text{mgE}$), catechin ($93.04 \pm 0.43 \mu\text{g}/\text{mgE}$), syringic acid ($55.63 \pm 0.35 \mu\text{g}/\text{mgE}$), emodin ($246.32 \pm 0.44 \mu\text{g}/\text{mgE}$), luteolin ($78.43 \pm 0.18 \mu\text{g}/\text{mgE}$), myricetin ($29.44 \pm 0.13 \mu\text{g}/\text{mgE}$), and quercetin ($97.45 \pm 0.22 \mu\text{g}/\text{mgE}$). Extracts presented prominent antifungal activity against dermatophytes and non-dermatophytes (MIC-31.25 $\mu\text{g}/\text{ml}$). The checkerboard method showed synergism with 4- and 8-fold reductions in the MICs of *A. sativum*, *Z. officinale*, *M. piperita*, *L. inermis*, and *C. longa* extracts and doses of amphotericin-B (Amp-B) and terbinafine (against non-dermatophytes and dermatophytes, respectively). Furthermore, the synergistic therapy showed a time-dependent decrease in fungal growth even after 9 and 12 h of treatment. The inhibition of fungal proteins was also observed to be higher with the treatment of synergistic combinations than with the extracts alone, along with the cell membrane damage caused by terbinafine and amp-B, thus making the resistant fungi incapable of subsisting. **Conclusion:** The extracts of *A. sativum*, *Z. officinale*, *M. piperita*, *L. inermis*, and *C. longa* have proven to be promising alternatives to combat oxidative stress, resistance, and other treatment challenges of onychomycosis.

Dose-dependent effects of neem crude extract on human dental pulp cell and murine osteoblast viability and mineralization

Patntirapong S, Aupaphong V, Pipatboonyarit P, Kritsuttsikun K, Phubai T.

Braz Dent J. 2022 Nov-Dec;33(6):56-64. doi: 10.1590/0103-6440202205207.PMID: 36477965

Neem products for oral application are on the rise. Before recommendation for therapeutic use in human, its effects on cellular activities need to be examined. Therefore, the aim of this study was to test the effects of the ethanolic neem crude extract on dental pulp cells and osteoblasts in terms of cell viability, mineralization, and gene expressions. The ethanolic neem extract derived from dry neem leaves was subjected to chemical identification using GC-MS. Human dental pulp stem cells (hDPSCs) and pre-osteoblasts (MC3T3) were treated with various concentrations of the neem crude extract. Cell viability, mineralization, and gene expressions were investigated by MTT assay, real-time PCR, and alizarin red S assay, respectively. Statistical analysis was performed by one-way ANOVA followed by Dunnett test. GC-MS detected several substance groups such as sesquiterpene. Low to moderate doses of the neem crude extract (4 - 16 µg/ml) did not affect hDPSC and MC3T3 viability, while 62.5 µg/ml of the neem extract decreased MC3T3 viability. High doses of the neem crude extract (250 - 1,000 µg/ml) significantly reduced viability of both cells. The neem crude extract at 1,000 µg/ml also decreased viability of differentiated hDPSC and MC3T3 and their mineralization. Furthermore, 4 µg/ml of neem inhibited viability of differentiated hDPSC. There is no statistical difference in gene expressions related to cell differentiation. In conclusion, the neem crude extract affected cell viability and mineralization. Cell viability altered differently depending on the doses, cell types, and cell stages. The neem crude extract did not affect cell differentiation. Screening of its effect in various aspects should be examined before the application for human use.

Evaluating neem gum-polyvinyl alcohol (NGP-PVA) blend nanofiber mat as a novel platform for wound healing in murine model.

Rajora AD, Bal T.

Int J Biol Macromol. 2022 Dec 6:S0141-8130(22)02908-7. doi: 10.1016/j.ijbiomac.2022.12.014. PMID: 36493923

Modern-day treatment demands scarless wound healing utilizing scaffolds in the form of nanofiber mats which are tissue and environment-friendly. Neem gum polysaccharide (NGP) in conjugation with Polyvinyl alcohol (PVA) in the form of nanofibers exhibits antimicrobial properties mimicking extracellular matrix for tissue growth. Different grades of nanofiber mats (NFM) were prepared by combining different ratios of NGP and PVA which were later crosslinked using glutaraldehyde vapors (25 % w/v in 0.5 M HCl), and optimized grade G14 exhibited maximum tensile strength with smooth surface morphology, hemocompatible properties, in-vitro biodegradability and antimicrobial action against *S. aureus* & *E. coli*. G14 was analytically characterized using different analytical techniques viz. Fourier-transform infrared spectroscopy (FTIR), Thermogravimetric analysis (TGA), which indicated polymer-polymer compatibility. The surface hydrophobicity as detected using Optical contact angle (OCA) confirmed the hydrophobicity of NFM with increased glutaraldehyde vapor for crosslinking when compared to non-crosslinked NFM. Histopathology slides indicated G14

CL-NFM accelerated the wound healing in mice with dense collagen and fibroblasts when compared to control mice suggesting the tissue engineering potential of the prepared device.

Antimicrobial effects of copper nanoparticles with green tea and neem formulation.

Anna Thomas A, Varghese RM, Rajeshkumar S.

Bioinformation. 2022 Mar 31;18(3):284-288. doi: 10.6026/97320630018284. eCollection 2022.PMID: 36518121

Nanotechnology is the science which is about manipulating matter, atom by atom and is associated with particles smaller than 100 nm in size. Copper nanoparticles are used mainly due to its surplus amount, low cost, easy availability and biocompatible property. Green synthesis of copper nanoparticles is very simple, economical and eco-friendly method that does not involve any toxic chemicals. The aim of our study is green synthesis of copper nanoparticles using green tea and neem formulation and assessment of its antimicrobial effects. 20mM of copper sulphate solution is mixed with 40mL of plant extract and 60 mL of distilled water was added and made it into 100 ml solution. Once the copper nanoparticles are synthesized the solution is characterized using UV- vis-spectroscopy and was scanned in double beam UV-vis- spectrophotometer from 300 nm to 700nm wavelength. The antimicrobial property of copper nanoparticle is evaluated by agar well diffusion method. The colour change from green to brown and peak observed in UV-vis- spectrophotometer was associated with the synthesis of copper nanoparticles. Copper nanoparticle from green tea and tea extract has good antimicrobial activity against *S.mutans*, *C.albicans*, *E.faecalis*, & *S.aureus*. Copper nanoparticles can be efficiently synthesised from green and neem formulation. These copper nanoparticles showed good antibacterial properties and are effective against oral pathogens.

A Split *Renilla* Luciferase Complementation Assay for the Evaluation of Hsp90/Aha1 Complex Disruptors and Their Activity at the Aha1 C-Terminal Domain.

Keegan BM, Blagg BSJ.

ACS Chem Biol. 2022 Dec 14. doi: 10.1021/acscchembio.2c00854. Online ahead of print. PMID: 36516069

Disruption of interactions between Hsp90 and the cochaperone protein, Aha1, has emerged as a therapeutic strategy to inhibit Aha1-driven cancer metastasis and tau aggregation in models of tauopathy. A combination of split *Renilla* luciferase assays was developed to screen and quantify the ability of small molecules to disrupt interactions between Hsp90 and both full length Aha1 protein (Aha1-FL) and the Aha1 C-terminal domain (Aha1-CTD). This luminescence-based approach was used to identify withaferin A and gedunin as disruptors of Hsp90/Aha1 interactions and provided insight into the binding regions for gambogic acid and gedunin on the Hsp90 homodimer. All compounds tested that disrupted Hsp90/Aha1-CTD interactions were found to disrupt interactions between Hsp90 and Aha1-FL, suggesting that interactions between Hsp90 and the Aha1-CTD play a key role in the stability of Hsp90/Aha1 complexes.

Glucosidase inhibitor, Nimbidol ameliorates renal fibrosis and dysfunction in type-1 diabetes.

Juin SK, Pushpakumar S, Tyagi SC, Sen U.

Sci Rep. 2022 Dec 15;12(1):21707. doi: 10.1038/s41598-022-25848-1.PMID: 36522378

Diabetic nephropathy is characterized by excessive accumulation of extracellular matrix (ECM) leading to renal fibrosis, progressive deterioration of renal function, and eventually to end stage renal disease. Matrix metalloproteinases (MMPs) are known to regulate synthesis and degradation of the ECM. Earlier, we demonstrated that imbalanced MMPs promote adverse ECM remodeling leading to renal fibrosis in type-1 diabetes. Moreover, elevated macrophage infiltration, pro-inflammatory cytokines and epithelial–mesenchymal transition (EMT) are known to contribute to the renal fibrosis. Various bioactive compounds derived from the medicinal plant, *Azadirachta indica* (neem) are shown to regulate inflammation and ECM proteins in different diseases. Nimbidol is a neem-derived diterpenoid that is considered as a potential anti-diabetic compound due to its glucosidase inhibitory properties. We investigated whether Nimbidol mitigates adverse ECM accumulation and renal fibrosis to improve kidney function in type-1 diabetes and the underlying mechanism. Wild-type (C57BL/6J) and type-1 diabetic (C57BL/6-Ins2^{Akita}/J) mice were treated either with saline or with Nimbidol (0.40 mg kg⁻¹ d⁻¹) for eight weeks. Diabetic kidney showed increased accumulation of M1 macrophages, elevated pro-inflammatory cytokines and EMT. In addition, upregulated MMP-9 and MMP-13, excessive collagen deposition in the glomerular and tubulointerstitial regions, and degradation of vascular elastin resulted to renal fibrosis in the Akita mice. These pathological changes in the diabetic mice were associated with functional impairments that include elevated resistive index and reduced blood flow in the renal cortex, and decreased glomerular filtration rate. Furthermore, TGF-β1, p-Smad2/3, p-P38, p-ERK1/2 and p-JNK were upregulated in diabetic kidney compared to WT mice. Treatment with Nimbidol reversed the changes to alleviate inflammation, ECM accumulation and fibrosis and thus, improved renal function in Akita mice. Together, our results suggest that Nimbidol attenuates inflammation and ECM accumulation and thereby, protects kidney from fibrosis and dysfunction possibly by inhibiting TGF-β/Smad and MAPK signaling pathways in type-1 diabetes.

EXPLORING the prophylactic potential of *Azadirachta indica* leaf extract against dyslipidemia.

Asghar HA, Syed QA, Shukat R, Israr B.

J Ethnopharmacol. 2022 Dec 12:116008. doi: 10.1016/j.jep.2022.116008. Online ahead of print. PMID: 36521768

Ethnopharmacological relevance: Several studies revealed that different parts of *Azadirachta indica* A. Juss, has therapeutic potential against inflammatory issues and dyslipidemia which is a major contributing cause to cardiovascular diseases, oxidative stress and serum glucose levels, etc. AIM OF STUDY: Present study was conducted to evaluate anti-dyslipidemic capacity of *Azadirachta indica* leaf extract in dyslipidemic rabbits.

Materials and methods: Ethanolic extract of *Azadirachta indica* leaves was obtained by using Soxhlet apparatus. This extract was used for efficacy study on rabbits. In this context, 25 healthy rabbits were selected for study, Efficacy trial involved five groups of rabbits, 5

rabbits in each group; NC (Negative Control); healthy rabbits received normal diet. In remaining 20 rabbits, dyslipidemia was induced by using high fat diet for 28 days followed by administration of *Azadirachta indica* leaf ethanolic extract for 60 days in a dose-dependent manner. PC (Positive Control) include dyslipidemic rabbits received normal diet while G₁, G₂, G₃ groups included dyslipidemic rabbits receiving different concentrations of *Azadirachta indica* leaf extract (i.e. 300, 500 and 700 mg/kg of body weight, respectively). Blood samples were analyzed for serum lipid profile after every 15 days to determine the effect of treatments. **Results:** Significant reduction in total cholesterol (60 ± 3.4 mg/dL), triglycerides (40.31 ± 2.5 mg/dL) and low-density lipoprotein (28.87 ± 2.1 mg/dL) was observed in G₂ ($P \leq 0.05$) while a significant increase was observed in high-density lipoprotein (60.47 ± 1.7 mg/dL) of G₂ ($P \leq 0.05$) as compared to other groups. **Conclusion:** Results revealed that ethanolic extract of *Azadirachta indica* leaves in G₂ group (@ 500 mg/kg of body weight) normalized lipid profile in dyslipidemic rabbits after 60 days of extract administration which significantly lowered TC, TG, LDL levels ($P \leq 0.05$) and improved HDL level.

Multifunctional Ternary NLP/ZnO@l-cysteine-grafted-PANI Bionanocomposites for the Selective Removal of Anionic and Cationic Dyes from Synthetic and Real Water Samples.

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The development of competent adsorbents based on agro-waste materials with multifunctional groups and porosity for the removal of toxic dyes from aqueous solutions is still a challenge. Herein, a bionanocomposite made up of neem leaf powder (NLP), zinc oxide (ZnO), and amino acid (l-cysteine)-functionalized polyaniline (PANI), namely, NLP/ZnO@l-cysteine-grafted-PANI (NZC-*g*-PANI), has been prepared by an in situ polymerization method. The as-prepared bionanocomposite was tested for the adsorptive removal of three anionic dyes, namely, methyl orange (MO), amido black 10B (AB 10B), and eriochrome black T (EBT), as well as three cationic dyes, namely, brilliant green (BG), crystal violet (CV), and methylene blue (MB), from synthetic aqueous medium. The morphological and structural characteristics of the NZC-*g*-PANI nanocomposite were examined with the help of HR field emission scanning electron microscopy (FESEM), transmission electron microscopy (TEM), X-ray diffraction (XRD), Fourier transform infrared (FTIR), and Raman spectroscopy. FTIR and Raman studies show that the formulated NZC-*g*-PANI have an ample number of functional moieties such as carboxyl (-COOH), hydroxyl (-OH), amines (-NH₂), and imines (-N=), thus demonstrating outstanding dye removal capacity. C-S linkage helps to attach l-cysteine with polyaniline. Moreover, the predominance of chemisorption via ionic/ π - π interaction and hydrogen bonding between the NZC-*g*-PANI nanocomposite and dyes (BG and MO) has been realized by FTIR and fitting of kinetics data to the PSO model. For both BG and MO dyes, the biosorption isotherm was precisely accounted for by the Langmuir isotherm with q_{\max} values of up to 218.27 mg g⁻¹ for BG at pH 6 and 558.34 mg g⁻¹ for MO at pH 1. Additionally, thermodynamic studies revealed the endothermic and spontaneous nature of adsorption. NZC-*g*-PANI showed six successive regeneration cycles for cationic (MO: from 96.3 to 90.4%) and anionic (BG: from 94.7 to 88.7%) dyes. Also, batch adsorption operations were validated to demonstrate dye biosorption from real wastewater, such as tap water, river water, and laundry wastewater. Overall, this study indicates that the prepared NZC-*g*-PANI biosorbent could be used as an effective adsorbent for the removal of various types of anionic as well as cationic dyes from different aqueous solutions.

Special wettable *Azadirachta indica* leaves like microarchitecture mesh filtration membrane produced by galvanic replacement reaction for layered oil/water separation.

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The oil/water separation has received significant attention due to its critical environmental impact. The special wettable surfaces are highly desired to deal with the oil/water mixtures. This work demonstrates a simple two-step method to develop a superhydrophobic *Azadirachta indica* leaves like Ag-decorated electrochemically copper-coated stainless-steel mesh (SH-AIL-Ag-EC-Cu-Mesh) for efficient separation of oil/water mixtures. In the first step, the electrodeposition of the copper took place on the mesh surface at a suitable applied potential. In the second step, the galvanic replacement reaction between the Ag^+ and electrodeposited Cu produced the fascinating superhydrophobic Ag leaves on the mesh surface. The SH-AIL-Ag-EC-Cu-Mesh was thoroughly characterized by the X-ray photoelectron spectroscopy (XPS), Energy Dispersive X-Ray Spectroscopy (EDX), elemental mapping, surface wettability analysis, and the contact analyzer. The morphological analysis has shown the unique leafy structures of the reduced Ag on the surface of the mesh. The XPS analysis has confirmed that most of the Ag present on the surface is in zerovalent form. The combination of the electrodeposition and the displacement reaction between the copper and the silver turned the surface superhydrophobic, and the water contact angle was significantly improved from 115° to 158° . The designed SH-AIL-Ag-EC-Cu-Mesh has shown excellent selectivity for oil in oil/water mixtures with a separation efficiency of 99.1% with an exceptionally high flux of $8963 \text{ L m}^{-2}\text{h}^{-1}$. The SH-AIL-Ag-EC-Cu-Mesh has shown excellent reusability, and after 15 cycles of separation, no significant decrease in the oil/water separation efficiency was observed.

