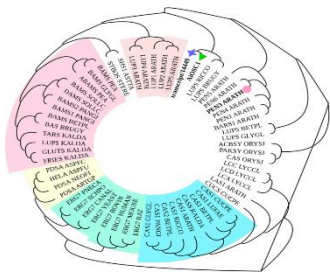




# Neem Research Newsletter

## Volume 3, Issue 12, 2023



**WORLD NEEM ORGANISATION (WNO)**

From

The Editor's Desk.....

As the year draws to a close, it is time to recapitulate interesting research findings on neem in diverse areas and look forward to many more exciting developments in the coming year. Although research articles published on neem during this month are much less relative to the previous issues, interest in investigating the vast potential of neem continues unabated. The antihelminthic properties of neem extract have been analysed with reference to fish host safety. Based on research findings, neem seed oil has been highly recommended as feedstock for biodiesel production, effective for high-quality liquid fuel synthesis and could be an optional green route to cleaner production of bioenergy, eventually leading to sustenance, robustness, and resilience that will aid in developing a holistic framework for integrated waste management. Triterpenoids from neem such as azadirachtin have been identified as effective antiviral molecules that target SARS-CoV-2-specific enzymes and also host immune pathways involved in virus-mediated inflammation. Neem with zinc oxide was found to be useful as a root canal filling material in primary teeth. In experimental rabbits, neem formulation proved to be efficacious in enhancing wound contraction of oral ulcers. Silver nanoparticles biosynthesized through the green synthesis method using neem seed extract exhibited potent anti-diabetic activity. Azadiradione, a neem limonoid, was shown to be promising in restoring synaptic dysfunction in neuropsychiatric/neurodegenerative disorders.

I take this opportunity to wish all members of WNO and neem enthusiasts world over a very Happy, Productive, Peaceful, and Healthy New Year.

S. Nagini

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



## Neem in Agriculture & Aquaculture

### Phytochemical profiling and anthelmintic potential of extracts of selected tropical plants on parasites of fishes in Epe Lagoon.

Ukwa UD, Saliu JK, Akinsanya B.

*Sci Rep. 2023 Dec 20;13(1):22727. doi: 10.1038/s41598-023-48164-8.PMID: 38123590*

This research aims to study the anthelmintic properties of selected five (5) tropical plant extracts, ascertained margin of fish host safety in reference with praziquantel, a commonly used chemo-therapeutics. Qualitative and quantitative analysis of Alligator pepper seeds (*Aframomum melegueta*), Moringa leaves (*Moringa oleifera*), Neem leaves (*Azadirachta indica*), Ginger bulbs (*Zingiber officinale*) and Garlic (*Allium sativum*) and their potencies in reference to praziquantel against *Clarias gariepinus* and different classes of helminth parasites were investigated. The results obtained show that the 70% ethanol extract had 80 to 100% presence of the phytochemical content, compared with the 100% aqueous and 100% ethanol extracts with 50 to 80% and 50 to 90%, respectively. Among the five tropical plants, the richest in saponin and flavonoids are alligator pepper and neem with alkaloids, tannin, flavonoid and saponin in ratios 1:1:3:9 and 1:1:4:3 respectively. While, moringa, garlic and ginger are rich in alkaloids with alkaloids, tannin, flavonoid and saponin in ratios, 8:1:10:1, 6:2:1:4 and 6:3:2:1, respectively. *Aframomum melegueta* and praziquantel showed above 70% potency (at 96 h LC<sub>5</sub>) against all the classes of parasites; *Wenyonia* spp (cestode), *Procamallanus* spp (nematode), *Tenuisentis* spp (acanthocephalan), and *Electrotaenia* sp (cestode) as compared to the other plant extracts that showed above 70% potency (at 96 h LC<sub>5</sub>) only against *Electrotaenia* spp. Sub-lethal Concentrations (96 h LC<sub>5</sub>) of praziquantel and *Aframomum melegueta* on the juvenile fish host (12.36 mg/l and 9.9 mg/l respectively) were found to be 90.9% and 93.5% effective against adult *Electrotaenia* spp after 8 to 10 min of exposure. These concentrations were 78 to 85.7% and 89.7 to 88.4%, respectively, effective against the other classes of parasites after 18 to 25 min and 15 to 21 min of exposure. These concentrations were tested on the post juvenile of the fish to determine behavioral changes; there were no significant behavioral responses after 24 h of exposure. The effective concentrations indicate the widest margin of safety for the fish host.



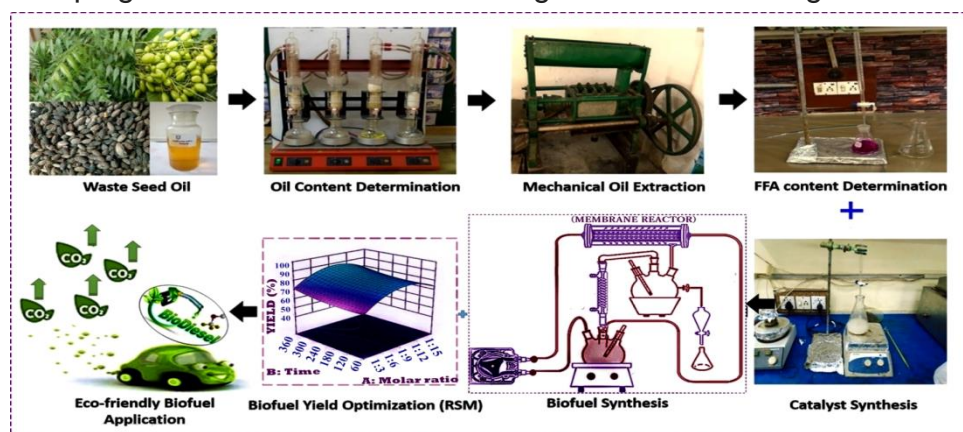
# Neem for Sustainable Environment & Green Synthesis

## Cleaner Biofuel Production via Process Parametric Optimization of Nonedible Feedstock in a Membrane Reactor Using a Titania-Based Heterogeneous Nanocatalyst: An Aid to Sustainable Energy Development.

Ameen M, Zafar M, Ahmad M, Munir M, Abid I, Mustafa AEMA, Athar M, Makhkamov T, Mamarakhimov O, Yuldashev A, Khaydarov K, Mammadova AO, Botirova L, Makkamov Z.

*Membranes (Basel)*. 2023 Nov 27;13(12):889. doi: 10.3390/membranes13120889. PMID: 38132893

Membrane technology has been embraced as a feasible and suitable substitute for conventional time- and energy-intensive biodiesel synthesis processes. It is ecofriendly, easier to run and regulate, and requires less energy than conventional approaches, with excellent stability. Therefore, the present study involved the synthesis and application of a highly reactive and recyclable Titania-based heterogeneous nanocatalyst ( $\text{TiO}_2$ ) for biodiesel production from nonedible *Azadirachta indica* seed oil via a membrane reactor, since *Azadirachta indica* is easily and widely accessible and has a rich oil content (39% w/w). The high free fatty acids content (6.52 mg/g KOH) of the nonedible oil was decreased to less than 1% via two-step esterification. Following the esterification, transesterification was performed using a heterogeneous  $\text{TiO}_2$  nanocatalyst under optimum conditions, such as a 9:1 methanol-oil molar ratio, 90 °C reaction temperature, 2 wt.% catalyst loading, and an agitation rate of 600 rpm, and the biodiesel yield was optimized through response surface methodology (RSM). *Azadirachta indica* seed oil contains 68.98% unsaturated (61.01% oleic acid, 8.97% linoleic acid) and 31.02% saturated fatty acids (15.91% palmitic acid, 15.11% stearic acid). These fatty acids transformed into respective methyl esters, with a total yield up to 95% achieved. The biodiesel was analyzed via advanced characterization techniques like gas chromatography-mass spectrometry (GC-MS), Fourier transform infrared spectroscopy (FT-IR), and nuclear magnetic resonance (NMR), whereas the catalyst was characterized via X-ray diffraction (XRD), scanning electron microscopy (SEM), energy-dispersive X-ray (EDX), and Fourier transform infrared spectroscopy (FT-IR). Due to its physicochemical properties, *Azadirachta indica* seed oil is a highly recommended feedstock for biodiesel production. Moreover, it is concluded that the Titania-based heterogeneous nanocatalyst ( $\text{TiO}_2$ ) is effective for high-quality liquid fuel synthesis from nonedible *Azadirachta indica* seed oil in a membrane reactor, which could be an optional green route to cleaner production of bioenergy, eventually leading to sustenance, robustness, and resilience that will aid in developing a holistic framework for integrated waste management.



## Neem for Human Health

### Unraveling antiviral efficacy of multifunctional immunomodulatory triterpenoids against SARS-CoV-2 targeting main protease and papain-like protease.

Choudhary S, Nehul S, Singh A, Panda PK, Kumar P, Sharma GK, Tomar S.

IUBMB Life. 2023 Dec 7. doi: 10.1002/iub.2793. PMID: 38059400

The coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) may be over, but its variants continue to emerge, and patients with mild symptoms having long COVID is still under investigation. SARS-CoV-2 infection leading to elevated cytokine levels and suppressed immune responses set off cytokine storm, fatal systemic inflammation, tissue damage, and multi-organ failure. Thus, drug molecules targeting the SARS-CoV-2 virus-specific proteins or capable of suppressing the host inflammatory responses to viral infection would provide an effective antiviral therapy against emerging variants of concern. Evolutionarily conserved papain-like protease (PLpro) and main protease (Mpro) play an indispensable role in the virus life cycle and immune evasion. Direct-acting antivirals targeting both these viral proteases represent an attractive antiviral strategy that is also expected to reduce viral inflammation. The present study has evaluated the antiviral and anti-inflammatory potential of natural triterpenoids: azadirachtin, withanolide\_A, and isoginkgetin. These molecules inhibit the Mpro and PLpro proteolytic activities with half-maximal inhibitory concentrations (IC<sub>50</sub>) values ranging from 1.42 to 32.7 μM. Isothermal titration calorimetry (ITC) analysis validated the binding of these compounds to Mpro and PLpro. As expected, the two compounds, withanolide\_A and azadirachtin, exhibit potent anti-SARS-CoV-2 activity in cell-based assays, with half-maximum effective concentration (EC<sub>50</sub>) values of 21.73 and 31.19 μM, respectively. The anti-inflammatory roles of azadirachtin and withanolide\_A when assessed using HEK293T cells, were found to significantly reduce the levels of CXCL10, TNFα, IL6, and IL8 cytokines, which are elevated in severe cases of COVID-19. Interestingly, azadirachtin and withanolide\_A were also found to rescue the decreased type-I interferon response (IFN-α1). The results of this study clearly highlight the role of triterpenoids as effective antiviral molecules that target SARS-CoV-2-specific enzymes and also host immune pathways involved in virus-mediated inflammation.

### Clinical and Radiographic Evaluation of Various Herbal Products Used with Zinc Oxide as an Obturating Material in Primary Teeth: An *In Vivo* Study.

Sunil, Anand S, Ahmad A, Prakash R, Singh A, Megha V.

*J Contemp Dent Pract.* 2023 Sep 1;24(9):692-699. doi: 10.5005/jp-journals-10024-3559.PMID: 38152944

**Aim:** To compare the clinical and radiographically mixture of zinc oxide with *Aloe vera*, *Curcumin* and neem as an obturating material for pulpectomy. **Materials and methods:** The study comprised of age group 4-8 years children requiring endodontic treatment for at least a single primary molar tooth. Sixty primary molar teeth from 43 children were divided equally and randomly into four study groups. The materials used for obturation were zinc oxide powder (ZnO) and Eugenol (ZOE) (group I), ZnO and *Aloe*

vera Gel (group II), ZnO and *Curcumin* Powder (group III), ZnO and neem extract (group IV). They were evaluated clinically and radiographically at immediate postoperative and then at 1-, 3-, 6-, and 9-month intervals. **Results:** At the end of 9 months, the Chi-square test revealed 100% success rate for recovery of pain in group I and III, 66.66% in group II and 93.3% in group IV. The success rates for absence of abscess and for periradicular radiolucency in group I, III, and group IV were 100% and 66.6% for group II. The success rate for periapical radiolucency in group I and group III was 100%, in group II 66.6% and in group IV 93.35%. The success rate for all the groups shows 100% success in terms of pathological root resorption. **Conclusion:** Zinc oxide eugenol has proven to be the best obturating material. ZnO with *Aloe vera* showed a success rate which is significantly lower than the other medicaments. ZnO with *Curcumin* and ZnO with neem had shown promising clinical and radiographical results. **Clinical significance:** ZnO with *Curcumin* and ZnO with neem can be used as a root canal filling material in primary teeth with further follow-up studies.

### Evaluation of the Efficacy of a Neem Extract-Based Herbal Ointment, Herbal Antioxidant, and Propolis on Oxidative Stress Related to Oral Ulcers: An Interventional Animal Study.

Patel A, Patel SA, Mahapatra J, Mehta D, Murugesan S, Kamatchi Subramani S.

Cureus. 2023 Nov 8;15(11):e48542. doi: 10.7759/cureus.48542. eCollection 2023 Nov. PMID: 38073963

Traumatic oral ulcers are one of the most commonly encountered oral ulcers. Their healing may be delayed due to factors like the presence of opportunistic infectious microbes in the oral cavity, secondary trauma from sharp edges of teeth, and the systemic condition of the patient. **Aim:** To compare the efficacy of a newly developed neem extract containing herbal ointments (propolis and *Hemidesmus indicus*) in enhancing the wound contraction of traumatic oral ulcers and to determine the relationship between oxidative stress and oral ulcers. **Method:** Ulcers were inflicted by trauma in the mouths of experimental rabbits using a 5 mm punch biopsy device. Forty-eight animals were randomly put into six groups (n = 12). Group 1 was the control group that did not receive any intervention; Group 2 had a systemic treatment of *Hemidesmus indicus* extract; Group 3 received a topical application of propolis; Group 4 had a topical application of a neem extract-based herbal ointment; Group 5 was administered a combination of *Hemidesmus indicus* and propolis; and Group 6 had a combination of a neem-based herbal ointment and *Hemidesmus indicus*. Oxidative stress levels were calculated by measuring superoxide dismutases and malondialdehyde levels in the blood on days 0, one, seven, and 14. Wound contraction scores of ulcers were also assessed on days seven and 14. **Results:** Significantly higher wound contraction scores were seen in groups treated with herbal ointment in comparison to groups treated without herbal ointment. Oxidative stress levels increased in all groups after the infliction of ulcers (day one) and then declined as the ulcers healed, reaching near-normal levels on day 14. Groups containing *Hemidesmus indicus* showed a significant reduction in oxidative stress in comparison to groups without *Hemidesmus indicus*. A p-value of <0.05 was considered significant. **Conclusion:** A combined formulation of herbal ointment and *Hemidesmus indicus* proved to be the most efficacious in enhancing wound contraction of oral ulcers along with significantly reducing oxidative stress in experimental rabbits.

## Green Synthesis and Characterization of Silver Nanoparticles Using *Azadirachta indica* Seeds Extract: In Vitro and In Vivo Evaluation of Anti-Diabetic Activity.

Rehman G, Umar M, Shah N, Hamayun M, Ali A, Khan W, Khan A, Ahmad S, Alrefaei AF, Almutairi MH, Moon YS, Ali S.

*Pharmaceuticals (Basel)*. 2023 Dec 1;16(12):1677. doi: 10.3390/ph16121677.PMID: 38139804

**Background:** Diabetes mellitus (DM) is a non-communicable, life-threatening syndrome that is present all over the world. The use of eco-friendly, cost-effective, and green-synthesised nanoparticles as a medicinal therapy in the treatment of DM is an attractive option. **Objective:** In the present study, silver nanoparticles (AI-AgNPs) were biosynthesized through the green synthesis method using *Azadirachta indica* seed extract to evaluate their anti-diabetic potentials. **Methods:** These nanoparticles were characterized by using UV-visible spectroscopy, Fourier transform infrared spectrophotometers (FTIR), scanning electron microscopy (SEM), DLS, and X-ray diffraction (XRD). The biosynthesized AI-AgNPs and crude extracts of *Azadirachta indica* seeds were evaluated for anti-diabetic potentials using glucose adsorption assays, glucose uptake by yeast cells assays, and alpha-amylase inhibitory assays. **Results:** AI-AgNPs showed the highest activity ( $75 \pm 1.528\%$ ), while crude extract showed ( $63 \pm 2.5\%$ ) glucose uptake by yeast at 80  $\mu\text{g/mL}$ . In the glucose adsorption assay, the highest activity of AI-AgNPs was  $10.65 \pm 1.58\%$ , while crude extract showed  $8.32 \pm 0.258\%$  at 30 mM, whereas in the alpha-amylase assay, AI-AgNPs exhibited the maximum activity of  $73.85 \pm 1.114\%$  and crude extract  $65.85 \pm 2.101\%$  at 100  $\mu\text{g/mL}$ . The assay results of AI-AgNPs and crude showed substantial dose-dependent activities. Further, anti-diabetic potentials were also investigated in streptozotocin-induced diabetic mice. Mice were administered with AI-AgNPs (10 to 40 mg/kg b.w) for 30 days. **Conclusions:** The results showed a considerable drop in blood sugar levels, including pancreatic and liver cell regeneration, demonstrating that AI-AgNPs have strong anti-diabetic potential.

## Azadiradione up-regulates the expression of parvalbumin and BDNF via Ube3a.

Jana S, Giri B, Das S, Manna A, Mandal SC, Ranjan Jana N.

*Gene*. 2023 Dec 13;897:148081. doi: 10.1016/j.gene.2023.148081. Online ahead of print.PMID: 38101713

Azadiradione is a small bioactive limonoid found in the seed of *Azadirachta Indica*, an Indian medicinal plant commonly known as Neem. Recently, it has been shown to ameliorate the disease pathology in fly and mouse model of Huntington's disease by restoring impaired proteostasis. Here we report that the azadiradione could be involved in modulating the synaptic function through increased expression of Ube3a, a dual function protein having ubiquitin ligase and co-activator functions and associated with Angelman syndrome and autism. Treatment of azadiradione to HT22 hippocampal cell line and in adult mice induced the expression of Ube3a as well as two important synaptic function and plasticity regulating proteins, parvalbumin and brain-derived neurotropic factor (BDNF). Interestingly, another synaptic plasticity modulating protein Arc (activity-regulated cytoskeletal associated protein) was down-regulated by azadiradione. Partial knockdown

of Ube3a in HT22 cell abrogated azadiradione induced expression of parvalbumin and BDNF. Ube3a-maternal deficient mice also exhibited significantly decreased expression of parvalbumin and BDNF in their brain and treatment of azadiradione in these animals did not rescue the altered expression of either parvalbumin or BDNF. These results indicate that azadiradione-induced expression of parvalbumin and BDNF in the brain is mediated through Ube3a and suggest that azadiradione could be implicated in restoring synaptic dysfunction in many neuropsychiatric/neurodegenerative disorders.