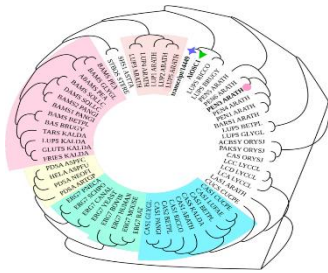
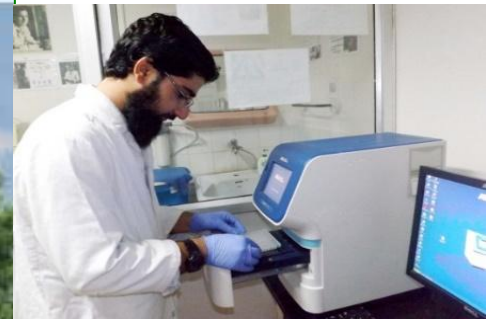




Neem Research Newsletter

Volume 4, Issue 8, 2024



WORLD NEEM ORGANISATION (WNO)



From

The Editor's Desk.....

This month's survey of research on neem has unveiled several known and hitherto unknown facets of the immense potential of neem. Thai neem seed extract has been reported to be a safe alternative to the synthetic insecticides commonly used in mulberry cultivation, which can harm silkworms. Deep learning-based models were developed using neem fruit and leaf images to predict the concentration of metabolites thus simplifying the process of choosing the right tree for fruit collection. Neem vegetable cake significantly enhanced productivity of corn crops. Neem extract was found to be promising in pest management strategies in sustainable arabica coffee crops. Ethanolic neem extract was shown to activate the immune system and antioxidant response in Nile tilapia fingerlings, improving growth and fish resistance to parasitic and bacterial infections. Oral supplementation of aqueous neem leaf extract protected against respiratory viral infections in broiler chickens. Although neem has multifarious beneficial effects, occupational exposure to neem oil dust and neem chemicals was found to induce respiratory symptoms emphasizing the need to wear protective gear. Nanoemulsions of neem extract were shown to be effective in the treatment of gonorrhoea. Silver nanoparticles of neem exerted antibacterial effects against oral pathogens. Neem Leaf Glycoprotein was demonstrated to disrupt exhausted CD8+ T-cell-mediated cancer stem cell aggression that offers immense potential in cancer therapeutics. The key genes/pathways responsible for inducing the death of breast cancer cells by the neem limonoid, epoxyazadiradione were identified using next-generation sequencing and bioinformatics analysis. Modular synthesis of nimbolide has been suggested to potentiate its anticancer properties. Based on experimental evidence, azadiradione has been suggested as a potential candidate agent for the treatment of neurological disorders.

S. Nagini

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



Neem in Agriculture & Aquaculture

[Impact of Neem Seed Extract on Mortality, Esterase and Glutathione-S-Transferase Activities in Thai Polyvoltine Hybrid Silkworm, *Bombyx mori* L.](#)

Rattanapan A, Sujayanont P.

Insects. 2024 Aug 3;15(8):591. doi: 10.3390/insects15080591.PMID: 39194796

Neem, a biopesticide, offers a safer alternative to the synthetic insecticides commonly used in mulberry cultivation, which can harm silkworms. This study aimed to investigate the effects of Thai neem seed extract on all instar larvae of the Thai polyvoltine hybrid silkworm, *Bombyx mori* L., Dok Bua strains, focusing on the mortality rate and the activities of esterase (EST) and glutathione S-transferases (GST) enzymes. Acute toxicity was assessed using the leaf-dipping method. Results showed that the mortality rate tended to be higher in younger instars than in older ones. The first instar larvae exhibited the highest mortality rate at 94%, whereas the LC_{50} was highest in the third instar at 5.23 mg L^{-1} at 72 h. This trend aligns with the activities of EST and GST, which were evaluated in the whole bodies of the first instar larvae and the midgut tissue of fifth instar larvae. As the extract concentration increased, EST activity decreased while GST activity increased in both the first and fifth instar larvae. These findings highlight that neem extract is toxic to all instar larvae, with GST playing a crucial role in detoxification, particularly in the whole body of the Thai polyvoltine hybrid silkworm.

[Fruit-In-Sight: A deep learning-based framework for secondary metabolite class prediction using fruit and leaf images](#)

Krishnan NM, Kumar S, Panda B.

PLoS One. 2024 Aug 8;19(8):e0308708. doi: 10.1371/journal.pone.0308708..PMID: 39116159

Fruits produce a wide variety of secondary metabolites of great economic value. Analytical measurement of the metabolites is tedious, time-consuming, and expensive. Additionally, metabolite concentrations vary greatly from tree to tree, making it difficult to choose trees for fruit collection. The current study tested whether deep learning-based models can be developed using fruit and leaf images alone to predict a metabolite's concentration class (high or low). We collected fruits and leaves ($n = 1045$) from neem trees grown in the wild across 0.6 million sq km, imaged them, and measured concentration of five metabolites (azadirachtin, deacetyl-salannin, salannin, nimbin and nimbolide) using high-performance liquid chromatography. We used the data to train deep learning models for metabolite class prediction. The best model out of the seven tested (YOLOv5, GoogLeNet, InceptionNet, EfficientNet_B0, Resnext_50, Resnet18, and SqueezeNet) provided a validation F1 score of 0.93 and a test F1 score of 0.88. The sensitivity and specificity of the fruit model alone in the test set were 83.52 ± 6.19 and 82.35 ± 5.96 , and 79.40 ± 8.50 and 85.64 ± 6.21 , for the low and the high classes, respectively. The sensitivity was further boosted to 92.67 ± 5.25 for the low class and 88.11 ± 9.17 for the high class, and the specificity to 100% for both classes, using a multi-analyte framework. We incorporated the multi-analyte model in an Android mobile App Fruit-In-Sight that uses fruit and leaf images to decide whether to 'pick' or 'not pick' the fruits from a specific tree based on the

metabolite concentration class. Our study provides evidence that images of fruits and leaves alone can predict the concentration class of a secondary metabolite without using expensive laboratory equipment and cumbersome analytical procedures, thus simplifying the process of choosing the right tree for fruit collection.

[Interaction of *Capnodium alfenasii* with extrafloral nectaries of *Azadirachta indica*.](#)

Mesquita NLS, Leitão CAE, de Souza Soares PP, de Novaes QS, de Melo MP, Bezerra JL, Santos A.

Protoplasma. 2024 Aug 7. doi: 10.1007/s00709-024-01977-4. PMID: 39112644

Sooty moulds are saprophytic epiphytic fungi that grow mostly on insect secretions, but they can also be associated with plant secretions. In this study, we aimed to describe the interaction of *Capnodium alfenasii* sooty mould with the extrafloral shoot nectaries of *Azadirachta indica*. Anatomical and histochemical studies were carried out on serial sections of extrafloral shoot nectaries of *A. indica* without and with *C. alfenasii* infestation. The total soluble sugar content of the secreted nectar was determined, and the conidial germination of the fungus in distilled water and in dextrose and nectar solutions was evaluated. The shoot nectaries of *A. indica* are elongated structures that occur in pairs near the base of the petiole. The exuded nectar contains an average of 534.8 µg of total soluble sugars per µL of nectar and provides ideal conditions for conidial germination and fungal growth. *C. alfenasii* hyphae grow on the nectary, penetrate through breaks in the cuticle, travel under the cuticle and penetrate the secretory tissue by inter- and intracellular routes. The present report is the first to describe the interaction of *C. alfenasii* with the *A. indica* nectary, including the penetration of hyphae into nectariferous tissues and the plant defence mechanisms.

[Use of neem vegetable cake \(*Azadirachta indica* A. Juss\) increases corn productivity.](#)

Silva JSL, Silva JHB, Silva AV, Silva LKP, Ferreira JS, Guedes JVS, Santiago MST, Costa EN, Freitas PVM, Mielezrski F.

Braz J Biol. 2024 Aug 5;84:e281515. doi: 10.1590/1519-6984.281515. PMID: 39109718

The need to transition to more sustainable agriculture that is adaptable to environmental challenges, reducing dependence on chemical fertilizers and minimizing environmental impact, represents the new paradigm of the moment. In this scenario, studies with the adoption of bioinputs in corn cultivation emerge as a viable option for the sustainability of agricultural activity. Therefore, the objective was to evaluate the effect of doses of neem vegetable cake on the yield components of corn crops. An experimental design was used of randomized blocks was used, consisting of four doses of neem vegetable rendering (3 kg ha⁻¹, 6 kg ha⁻¹, 9 kg ha⁻¹ and 12 kg ha⁻¹) and a treatment control without the presence of organic fertilizer. The result indicates the presence of a significant effect of treatments with the application of neem cake on the main components of corn yield, including grain productivity, suggesting that the high carbon content present in the organic product can induce phytochemical effects and biological changes in the soil, making it more productive. It was found that, when administering the maximum experimental dose, compared to the control group, there was a significant effect ($p \leq 0.01$) of 21.3% on grain productivity, jumping from 2,140 kg ha⁻¹, when did not apply organic fertilizer, to 2,596 kg

ha⁻¹ with the application of 12 kg of neem cake per hectare. It is noted that the increase in grain productivity was in the proportion of 38 kg ha⁻¹ of corn for each kilo of neem cake applied. To facilitate interpretation and decision-making, an analysis of the economic viability of neem cake for rainfed corn was also determined, also identifying the maximum experimental dose of 12 kg ha⁻¹, as the most economically viable, providing an increase in profit of around R\$ 119.92 per hectare, in relation to the control.

[Neem-Extract Formulation on Hypothenemus hampei Preference and Performance in Arabica Coffee Fruits and Artificial Diet.](#)

Padilla JJE, de Souza BHS, de Souza Carneiro F, Keller E.

Neotrop Entomol. 2024 Aug 14. doi: 10.1007/s13744-024-01186-x. PMID: 39141219

Coffee berry borer (CBB) *Hypothenemus hampei* is a major biotic threat to coffee production worldwide. Studies have reported negative effects on CBB by oil-based formulations of neem (*Azadirachta indica*), but little information is available for other neem-extract formulations. This study evaluated CBB preference and performance in arabica coffee fruits and artificial diet treated with a neem-extract formulation (Openeem Plus®) in the field and laboratory conditions. Field experiments were performed using CBB females artificially infested in cherry or green coffee fruits confined in voile-fabric cages tied to branches of neem-treated and control plants, recording the adult mortality and offspring production. Dual-choice and no-choice bioassays assessed CBB preference and development in fruits and artificial diet treated with the neem extract compared to controls in the laboratory, respectively. As main results obtained in the field and laboratory experiments, the neem extract significantly reduced CBB oviposition in both cherry and green fruits, as well as in artificial diet compared to controls. However, the botanical product did not affect CBB adult survival and preference in the laboratory bioassays. The neem extract is promising for use in pest management strategies in sustainable arabica coffee crops by reducing CBB oviposition and offspring. These effects can contribute to lowering the pest population buildup along the crop cycle and damage potential to coffee production.

[Molecular identification and eco-friendly management of rice brown planthoppers in Bangladesh.](#)

Kar M, Jahan SMH, Rahman MA, Datta SD. Heliyon. 2024 Jul 31;10(15):e35514. doi: 10.1016/j.heliyon.2024.e35514. eCollection 2024 Aug 15. PMID: 39166075

Infestation by various insect pests is the main constraint for growing rice where rice brown planthopper (*Nilaparvata lugens* Stål) can severely damage rice plants directly through feeding. Therefore, the study aims to detect rice brown planthoppers (BPH) and provide environment-friendly management tactics to mitigate the problem which caused by brown planthoppers. The BPH samples were collected from rice fields of different locations in the Patuakhali of Bangladesh for molecular identification. A molecularly single species of rice brown planthopper, *Nilaparvata lugens* was identified using mitochondrial cytochrome oxidase subunit I (mtCOI) universal marker. The nucleotide sequences of collected samples were compared with other nucleotide sequences from the GenBank database of NCBI, which make single clades in the phylogenetic tree at an insignificant distance.

Moreover, brown planthopper management observations were recorded in laboratory conditions after providing an artificial diet with different treatments of plant-based insecticides Neem oil (1 %, 5 %, and 10 %), Castor oil (1 %, 5 %, and 10 %) where only 20 % sucrose solution was used as negative control and Abamectin (1 %, 5 % and 10 %) were also used as a positive control for comparing the efficacy of plant-based insecticides on rice brown planthoppers. The results showed the highest mortality (100 %) of rice brown planthoppers was recorded by Abamectin 10 %, followed by Abamectin 5 %. Neem 10 % performed better than Abamectin 1 % during 1st hour. Initial after exposure of 2nd hour for Abamectin 1 % revealed greater mortality (59 %) than Neem 10 %. Neem 5 % showed less effect on mortality in brown planthopper than Neem 10 % but was higher than Neem 1 % during 6 h of observation. The Castor oil of 10 % caused higher mortality than the Castor of 5 % but not up to the marks of Abamectin and different concentrations of Neem oil. Castor oil of 1 % and control have shown no mortality of brown planthopper for 6 h of observation.

[Elucidating the effect of dietary neem \(*Azadirachta indica*\) on growth performance, haemato-biochemical, immunological response, and anti-pathogenic capacity of Nile tilapia juveniles.](#)

Radwan M, Manaa EA, El-Feky MMM, Mohammadein A, Al Malki JS, Badawy LA, Abbas MMM. *Vet Res Commun.* 2024 Aug 24. doi: 10.1007/s11259-024-10497-8. PMID: 39180602

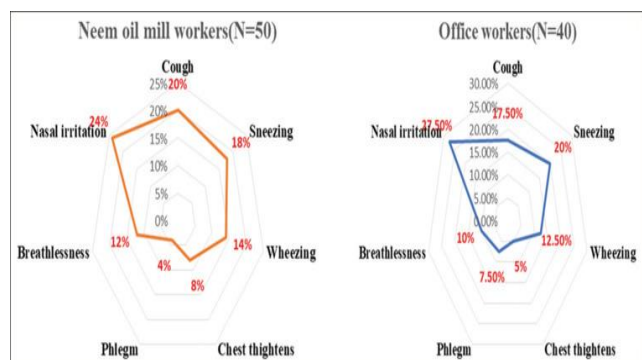
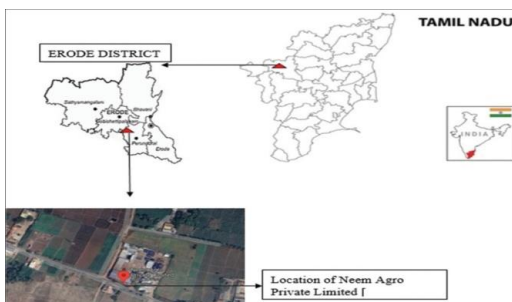
This investigation attempts to evaluate the effect of diet additives via aqueous or ethanolic herbal extracts from *Azadirachta indica* leaves on Nile tilapia, *Oreochromis niloticus*. Five dietary categories were assigned to the fish: the first category (N1, with no extract) was kept under control conditions; two categories contained aqueous extract (N2 (1.0 g/kg) and N3 (2.0 g/kg)); and two categories contained ethanolic extract, N4 (1.0 g/kg) and N5 (2.0 g/kg), with each group being fed for 60 days. After the feeding trial, *Aeromonas hydrophila* was injected intraperitoneally into fish for 14 days; fish mortality was recorded during this period. The results showed that the fish-fed dietary *A. indica* significantly improved growth performance and intestinal health (digestive enzymes and intestinal morphology), especially in the N4 and N5 categories. However, N4 and N5 categories demonstrated a significant decrease in AST and ALT activities and an increase in total protein, serum albumin, globulin, growth hormone (GH), leptin hormone (LEP), hemoglobin, white blood cells, and hematocrit ($P < 0.05$) in comparison with the control category (N1). Compared to the control category, the N4 and N5 categories have revealed a significant reduction in MDA activity and improvements in immunological activities (lysozyme, complement C3, and nitric oxide) and antioxidant enzymes (CAT, SOD, and GPX). Moreover, in tilapia-fed *A. indica*, the expression of IL-8, IL-1 β , and Nf-kb genes was downregulated partially in the N4 and N5 categories than the control category. In contrast, the lysozyme, C3, GPX, and CAT genes were upregulated partially at N4 and N5 compared to the control category. Following the bacterial challenge, fish in the N4 and N5 categories also displayed the lowest fish mortality compared to the control category. The ethanolic extract displayed a more potent resistance against the parasite *Cichlidogyrus tilapia* in vitro than the aqueous and control categories, partially at 2 g/L. According to these findings, an ethanolic neem extract (2.0 g/kg feed) activates the immune system and antioxidant response in Nile tilapia fingerlings, improving growth and fish resistance to parasitic and bacterial infections.

Neem- Occupational Exposure

Study of Respiratory Symptoms and Pulmonary Function among **Neem** Oil Industry Workers.

Subramaniam S, Ganesan A, Raju N, Rajavel N, Chenniappan M, Surendra Mohanty RM, Angamuthu S, Sivakumar K, Prakash C, Pramanik A, Basak AK. *Indian J Occup Environ Med.* 2024 Apr-Jun;28(2):163-169. doi: 10.4103/ijoem.ijoem_250_23. Epub 2024 Jun 28. PMID: 39114101

This study aims to investigate the pulmonary functions and respiratory symptoms of workers in the neem oil extraction industry in Tamil Nadu, India, who are exposed to neem oil dust and chemicals in their occupational environment. Fifty male workers from the exposed group and 50 male workers from the non-exposed group to neem dust and chemicals were investigated for this study. A modified respiratory assessment questionnaire based on the American Thoracic Society (ATS) standard and portable hand-held spirometry were used to assess their respiratory symptoms and pulmonary function. Respiratory symptoms such as coughing, sneezing, wheezing, and nasal irritation are found to be higher in the exposed groups than in the controlled groups. The pulmonary function of exposed workers had doubled respiratory problems than the controlled groups, which indicates the impacts of dust and chemicals generated during neem oil extraction on workers' health. Forced expiratory volume in one second (FEV₁)/forced vital capacity (FVC)% was noted regarding the duration of exposure to neem oil dust ($P < 0.001$). Also, there was a high difference between the heavily exposed and the lightly exposed ($P < 0.001$). Hence, to mitigate these problems, the oil mill workers should be cautious and wear personal protection equipment during working hours, and it is recommended to have an exhaust ventilation system.



Neem for Human Health

[Synthesis of Silver Nanoparticles Using *Azadirachta indica* and *Syzygium aromaticum* Extract and Its Antibacterial Action Against *Enterococcus faecalis*: An In Vitro Study.](#)

Chandran N, Ramesh S, Shanmugam R.

Cureus. 2024 Jul 21;16(7):e65044. doi: 10.7759/cureus.65044. PMID: 39165463

Introduction Nanotechnology is the study of manipulating matter at the atomic scale involving particles smaller than 100 nm. Silver nanoparticles (AgNPs) are gaining popularity across diverse sectors including medical, food, healthcare, consumer goods, and industrial fields due to their distinctive physical and chemical characteristics. The eco-friendly synthesis of AgNPs offers a straightforward, cost-effective, and environmentally benign method devoid of hazardous chemicals. **Methodology** Eighty milliliters (mL) of silver nitrate mixed with 20 mL of *Azadirachta indica* and *Syzygium aromaticum* plant extract underwent two days of magnetic stirring for AgNP synthesis. Characterization was done via ultraviolet-visible (UV-vis)-spectroscopy (300-700 nm), and antimicrobial properties, which were checked with *Enterococcus faecalis*, were assessed using the agar-well diffusion method. **Results** The change in color and peak observed in the UV-vis spectrum confirmed the successful synthesis of AgNPs. Both neem and clove extract-mediated synthesis of AgNPs exhibited antibacterial activity against *E. faecalis*. However, neem extract synthesized AgNPs displayed a larger inhibitory zone diameter and lower minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values compared to those synthesized using clove extract. **Conclusion** Incorporating neem and clove extracts in AgNP synthesis offers a practical, eco-friendly, and cost-efficient method with notable efficacy. These AgNPs exhibit antibacterial activity against *E. faecalis*, suggesting their viability as potent antibacterial agents for addressing oral pathogens. Their sustainable synthesis underscores a promising avenue for developing effective antimicrobial solutions in oral healthcare.

[Assessment of Smear Layer Removal and Push-Out Bond Strength Efficacy of Traditional and Herbal Root Canal Irrigants Across Different Root Levels: An In Vitro Study.](#)

Shah M, Patel C, Attur K, Patel N, Attur S, Solanki M, Patel A, Chhaya P, Pandya D, Maheshwari H.

Cureus. 2024 Jul 14;16(7):e64511. doi: 10.7759/cureus.64511. PMID: 39139342

Introduction: Endodontic therapy requires meticulous root canal debridement, pathogen elimination, and effective obturation to prevent microbial intrusion. The presence of the smear layer hinders sealer penetration, compromising sealing effectiveness. Sodium hypochlorite and chlorhexidine are esteemed endodontic irrigants. Herbal extracts like neem and tulsi, with antimicrobial and anti-inflammatory properties, show promise for root canal irrigation. The study aimed to evaluate the efficacy of various irrigants in removing the smear layer and enhancing push-out bond strength at different root canal levels. **Materials and methods:** One hundred mandibular premolars with single canals were collected, and 50 samples each were used for the smear layer and push-out bond strength

analysis. Neem and tulsi extracts were prepared for irrigation. Teeth were decoronated, and up to 30 (6%) canals were prepared and were randomly divided into five groups based on irrigants used. A smear layer examination was conducted after longitudinally sectioning the tooth and sections were observed in a scanning electron microscope (SEM). Obturation was done in the remaining samples, and the push-out bond strength was assessed using a universal test machine. **Results:** Sodium hypochlorite showed the highest smear layer removal efficacy followed by chlorhexidine, neem, tulsi leaves with rose water extract, and normal saline. Chlorhexidine exhibited the highest push-out bond strength, with the coronal third presenting the strongest values, followed by neem, tulsi with rose water, normal saline, and sodium hypochlorite. **Conclusion:** The study underscores the potential of herbal irrigants in endodontic therapy, indicating promising results while emphasizing the necessity for further clinical trials to validate their efficacy and other properties.

[Comparative Evaluation of Cytotoxicity of Zinc Oxide Nanoparticles Mixed With Herbal Extract as an Intracanal Medicament: A Zebrafish Model Study.](#)

Manoj A, Ranjan M, Singh S, Ragavendran C.

Cureus. 2024 Jul 9;16(7):e64131. doi: 10.7759/cureus.64131. PMID: 39119434

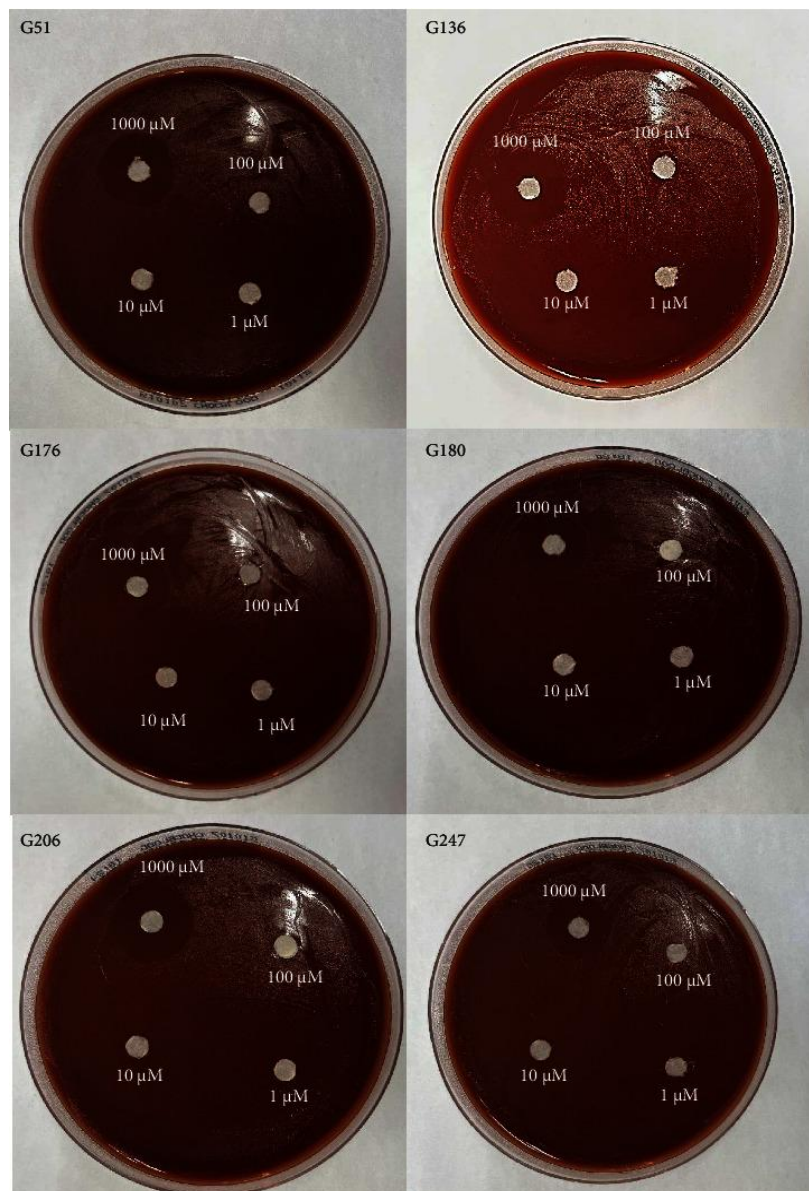
Objective In this study, zebrafish embryos are used to study the cytotoxic effects of a novel intracanal medication (ICM) based on zinc oxide nanoparticles (ZnO NPs) loaded with polyherbal extracts (*Azadirachta indica* and *Solanum xanthocarpum*). **Material and methods** In the present study, a green and sustainable method was employed for the synthesis of ZnO NPs mixed with bark and seed extracts of *Azadirachta indica* and *Solanum xanthocarpum* to be used as a polyherbal ICM. Formulation of ZnO NPs was confirmed with color change in mixture produced upon dissolving zinc acetate dihydrate in distilled water followed by slow addition of sodium hydroxide solution and herbal extracts. The effects of these green synthesized ZnO NPs were evaluated through a zebrafish embryo toxicity test. Embryos were exposed to different concentrations (25, 50, and 100 µg/mL) of synthesized experimental doses of ZnO NP and compared with the control embryos. Toxicological endpoints, such as the zebrafish embryo's survival rate, hatching rate, and heart rate, were noted and described. **Results** A concentration-dependent increase in mortality rate and hatching delay followed by declined heart rate was observed in green synthesized ZnO NP-treated embryos. The maximum toxicity was observed with an increase in the concentration of 100 µg/mL of the experimental dose, and at a low concentration of 25 µg/mL, it does not effectively show any developmental alteration in zebrafish embryos. **Conclusion** A novel polyherbal ICM loaded with ZnO NPs exhibited a dose-dependent effect on the heart rate, hatching, and mortality rate of the embryos. At optimal concentrations, the medication demonstrated minimal developmental malformations and cytotoxic effects, indicating its safety for use. However, increasing concentrations of the medication resulted in severe developmental malformations.

[Busting the Resistance: Antimicrobial Activity of Plant-Infused Nanoemulsions against *Neisseria gonorrhoeae*.](#)

Naicker D, Govender R, Abbai NS.

Int J Microbiol. 2024 Jul 30;2024:7084347. doi: 10.1155/2024/7084347. PMID: 39109214

The escalating antibiotic resistance rates in *Neisseria gonorrhoeae* (*N. gonorrhoeae*) are now a grave concern. There is a critical need for alternative treatment options for infection since *N. gonorrhoeae* has developed resistance to multiple antibiotics used for treatment. In this study, plant nanoemulsions from *Ocimum tenuiflorum*, *Moringa oleifera*, and *Azadirachta indica* were tested for their antimicrobial properties against *N. gonorrhoeae*. The study also assessed the toxicity of these plant nanoemulsions using human erythrocytes. The plants were sourced from the Botanical Gardens in Durban, South Africa. Nanoemulsions were produced from the dried plants using established methods. The nanoemulsion-based plant extracts were tested against laboratory (World Health Organization (WHO) strains) and clinical isolates of *N. gonorrhoeae* using the disk diffusion method. All six isolates had zones of inhibition for the 1000 μM concentration for all three nanoemulsion-based plant extracts. No zones of inhibition were observed for 100 μM , 10 μM , and 1 μM nanoemulsion concentrations for five of the isolates. Isolate G176 had zones of inhibition at 1000 μM and 100 μM concentrations for the nanoemulsions of *Ocimum tenuiflorum*. Both the WHO strains had zones of inhibition appearing at the 1000 μM concentration. For the WHO Y strain, zones of inhibition for both 1000 μM and 100 μM concentrations were observed for the nanoemulsions of *Ocimum tenuiflorum* and *Azadirachta indica*. According to the analysis, there was 0% haemolytic activity observed which suggests the nontoxic nature of the extracts. This study showed that the nanoemulsion and plant mix may potentially be used as a safer alternative to treat gonorrhoea.



[Retention of methicillin susceptibility in Staphylococcus aureus using natural adjuvant as an allosteric modifier of penicillin-binding protein 2a.](#)

Islam MR, Azmal M, Prima FS, Zaman B, Hossain MM, Mishu MA, Ghosh A. *Comput Biol Med.* 2024 Aug 27;181:109070. doi: 10.1016/j.combiomed.2024.109070. PMID: 39205340

The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) poses a significant global public health challenge due to its resistance to conventional antibiotics, primarily mediated by the mutated penicillin-binding protein, PBP2a. This study aims to investigate the potential of phytochemicals derived from medicinal plants in the Indian subcontinent to serve as adjuvants, enhancing the efficacy of methicillin against MRSA through allosteric modification of PBP2a using molecular docking and molecular dynamics (MD) simulation. After comprehensive Absorption, Distribution, Metabolism, and Excretion (ADME) profiling, along with AMES and hepatotoxicity tests, 9 compounds were shortlisted as suitable adjuvant candidates. Among them, nimbolide, quercetin, emodin, daidzein, eriodictyol, luteolin, and apigenin exhibited strong binding affinity to the allosteric site of PBP2a, with docking scores ranging from -8.7 to -7.3 kcal/mol. These phytochemicals facilitated enhanced methicillin binding, as evidenced by improved docking scores ranging from -6.1 to -6.8 kcal/mol, compared to -5.6 kcal/mol for methicillin alone. Molecular dynamics simulations confirmed the stability and favorable conformations of phytochemical-PBP2a complexes. Quercetin and daidzein were identified as the most promising adjuvant candidates, forming stable and energetically favorable complexes with PBP2a. Experimental validation showed that quercetin, at 30 mg/mL, effectively retained methicillin's antibacterial efficacy against MRSA. This study underscores the potential of natural compounds in overcoming antibiotic resistance and suggests that phytochemical-antibiotic synergism could be a viable strategy to combat multidrug-resistant bacterial infections.

[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.* 2024 Mar 1;897:148081. doi: 10.1016/j.gene.2023.148081. Epub 2023 Dec 13. PMID: 38101713

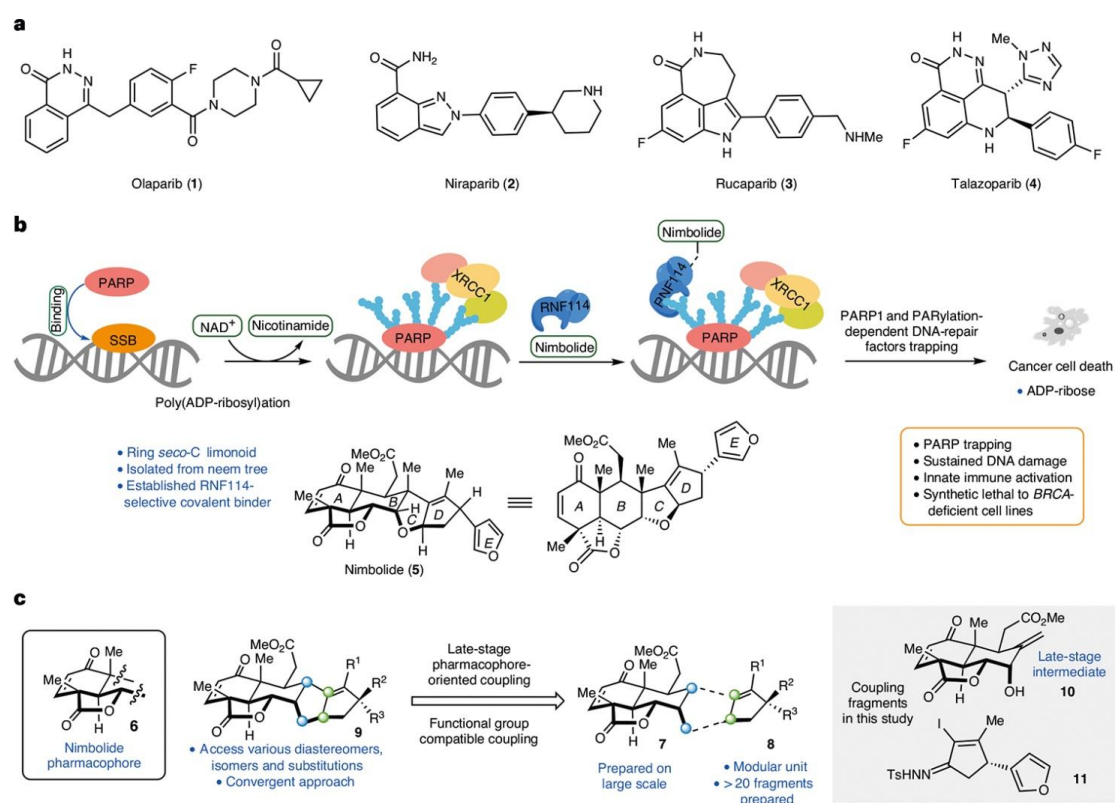
Azadiradione is a small bioactive limonoid found in the seed of *Azadirachta Indica*, an Indian medicinal plant commonly known as Neem. Recently, it has been shown to ameliorate the disease pathology in fly and mouse model of Huntington's disease by restoring impaired proteostasis. Here we report that 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.

Synthesis of nimbolide and its analogues and their application as poly(ADP-ribose) polymerase-1 trapping inducers.

Deng H, Deng H, Kim C, Li P, Wang X, Yu Y, Qin T. *Nat Synth.* 2024 Mar;3(3):378-385. doi: 10.1038/s44160-023-00437-w. PMID: 39119242

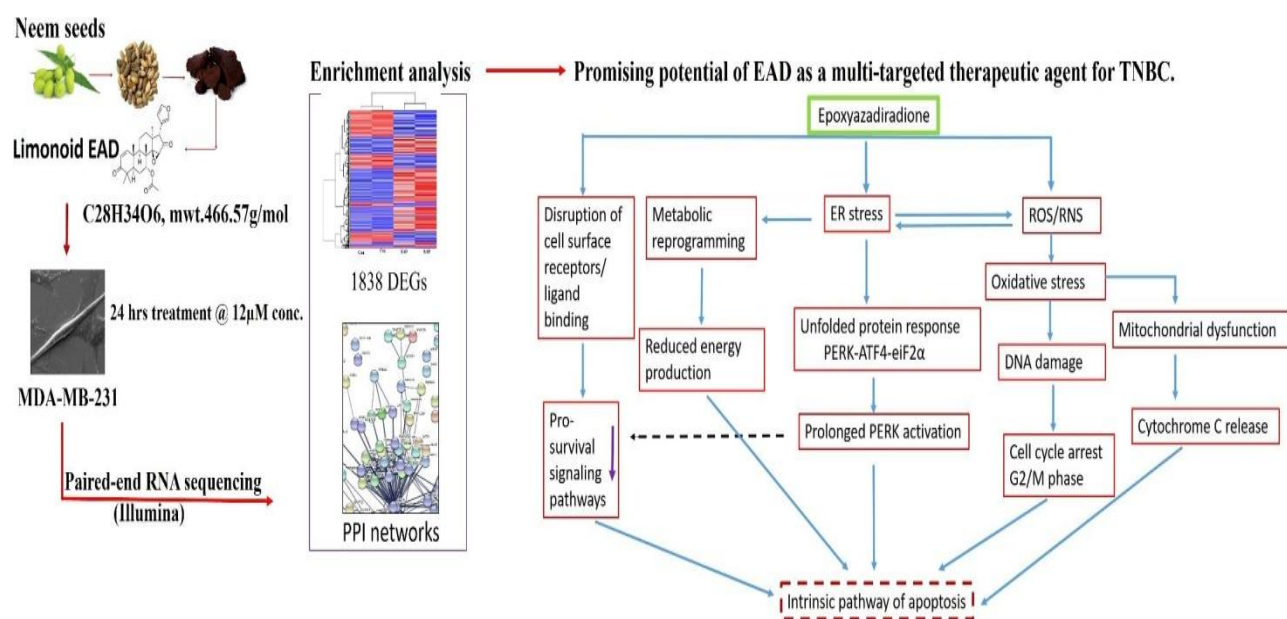
Nimbolide, a ring seco-C limonoid natural product, was recently found to inhibit the poly(ADP)-ribosylation (PARylation)-dependent ubiquitin E3 ligase RNF114. In doing so, it induces the 'supertrapping' of both PARylated PARP1 and PAR-dependent DNA-repair factors. PARP1 inhibitors have reshaped the treatment of cancer patients with germline *BRCA1/2* mutations partly through the PARP1 trapping mechanism. To this end, modular access to nimbolide analogues represents an opportunity to develop cancer therapeutics with enhanced PARP1 trapping capability. Here we report a convergent synthesis of nimbolide through a late-stage coupling strategy. Through a sulfonyl hydrazone-mediated etherification and a radical cyclization, this strategy uses a pharmacophore-containing building block and diversifiable hydrazone units to enable the modular synthesis of nimbolide and its analogues. The broad generality of our synthetic strategy allowed access to a variety of analogues with their preliminary cellular cytotoxicity and PARP1 trapping activity reported.



Molecular insights into epoxyzadiradione induced death in triple-negative breast cancer cells: A system biology approach.

Lakshmi S, Priya S. *Gene*. 2024 Aug 6;930:148814. doi: 10.1016/j.gene.2024.148814. Online ahead of print. PMID: 39116958

Epoxyzadiradione is an important limonoid with immense pharmacological potential. We have reported previously that epoxyzadiradione (EAD) induces apoptosis in triple negative breast cancer cells (MDA-MB 231) by modulating diverse cellular targets. Here, we identify the key genes/pathways responsible for this effect through next-generation sequencing of the transcriptome from EAD treated cells and integrated molecular data analysis using bioinformatics. In silico analysis indicated that EAD displayed favourable drug-like properties and could target multiple macromolecules relevant to TNBC. RNA sequencing revealed that EAD treatment results in the differential expression of 1838 genes in MDA-MB 231 cells, with 752 downregulated and 1086 upregulated. Gene set enrichment analysis of these genes suggested that EAD disrupts protein folding in the endoplasmic reticulum, triggering the unfolded protein response (UPR) and potentially leading to cell death. EAD also induced oxidative stress and DNA damage, downregulated pathways linked to metabolism, cell cycle progression, pro-survival signalling, cell adhesion, motility and inflammatory response. The identification of protein cluster and hub genes were also done. The validation of the identified hub genes gave an inverse correlation between their expression in EAD treated cells and TNBC patient samples. Thus, the identified hub genes could be explored as therapeutic or diagnostic markers for TNBC. Hence, EAD appears to be a promising therapeutic candidate for TNBC by targeting various hallmarks of cancer, including cell death resistance, uncontrolled proliferation and metastasis. To conclude, the identified pathways and validated targets for EAD will provide a roadmap for further in vivo studies and preclinical/clinical validation required for potential drug development.

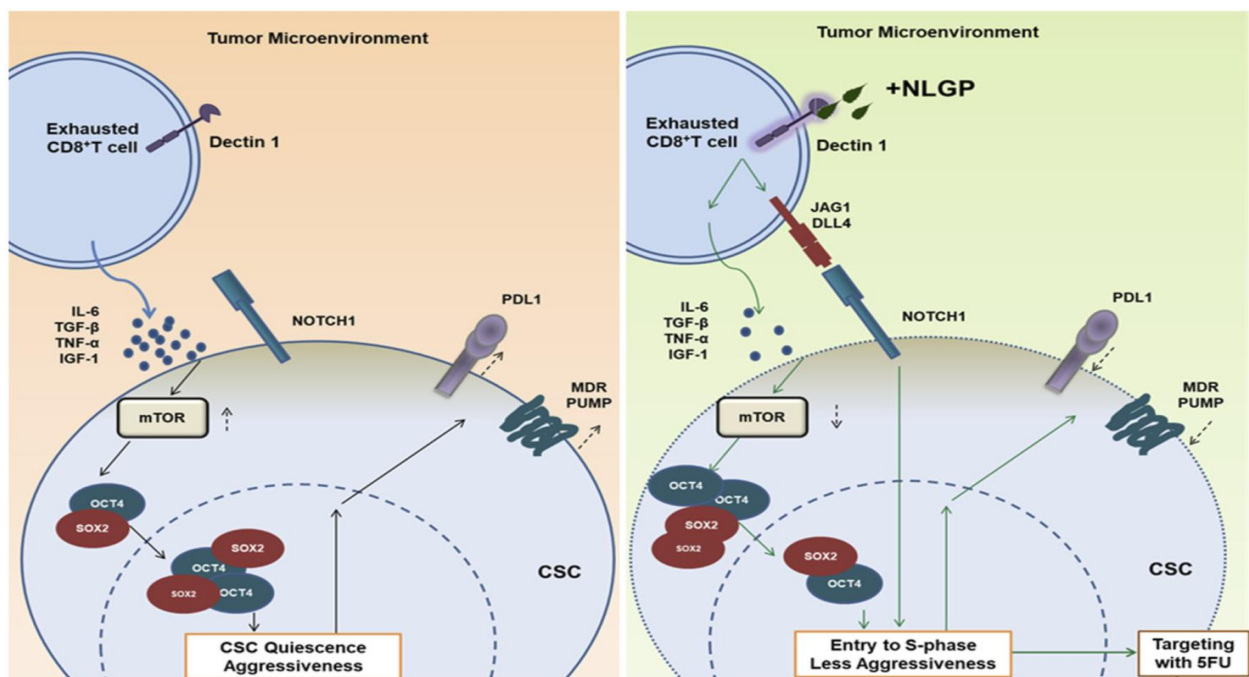


□ Identified hub genes have the potential to be developed as therapeutic targets/ diagnostic markers

[Neem Leaf Glycoprotein Disrupts Exhausted CD8+ T-Cell-Mediated Cancer Stem Cell Aggression.](#)

Chakravarti M, Bera S, Dhar S, Sarkar A, Choudhury PR, Ganguly N, Das J, Sultana J, Guha A, Biswas S, Das T, Hajra S, Banerjee S, **Baral R**, Bose A. *Mol Cancer Res.* 2024 Aug 2;22(8):759-778. doi: 10.1158/1541-7786.MCR-23-0993.PMID: 38743057

Targeting exhausted CD8⁺ T-cell (T_{EX})–induced aggravated cancer stem cells (CSC) holds immense therapeutic potential. In this regard, immunomodulation via Neem Leaf Glycoprotein (NLGP), a plant-derived glycoprotein immunomodulator is explored. Since former reports have proven immune dependent–tumor restriction of NLGP across multiple tumor models, we hypothesized that NLGP might reprogram and rectify T_{EX} to target CSCs successfully. In this study, we report that NLGP’s therapeutic administration significantly reduced T_{EX}-associated CSC virulence in *in vivo* B16-F10 melanoma tumor model. A similar trend was observed in *in vitro* generated T_{EX} and B16-F10/MCF7 coculture setups. NLGP rewired CSCs by downregulating clonogenicity, multidrug resistance phenotypes and PDL1, OCT4, and SOX2 expression. Cell cycle analysis revealed that NLGP educated–T_{EX} efficiently pushed CSCs out of quiescent phase (G₀G₁) into synthesis phase (S), supported by hyper-phosphorylation of G₀G₁–S transitory cyclins and Rb proteins. This rendered quiescent CSCs susceptible to S-phase–targeting chemotherapeutic drugs like 5-fluorouracil (5FU). Consequently, combinatorial treatment of NLGP and 5FU brought optimal CSC-targeting efficiency with an increase in apoptotic bodies and proapoptotic BID expression. Notably a strong nephron-protective effect of NLGP was also observed, which prevented 5FU-associated toxicity. Furthermore, Dectin-1–mediated NLGP uptake and subsequent alteration of Notch1 and mTOR axis were deciphered as the involved signaling network. This observation unveiled Dectin-1 as a potent immunotherapeutic drug target to counter T-cell exhaustion. Cumulatively, NLGP immunotherapy alleviated exhausted CD8⁺ T-cell-induced CSC aggravation.



Neem in Veterinary Science

The therapeutic efficacy of neem (*Azadirachta indica*) leaf extract against coinfection with *Chlamydophila psittaci* and low pathogenic avian influenza virus H9N2 in broiler chickens.

Hegazy AME, Morsy AM, Salem HM, Al-Zaban MI, Alkahtani AM, Alshammari NM, El-Saadony MT, Altarjami LR, Bahshwan SMA, Al-Qurashi MM, El-Tarabily KA, Tolba HMN. *Poult Sci.* 2024 Jul 10;103(10):104089. doi: 10.1016/j.psj.2024.104089. PMID: 39142030

Avian chlamydiosis is a serious avian infection that carries a significant zoonotic danger to the poultry industry. The respiratory co-infections caused by the low pathogenic avian influenza virus H9N2 (LPAIV H9N2) also cause significant financial losses in the poultry industry. The purpose of this study was to examine the pathogenicity of *Chlamydophila psittaci*, and LPAIV H9N2 individually and in combination in broiler chickens, as well as to determine whether or not aqueous neem (*Azadirachta indica*) leaf extract is effective against infections caused by these pathogens. Therefore, 120 broiler cobb chicks were equally divided into 4 groups (30 birds each) with triplicates with 10 birds. Broilers in group 1 (G1) were infected with only *C. psittaci*, broilers in group 2 (G2) were infected with only LPAIV H9N2, broilers in group 3 (G3) were infected with *C. psittaci* and LPAIV H9N2, and broilers in group 4 (G4) remained not challenged and non-treated with any therapeutic or preventive treatment (negative control). At 21 d postinfection (dpi), birds in G1, G2, and G3 were divided into 3 subgroups of 10 birds each: subgroup (A) remained infected and untreated (positive control), subgroup (B) infected and received oxytetracycline for 5 consecutive d, and subgroup (C) infected and received 8% aqueous neem leaf extract for 5 consecutive d. The multiplication of *C. psittaci* in birds in G1, in various tissues was evaluated using Giemsa staining and the data showed that multiplication was much higher in the lung, spleen, and liver from 6 h to 21 dpi, but low in the heart from 8 to 21 dpi. During simultaneous co-infection in G3, the birds developed significant clinical symptoms and postmortem lesions (PM). Quantitative real-time polymerase chain reaction (qRT-PCR) was used to detect viral shedding from oropharyngeal and cloacal swabs between 2 dpi and 8 dpi, with cycle threshold (CT) values ranging from 22 to 24. In contrast, bacterial shedding began 6 h after infection and continued until 21 dpi, with CT values ranging from 23 to 26. Administration of an aqueous neem leaf extract at an 8% concentration (Group C) resulted in a numerical rise in average body weight across all treatment groups in the third and fourth week, as well as a reduction in LPAIV H9N2 and *C. psittaci* replication in the respiratory and gut of treated birds compared to those treated with oxytetracycline (Group B). Overall, respiratory co-infections pose a considerable risk to the poultry business, which is a big threat. To control *C. psittaci* and LPAIV H9N2 in broiler chickens, oral supplementation of 8% aqueous neem leaf extract is recommended. This treatment improves the birds' performance, as evidenced by an increase in their average body weight. In addition, the application of 8% aqueous neem leaf extract lowers *C. psittaci* replication within tissues and diminishes LPAIV H9N2 shedding.

[The Anticoccidial In Vitro Effects and Antioxidant Properties of Several Plants Traditionally Used for Coccidiosis in Togo.](#)

Tchodo FG, Dakpogan HB, Sanvee S, Adjei-Mensah B, Kpomasse CC, Karou S, Pitala W, Tona K, Bakoma B. *Vet Sci.* 2024 Jul 31;11(8):345. doi: 10.3390/vetsci11080345. PMID: 39195799

Coccidiosis is a parasitic disease that often affects livestock. Identifying plants with inhibitory effects on the development of the parasite could help in finding new natural treatments. This study aimed to evaluate the anticoccidial potentials of extracts from *Azadirachta indica* leaves (AILs), *Combretum micranthum* leaves (CMLs), *Carica papaya* seeds (CPSs), *Sarcocephalus latifolius* roots (SLRs), and *Vernonia amygdalina* leaves (VALs). The in vitro anticoccidial efficacy of the extracts was evaluated through oocyst sporulation inhibition and sporozoite viability inhibition assays of *Eimeria* oocysts. The setup was examined for 72 h (every 24 h) of incubation. The DPPH radical scavenging activity and ferric reducing antioxidant power were used to evaluate the antioxidant potential of the extracts. Among the tested extracts, the SLR, CPS, and AIL extracts exhibited the maximum oocyst sporulation inhibition ($75.85 \pm 1.21\%$, $74.53 \pm 1.65\%$, and $71.58 \pm 0.24\%$, respectively) at a concentration of 75 mg/mL of plant extracts against the *Eimeria* species. The *Sarcocephalus latifolius* root extract showed the highest radical scavenging capacity (76.25 ± 0.53) and reducing power (86.21 ± 4.28). The biochemical screening of the selected plant extracts revealed the presence of antioxidant compounds such as phenols, flavonoids, alkaloids, saponins, and carbohydrates. The SLR extract contained the highest amounts of phenols (56.11 ± 0.33 $\mu\text{g/mL}$) and flavonoids (36.65 ± 1.85 $\mu\text{g/mL}$). In conclusion, the selected hydro-ethanolic extracts from these plants possess excellent anticoccidial and antioxidant activities, which can be attributed to the presence of medicinally important phytochemicals. Further research is needed to identify and isolate the active anticoccidial compounds from these plants, which could be utilized in the development of drugs against coccidiosis.