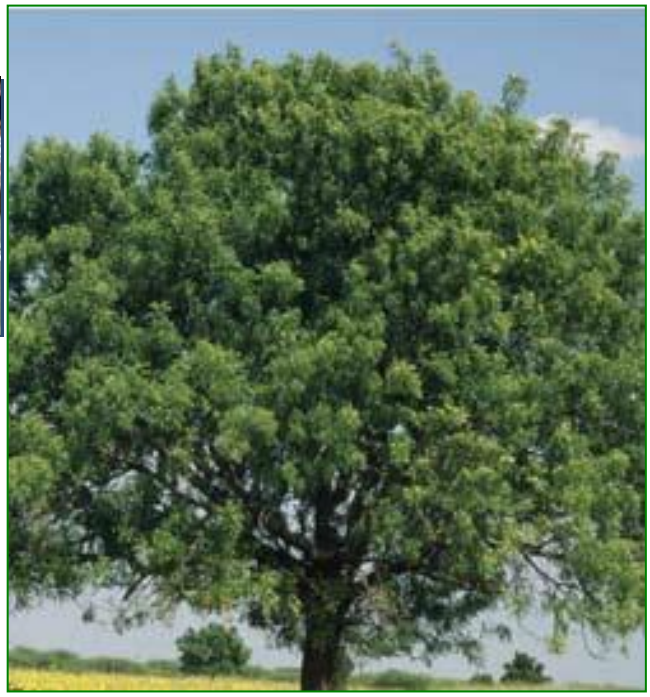
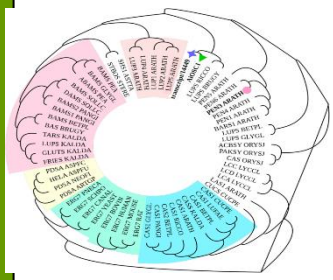
Neem Research Newsletter

Volume 3, Issue 3, 2023





WNO
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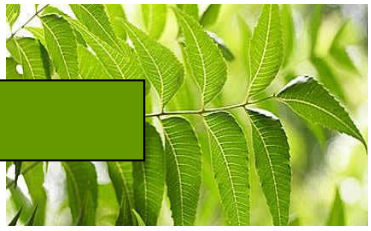


WORLD NEEM ORGANISATION (WNO)

From The Editor's Desk.....

In this issue of the newsletter, we see a continued interest in neem research in agriculture, human and animal health as well as industrial applications. Integration of azadirachtin with a synthetic nematicide or nematode-suppressive agronomical techniques, has been suggested to be more suitable to long-cycle crops. The microencapsulation by complex coacervation of neem leaf extract was shown to be an alternative for the preservation of insecticidal compounds extracted from neem leaves. Dissipation of neem oil was observed when applied alone or together with *Bt*. Neem oil, has been proved to be an excellent candidate against a wide range of vectors of medical and veterinary importance. LC-MS analysis, computational investigation, and antimalarial studies were carried out on neem fruit. Based on experimental analysis, neem oil components have been identified as potential therapeutic agents in the treatment of acne. Genotoxicity studies revealed that dried extract of neem leaves did not cause maternal toxicity or adversely affect fetal development. Nimbolide has been suggested as a potential candidate for therapeutic targeting of the toll-like receptors pathway in rheumatoid arthritis. Neem leaves were found to be effective in lowering neuropsychological decline in type 2 diabetes. Stigmasterol, a compound isolated from neem flowers was shown to ameliorate glutamate-induced neuronal cell death. Incorporation of neem wood ash into a clay material was demonstrated to reduce environmental problems and the total cost of raw material disposition. Neem leaf extract exerted antiviral activity against caprine lentivirus in colostrum and milk of goat nannies.

S. Nagini

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

Neem in Agriculture

Efficacy of Azadirachtin in the Integrated Management of the Root Knot Nematode *Meloidogyne incognita* on Short- and Long-Cycle Crops.

d'Errico G, Sasanelli N, Guastamacchia F, Stillittano V, D'Addabbo T.

Plants (Basel). 2023 Mar 17;12(6):1362. doi: 10.3390/plants12061362.PMID: 36987049

Activity of azadirachtin on phytoparasitic nematodes has been documented for some decades, but the relationship between its nematicidal efficacy and crop cycle length has not yet been clarified. This study aimed to assess the efficacy of an azadirachtin-based nematicide, for controlling the infestation of the root-knot nematode *Meloidogyne incognita*, on the short- and long-cycle crops, lettuce and tomato, respectively. Experiments on lettuce and tomato were carried out in a greenhouse infested by *M. incognita*, including non-treated soil, or treated with the nematicide fluopyram, as controls. In the experiment on the short-cycle lettuce crop, the azadirachtin product effectively suppressed *M. incognita* infestation and increased crop yield, without significant differences from fluopyram. In the tomato crop, both azadirachtin and fluopyram were not able to control nematode infestation, but resulted in significantly higher yields. Data from this study indicated that azadirachtin can be a valid alternative to fluopyram and other nematicides, for root-knot nematode control in short-cycle crops. Integration of azadirachtin with a synthetic nematicide or nematode-suppressive agronomical techniques, should be more suitable to long-cycle crops.

The Insecticidal Activity of *Azadirachta indica* Leaf Extract: Optimization of the Microencapsulation Process by Complex Coacervation.

Michel MR, Aguilar-Zárate M, Rojas R, Martínez-Ávila GCG, Aguilar-Zárate P.

Plants (Basel). 2023 Mar 14;12(6):1318. doi: 10.3390/plants12061318.PMID: 36987005

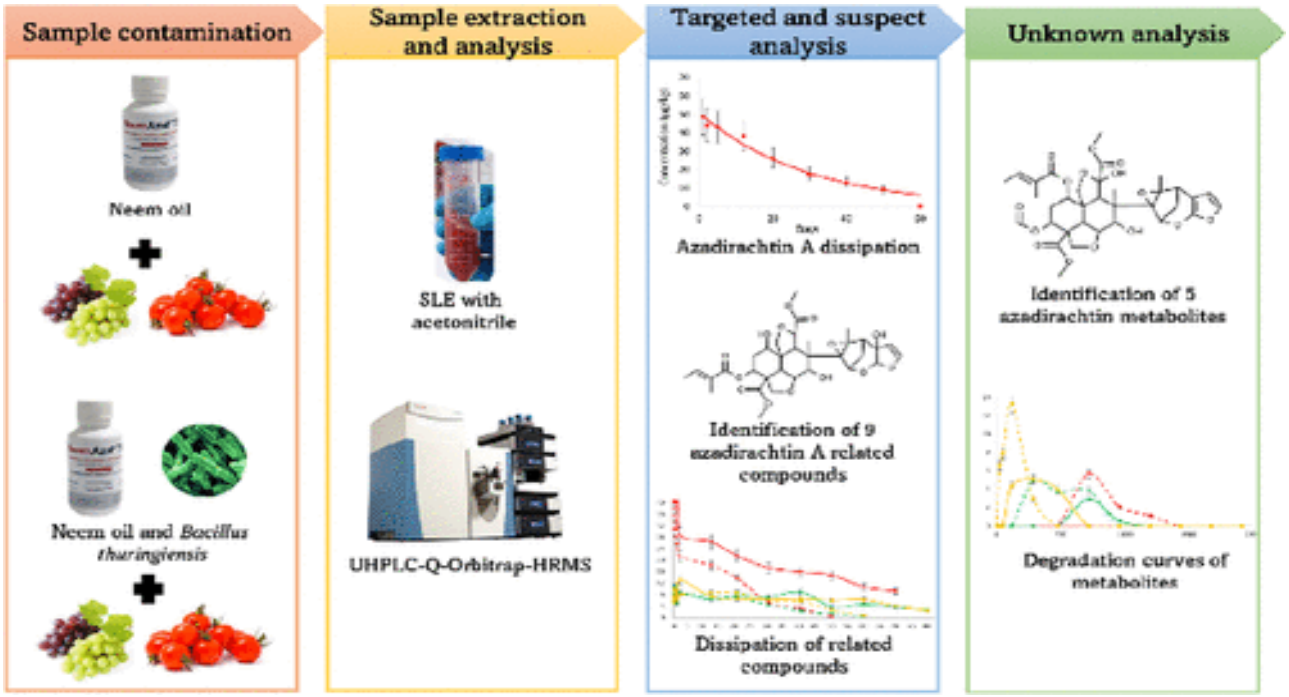
The objective of the present work was to optimize the microencapsulation conditions of neem (*Azadirachta indica* A. Juss) leaf extracts for the biocontrol of *Tenebrio molitor*. The complex coacervation method was used for the encapsulation of the extracts. The independent factors considered were the pH (3, 6, and 9), pectin (4, 6, and 8% *w*/*v*), and whey protein isolate (WPI) (0.50, 0.75, and 1.00% *w*/*v*). The Taguchi L9 (3^3) orthogonal array was used as the experimental matrix. The response variable was the mortality of *T. molitor* after 48 h. The nine treatments were applied by immersion of the insects for 10 s. The statistical analysis revealed that the most influential factor on the microencapsulation was the pH (73% of influence), followed by the pectin and WPI (15% and 7% influence, respectively). The software predicted that the optimal microencapsulation conditions were pH 3, pectin 6% *w*/*v*, and WPI 1% *w*/*v*. The signal-to-noise (S/N) ratio was predicted as 21.57. The experimental validation of the optimal conditions allowed us to obtain an S/N ratio of 18.54, equivalent to a *T. molitor* mortality of 85 ± 10.49%. The microcapsules had a diameter ranging from 1-5 µm. The microencapsulation by complex coacervation of neem leaf extract is an alternative for the preservation of insecticidal compounds extracted from neem leaves.

Comprehensive Dissipation of Azadirachtin in Grapes and Tomatoes: The Effect of *Bacillus thuringiensis* and Tentative Identification of Unknown Metabolites.

Marín-Sáez J, López-Ruiz R, Romero-Gonzalez R, Garrido Frenich A.

J Agric Food Chem. 2023 Mar 7. doi: 10.1021/acs.jafc.2c07077. PMID: 36881717

Neem oil is a biopesticide normally applied together with *Bacillus thuringiensis* (*Bt*). However, neither its dissipation nor the influence of *Bt* has been previously evaluated. In this study, dissipation of neem oil was investigated when it was applied alone or together with *Bt* at 3 and 22 °C. A methodology involving solid-liquid extraction and liquid chromatography-high-resolution mass spectrometry was developed for that purpose. The method was validated obtaining recoveries from 87 to 103%, with relative standard deviations lower than 19% and limits of quantification from 5 to 10 μg/kg. Azadirachtin A (AzA) dissipation was fit to a single first order, being faster when neem oil was applied together with *Bt* and at 22 °C (RL_{50} = 12-21 days) than alone and at 3 °C (RL_{50} = 14-25 days). Eight related compounds were found in real samples with similar dissipation curves compared to AzA, and five unknown metabolites were identified in degraded samples, with increasing concentrations during parent compound degradation.



Biopesticide efficacy of four plant essential oils against papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococcidae).

Mwanauta RW, Ndakidemi PA, Venkataramana PB.

Heliyon. 2023 Mar 1;9(3):e14162. doi: 10.1016/j.heliyon.2023.e14162. eCollection 2023 Mar.PMID: 36923866

Worldwide, *P. marginatus* causes 75% of estimated economic loss in papaya farming, with an increase in production costs. The extract of plant essential oils (PEO) has the potential

to control *P. marginatus* by degrading its wax coatings to death; however, it is less studied in the East African agroecosystem. Therefore, this study was conducted to evaluate the efficacy of four PEO from (neem, citrus, garlic, and castor) against *P. marginatus* at different concentrations (0.5%, 1%, and 1.5%) with and without 0.2% adjuvants separately as a biopesticide. The experiment was conducted in a completely randomized design with four replications per treatment concentration. The papaya seeds (Carina variety) were used in the experiment. After 3 weeks from transplanting, 50 *P. marginatus* specimens were inoculated in each plant. Before treatment application, insect abundance, leaf curling, yellowing, and soot mold were assessed. Then, 24 h, 48 h, and 72 h after biopesticide application, insect mortality was assessed. The results showed a significant difference (p = 0.001) for all assessment intervals in PEOs. However, for the PEOs in combination with the adjuvants, the results were significantly different (p = 0.001) only at 24 h. It was found that among the biopesticides, neem oil (1.5%) + isopropyl alcohol was highly effective (95.5%) after 72 h followed by (Imidacloprid (91%), citrus oil 1.5% (90.7%) and neem oil (1.5%) + paraffin oil (81.0%). But also, there were significant differences among treatments on leaf curling, yellowing, and soot mold reduction in papaya plants 21 days after spray. We conclude that neem oil (1.5%) + 0.2% isopropyl alcohol, neem oil (1.5%) + paraffin oil, and citrus oil (1.5%) significantly controlled *P. marginatus*. Thus, we recommend adopting these formulations for papaya farmers to control *P. marginatus* in their farms; however, simple formulations which can be easily accessed by smallholder farmers are essential.

Selectivity of Insecticides to a Pupal Parasitoid, *Trichospilus diatraeae* (Hymenoptera: Eulophidae), of Soybean Caterpillars.

Pereira HC, Pereira FF, Insabrald VB, Rodrigues A, Lucchetta JT, Silva FWS, Fernandes WC, Carneiro ZF, Périgo PHB, Zanuncio JC.

Insects. 2023 Feb 22;14(3):217. doi: 10.3390/insects14030217.PMID: 36975902

Selectivity is an important aspect of modern insecticides to be able to target pests whilst maintaining beneficial entomofauna in the crop. The present objective was to assess the selectivity of different insecticides for the pupal parasitoid of soybean caterpillars, i.e., *Trichospilus diatraeae* Cherian & Margabandhu, 1942 (Hymenoptera: Eulophidae). Acephate, azadirachtin, *Bacillus thuringiensis* (*Bt*), deltamethrin, lufenuron, teflubenzuron and thiamethoxam + lambda-cyhalothrin at the highest recommended concentrations for the soybean looper *Chrysodeixis includens* (Walker, [1858]) (Lepidoptera: Noctuidae), as well as water in the control, were used against the pupal parasitoid *T. diatraeae*. The insecticides and the control were sprayed on the soybean leaves, which were left to dry naturally and placed in cages with *T. diatraeae* females in each one. Survival data were submitted to analysis of variance (ANOVA) and the means were compared using Tukey's HSD test (α = 0.05). Survival curves were plotted according to the Kaplan-Meier method, and the pairs of curves were compared using the log-rank test at 5% probability. The insecticides azadirachtin, *Bt*, lufenuron and teflubenzuron did not affect *T. diatraeae* survival, while deltamethrin and thiamethoxam + lambda-cyhalothrin presented low toxicity and acephate was highly toxic, causing 100% mortality in the parasitoid. Azadirachtin, *Bt*, lufenuron and teflubenzuron are selective for *T. diatraeae* and could be used in IPM programs.

Neem-based products as potential eco-friendly mosquito control agents over conventional eco-toxic chemical pesticides-A review.

Chatterjee S, Bag S, Biswal D, Sarkar Paria D, Bandyopadhyay R, Sarkar B, Mandal A, Dangar TK.

Acta Trop. 2023 Apr;240:106858. doi: 10.1016/j.actatropica.2023.106858. Epub 2023 Feb 5. PMID: 36750152

Mosquitoes cause serious health hazards for millions of people across the globe by acting as vectors of deadly communicable diseases like malaria, filariasis, dengue and yellow fever. Use of conventional chemical insecticides to control mosquito vectors has led to the development of biological resistance in them along with adverse environmental consequences. In this light, the recent years have witnessed enormous efforts of researchers to develop eco-friendly and cost-effective alternatives with special emphasis on plant-derived mosquitocidal compounds. Neem oil, derived from neem seeds (Azadirachta indica A. Juss, Meliaceae), has been proved to be an excellent candidate against a wide range of vectors of medical and veterinary importance including mosquitoes. It is environment-friendly, and target-specific at the same time. The active ingredients of neem oil include limonoids like azadirachtin A, nimbin, salannin and numerous other substances that are still waiting to be discovered. Of these, azadirachtin has been shown to be very effective and is mainly responsible for its toxic effects. The quality of the neem oil depends on its azadirachtin content which, in turn, depends on its manufacturing process. Neem oil can be used directly or as nanoemulsions or nanoparticles or even in the form of effervescent tablets. When added to natural breeding habitat waters they exert their mosquitocidal effects by acting as ovicides, larvicides, pupicides and/or oviposition repellents. The effects are generated by impairing the physiological pathways of the immature stages of mosquitoes or directly by causing physical deformities that impede their development. Neem oil when used directly has certain disadvantages mainly related to its disintegration under atmospheric conditions rendering it ineffective. However, many of its formulations have been reported to remain stable under environmental conditions retaining its efficiency for a long time. Similarly, neem seed cake has also been found to be effective against the mosquito vectors. The greatest advantage is that the target species do not develop resistance against neem-based products mainly because of the innumerable number of chemicals present in neem and their combinations. This makes neem-based products highly potential yet unexplored candidates of mosquito control agents. The current review helps to elucidate the roles of neem oil and its various derivatives on mosquito vectors of public health concern.

LC-MS Analysis, Computational Investigation, and Antimalarial Studies of *Azadirachta indica* Fruit.

Faloye KO, Adesida SA, Oguntimehin SA, Adewole AH, Omoyeni OB, Fajobi SJ, Ugwo JP, Asiyanbola ID, Bamimore VO, Fakola EG, Oladiran OJ, Spiteller M.

Bioinform Biol Insights. 2023 Feb 25;17:11779322231154966. doi: 10.1177/11779322231154966. eCollection 2023.

Malaria is a deadly disease that continues to pose a threat to children and maternal well-being. This study was designed to identify the chemical constituents in the ethanolic fruit extract of *Azadirachta indica*, elucidate the pharmacological potentials of identified phytochemicals through the density functional theory method and carry out the antimalarial activity of extract using chemosuppression and curative models. The liquid chromatography-mass spectrometry (LC-MS) analysis of the ethanolic extract was carried out, followed by the density functional theory studies of the identified phytochemicals using B3LYP and 6-31G (d, p) basis set. The antimalarial assays were performed using the chemosuppression (4 days) and curative models. The LC-MS fingerprint of the extract led to the identification of desacetylnimbinolide, nimbidiol, O-methylazadironolide, nimbidic acid, and desfurano-6α-hydroxyazadiradione. Also, the frontier molecular orbital properties, molecular electrostatic potential, and dipole moment studies revealed the identified phytochemicals as possible antimalarial agents. The ethanolic extract of *A indica* fruit gave 83% suppression at 800 mg/kg, while 84% parasitaemia clearance was obtained in the curative study. The study provided information about the phytochemicals and background pharmacological evidences of the antimalarial ethnomedicinal claim of *A indica* fruit. Thus, isolation and structure elucidation of the identified phytochemicals from the active ethanolic extract and extensive antimalarial studies towards the discovery of new therapeutic agents is recommended for further studies.

Bioactive Azadirachta indica and Melia azedarach leaves extracts with anti-SARS-CoV-2 and antibacterial activities.

Hemdan BA, Mostafa A, Elbatanony MM, El-Feky AM, Paunova-Krasteva T, Stoitsova S, El-Liethy MA, El-Taweel GE, Abu Mraheil M.

PLoS One. 2023 Mar 8;18(3):e0282729. doi: 10.1371/journal.pone.0282729. eCollection 2023.PMID: 36888689

The leaves of Azadirachta indica L. and Melia azedarach L., belonging to Meliaceae family, have been shown to have medicinal benefits and are extensively employed in traditional folk medicine. Herein, HPLC analysis of the ethyl acetate fraction of the total methanolic extract emphasized the enrichment of both A. indica L., and M. azedarach L. leaves extracts with phenolic and flavonoids composites, respectively. Besides, 4 limonoids and 2 flavonoids were isolated using column chromatography. By assessing the in vitro antiviral activities of both total leaves extracts against Severe Acute Respiratory Syndrome Corona virus 2 (SARS-CoV-2), it was found that A. indica L. and M. azedarach L. have robust anti-SARS-CoV-2 activities at low half-maximal inhibitory concentrations (IC50) of 8.451 and 6.922 μg/mL, respectively. Due to the high safety of A. indica L. and M. azedarach L. extracts with

half-maximal cytotoxic concentrations (CC50) of 446.2 and 351.4 µg/ml, respectively, both displayed extraordinary selectivity indices (SI>50). A. indica L. and M. azedarach L. leaves extracts could induce antibacterial activities against both Gram-negative and positive bacterial strains. The minimal inhibitory concentrations of A. indica L. and M. azedarach L. leaves extracts varied from 25 to 100 mg/mL within 30 min contact time towards the tested bacteria. Our findings confirm the broad-spectrum medicinal value of A. indica L. and M. azedarach L. leaves extracts. Finally, additional in vivo investigations are highly recommended to confirm the anti-COVID-19 and antimicrobial activities of both plant extracts.

Network Pharmacology and Molecular Modeling to Elucidate the Potential Mechanism of Neem Oil against *Acne vulgaris*.

Kola-Mustapha AT, Raji MA, Adedeji O, Ambrose GO.

Molecules. 2023 Mar 21;28(6):2849. doi: 10.3390/molecules28062849.PMID: 36985821

Acne vulgaris is a common skin disorder with a complicated etiology. Papules, lesions, comedones, blackheads, and other skin lesions are common physical manifestations of *Acne vulgaris*, but the individual who has it also regularly has psychological repercussions. Natural oils are being utilized more and more to treat skin conditions since they have fewer negative effects and are expected to provide benefits. Using network pharmacology, this study aims to ascertain if neem oil has any anti-acne benefits and, if so, to speculate on probable mechanisms of action for such effects. The neem leaves (*Azadirachta indica*) were collected, verified, authenticated, and assigned a voucher number. After steam distillation was used to extract the neem oil, the phytochemical components of the oil were examined using gas chromatography-mass spectrometry (GC-MS). The components of the oil were computationally examined for drug-likeness using Lipinski's criteria. The Pharm Mapper service was used to anticipate the targets. Prior to pathway and protein-protein interaction investigations, molecular docking was performed to predict binding affinity. Neem oil was discovered to be a potential target for STAT1, CSK, CRABP2, and SYK genes in the treatment of *Acne vulgaris*. In conclusion, it was discovered that the neem oil components with PubChem IDs: ID_610088 (2-(1-adamantyl)-*N*-methylacetamide), ID_600826 (*N*-benzyl-2-(2-methyl-5-phenyl-3*H*-1,3,4-thiadiazol-2-yl)acetamide), and ID_16451547 (*N*-(3-methoxyphenyl)-2-(1-phenyltetrazol-5-yl)sulfanylpropanamide) have strong affinities for these drug targets and may thus be used as therapeutic agents in the treatment of acne.

Combination of St. John's Wort Oil and Neem Oil in Pharmaceuticals: An Effective Treatment Option for Pressure Ulcers in Intensive Care Units.

Özdemir S, Bostanabad SY, Parmaksız A, Canatan HC.

Medicina (Kaunas). 2023 Feb 27;59(3):467. doi: 10.3390/medicina59030467.PMID: 36984468

Background and Objectives: Phytotherapeutically, various herbal remedies, such as St. John's wort oil, have been introduced as wound care options. Recently, Neem oil has been considered a herbal option for the management of superficial wounds. Wound care is a complex process that involves several factors including the patient, caregiver, and medications. Herbal combinations could be an alternative to the chemical counterparts in

the wound care area. This report includes an investigation of the possible supportive impacts of the St. John's wort and Neem oil containing ointment (W Cura G Plus ®) in the management of pressure ulcers (PUs) in three intensive care unit (ICU) patients. *Materials and Methods*: The ointment was administered to individuals once daily for 42 consecutive days. The status of individuals was macroscopically monitored by measuring the PU area and histopathological assessment of the tissue sections taken on the first and last days of wound treatment. *Results*: The outcomes of the macroscopic and histopathological techniques exhibited that St. John's wort and Neem oil containing ointment provided a remarkable supportive impact on the patients that suffered from PUs in the ICUs. *Conclusions*: The combination of St. John's wort and Neem oil could be suggested as an efficient active phytoconstituent for the management of PUs. The herbal ointments may be suggested as an alternative for the patients that have PUs in the ICUs.

Genotoxicity and maternal-fetal safety of the dried extract of leaves of Azadirachta indica A. Juss (Meliaceae) in Wistar rats.

Lacerda Ramalho CE, Reis DDS, Caixeta GAB, Oliveira MC, Silva DMFD, Cruvinel WM, Teófilo MNG, Gomes CM, Sousa PA, Soares LF, Melo AM, Rocha JD, Bailão EFLC, Amaral VCS, Paula JAM.

J Ethnopharmacol. 2023 Mar 22:116403. doi: 10.1016/j.jep.2023.116403. Online ahead of print. PMID: 36963474

Ethnopharmacological relevance: Azadirachta indica A. Juss (Meliaceae), popularly known as "neem", is used for the treatment of rheumatism, cancer, ulcers, diabetes, respiratory problems, among others. This species is present on six continents and contains more than 400 bioactive compounds. Practically all parts of the plant are used in the treatment of diseases. Although it is widely used, no study has evaluated the safety of this species throughout the gestational period in Wistar rats. **Aim of the study:** To evaluate the genotoxicity and the effect of treatment with dried extract of leaves of Azadirachta indica on maternal toxicity and fetal development. **Materials and methods:** The dried extract of leaves of A. indica was obtained by spray drying after percolation of the plant material in 30% ethanol (w/w). The total flavonoids and rutin contents of the extract were determined by spectrophotometric method and HPLC-DAD, respectively. Pregnant Wistar rats (n = 40) were divided into four groups (n = 10/group): one control and three groups treated with dried extract of leaves of A. indica at doses of 300, 600 or 1200 mg/kg. Treatments were carried out from gestational day (GD) 0-20. During gestation, clinical signs of toxicity, weight gain, feed and water consumption of the dams were evaluated. On GD 21, rats were euthanized and cardiac blood was collected. Liver, kidneys, lung, heart, uterus, ovaries and bone marrow were collected. Reproductive performance parameters, histopathological analysis, biochemistry and genotoxicity were evaluated. Fetuses were evaluated for external morphology, skeletal and visceral changes. **Results:** The total flavonoid content of the extract ranged from 2.64 to 3.01%, and the rutin content was 1.07%. There was no change in body mass gain, food and water consumption between the evaluated groups. There was also no difference between the groups in terms of biochemical parameters, reproductive performance, histopathological analysis of the mother's organs and genotoxicity. Supernumerary ossification sites of the sternum were observed, and other skeletal and visceral alterations were not significant. **Conclusions:** The treatment did not induce

maternal toxicity, it was neither embryotoxic nor fetotoxic. The extract was not potentially genotoxic, and at a dose of 1200 mg/kg, it caused changes in the ossification of the sternum.

Production of herbal toothpaste: Physical, organoleptic, phyto-compound, and antimicrobial properties.

Oluwasina OO, Idris SO, Ogidi CO, Igbe FO.

Heliyon. 2023 Feb 28;9(3):e13892. doi: 10.1016/j.heliyon.2023.e13892. eCollection 2023 Mar. PMID: 36923892

Objective: To investigate the possibility of producing dental antimicrobial toothpaste from *Allium cepa* L skin chaff, *Azadirachta indica A.* seed, and *Tetrapleura tetraptera* pod extracts. **Methods:** Ethanolic extracts of the three plant materials were obtained. These were subjected to phytochemical and GC-MS analyses. The different extract combinations were used for the production of various toothpaste. The toothpaste's physical, organoleptic, and antimicrobial properties were determined. **Results:** From the phytochemical analysis, *Allium cepa* has the highest phenolic (1.20 mgGAE/g), saponin (14.80%), tannin (0.11 mg/g) and DPPH (82.80%), *Tetrapleura tetraptera* has the highest flavonoid (0.33 mg RE/g), and alkaloid (20.50 mg/g) while, *Azadirachta indica* has the highest oxalate (77.50 mg/g). The GC-MS revealed significant chemical components of *Allium cepa* as 1-heptatriacotanol, germacra-1(10),4,11(13)-trien-12-oic acid, 6-alpha-hydroxy-,gamma-lactone, (E,E)-, 11H-Indeno [1,2b] quinoxaline, 2-methyl- while *Azadirachta indica* have butyl benzoate, benzoic acid, hexyl ester, hexadecanoic acid, methyl ester and *Tetrapleura tetraptera* have the following 15-hydroxypentadecanoic acid, *cis*-9-hexadecenal, and 11,13-dimethyl-12-tetradecen-1-ol acetate. All the produced toothpaste has a brown colour and a pleasant smell, with pH from 7.30 to 8.10 and foamability from 19.23% of stand-alone toothpaste to 44.44% of *Allium cepa*-based. Amongst the produced toothpaste *Allium cepa*-based toothpaste has the best antimicrobial activities against the tested bacteria (*Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae*, *Klebsiella oxytoca*) and fungi (*Candida albicans* and *Candida parapsilosis*). The stand-alone toothpaste has the lowest minimum inhibitory concentration of 1.25 mg/mL against bacteria and fungi. **Clinical significance:** The study provides information on the production of human health-friendly dental antimicrobial toothpaste from plant materials.

Nimbolide attenuates complete Freund's adjuvant induced arthritis through expression regulation of toll-like receptors signaling pathway.

Israr M, Naseem N, Akhtar T, Aftab U, Zafar MS, Faheem MA, Shahzad M.

Phytother Res. 2023 Mar;37(3):903-912. doi: 10.1002/ptr.7672. Epub 2022 Nov 27.PMID: 36437579

Nimbolide is an active constituent of Azadirachta indica and is known for its anti-inflammatory, anti-oxidant, immune-modulatory, and anti-cancer effects. Few studies suggest that nimbolide treatment influences the responses to rheumatoid arthritis, but the underlying molecular mechanisms involved are not yet well established. Therefore, the present study was designed to determine the effect of nimbolide on expression regulation of toll-like receptors to attenuate rheumatoid arthritis. The rheumatoid arthritis model was established by injecting complete Freund's adjuvant (CFA) intra-dermally into the sub-

plantar region of the left hind paw of rats. Nimbolide (20 mg/kg) and piroxicam (10 mg/kg) were given to arthritic rats. Rats treated with nimbolide showed a significant reduction in inflammatory cells, rheumatoid factor, ESR, and improved the body weight. The results indicated that nimbolide possesses the capacity to attenuate rheumatoid arthritis by downregulating toll-like receptors, IL-17, IL-23, HSP70, and IFN-γ expression levels. Nimbolide treatment showed significant reduction in the severity of inflammation and destruction of joints and showed comparable effects to piroxicam, which is a standard non-steroidal anti-inflammatory drug used for the treatment of rheumatoid arthritis. It can be concluded that nimbolide can be considered as a potential candidate for therapeutic targeting of the toll-like receptors pathway in rheumatoid arthritis.

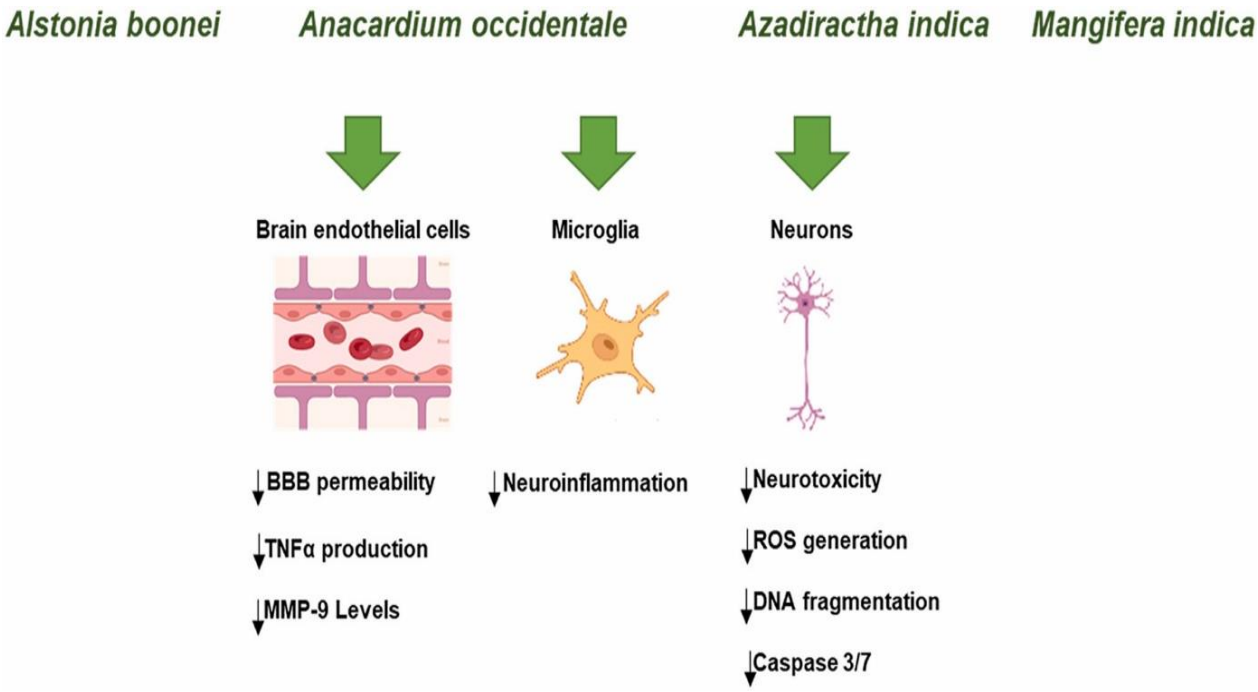
Neuroprotection by Alstonia boonei De Wild., Anacardium occidentale L., Azadirachta indica A.Juss. and Mangifera indica L.

Iwuanyanwu VU, Banjo OW, Babalola KT, Olajide OA.

J Ethnopharmacol. 2023 Mar 23:116390. doi: 10.1016/j.jep.2023.116390. PMID: 36965546

Ethnopharmacology relevance: Alstonia boonei De Wild. (stem bark), Anacardium occidentale L. (stem bark), Azadirachta indica A.Juss (leaves), Enantia chlorantha Oliv. (stem bark), Khaya senegalensis A.Juss (stem bark) Mangifera indica L. (stem bark), and Nauclea latifolia Sm. (stem bark) are used for treating malaria in southwest Nigeria. Surveys also revealed that these plants are also employed for treating symptoms of malaria and cerebral malaria in the region. **Aim of the study:** In this study, the effects of freeze-dried extracts of these plants were investigated on synthetic hemozoin (HZ)-induced neuroinflammation, neuronal damage, and increased permeability of brain microvascular endothelial cells. **Materials and methods:** Effects of freeze-dried plant extracts were investigated on neuroinflammation by measuring levels of pro-inflammatory mediators in culture supernatants, while in-cell western assays were used to measure protein levels of iNOS and NLRP3. Effects on HZ-induced neurotoxicity and ROS generation was measured using MTT and DCFDA assays, respectively. HZ-induced permeability of hCMEC/D3 endothelial cells was determined using the in vitro vascular permeability assay kit. **Results:** The extracts produced significant ($p < 0.05$) reduction in TNFα, IL-6, IL-1β, MCP-1, RANTES and iNOS/NO production in HZ-stimulated BV-2 microglia. Pre-treatment with 50 μg/mL of A. boonei, A. indica, A. occidentale, E. chlorantha and M. indica also resulted in the inhibition of NF-κB activation. Pre-treatment with A. indica produced, A. occidentale, M. indica and A. boonei reduced HZ-induced increased NLRP3 protein expression. HZ-induced increased caspase-1 activity was also reduced by A. boonei, A. occidentale, A. indica, E. chlorantha, and M. indica. Freeze-dried extracts of A. boonei, A. occidentale, A. indica and M. indica produced a neuroprotective effect in HT-22 neuronal cells incubated with HZ by preventing HZ-induced neurotoxicity, ROS generation, DNA fragmentation and caspase 3/7 activity. Inhibition of HZ-induced increase in permeability of human hCMEC/D3 brain endothelial cells was also observed with A. boonei, A. occidentale, A. indica and M. indica, while reducing the release of TNFα and MMP-9. **Conclusions:** These results suggest that A. boonei, A. occidentale, A. indica and M. indica are neuroprotective through inhibition of neuroinflammation, neuronal damage and increased permeability of blood brain

barrier. The outcome of the study provides pharmacological evidence for the potential benefits of plants as herbal treatments for cerebral malaria symptoms.



Combating effects of Azadirachta indica leaves extract on biochemical and neuropsychological decline observed in diabetes.

Khaliq S, Ruby T, Samad N, Ahmad S, Alam M, Ameer Rehman S, Azizuddin -, Shahroz M, Rizvi S.

Pak J Pharm Sci. 2022 Nov;35(6(Special)):1725-1731.PMID: 36861235

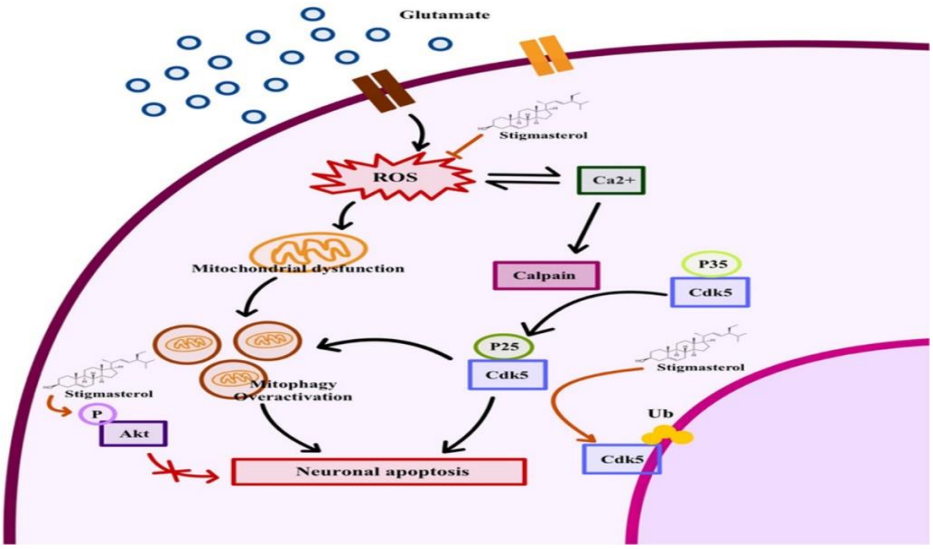
Diabetes is a group of metabolic disorder effecting health of wide number of population and cause neuropsychological decline. In the present study, effect of AI leaves extract on neuropsychological behaviors was observed in diabetic rat's model. Rats were divided into 4 groups as control (saline treated healthy rats), positive control (pioglitazone treated diabetic rats), diabetic control (untreated diabetic rats) and AI leaves extract treated diabetic rats. Diabetes was induced by giving 35% fructose for 6 weeks and a single dose of Streptozotocin (40 mg/kg). After 3 weeks of treatment behavioral and biochemical analysis were done. Behavioral results revealed that induction of type 2 diabetes produced anxiety, depression, decreased motor activity and impaired recognition memory in rats. Treatment with AI leaves extract in diabetic rats significantly decreased anxiety, depression, increased motor activity, enhanced recognition memory. Biochemical investigation revealed that AI leaves extract treat diabetes via improving the levels of fasting insulin and HbA1c and a significant decrease in CK and SGPT levels were observed in AI leaves treated diabetic rats. So, AI besides treating diabetes, helps in lowering the risk of co-occurring diabetic diseases and found effective in lowering neuropsychological decline observed in type 2 diabetes.

Stigmasterol isolated from Azadirachta indica flowers attenuated glutamate-induced neurotoxicity via downregulation of the Cdk5/p35/p25 signaling pathway in the HT-22 cells.

Mongkolpobsin K, Sillapachaiyaporn C, Nilkhet S, Tencomnao T, Baek SJ.

Phytomedicine. 2023 Feb 24;113:154728. doi: 10.1016/j.phymed.2023.154728. PMID: 36898255

Background: Glutamate, an excitatory neurotransmitter, was elevated in the brain of neurodegenerative disease (ND) patients. The excessive glutamate induces Ca^{2+} influx and reactive oxygen species (ROS) production which exacerbates mitochondrial function, leading to mitophagy aberration, and hyperactivates Cdk5/p35/p25 signaling leading to neurotoxicity in ND. Stigmasterol, a phytosterol, has been reported for its neuroprotective effects; however, the underlying mechanism of stigmasterol on restoring glutamate-induced neurotoxicity is not fully investigated. **Purpose:** We investigated the effect of stigmasterol, a compound isolated from Azadirachta indica (AI) flowers, on ameliorating glutamate-induced neuronal apoptosis in the HT-22 cells. **Study design:** To further understand the underlying molecular mechanisms of stigmasterol, we investigated the effect of stigmasterol on Cdk5 expression, which was aberrantly expressed in glutamate-treated cells. Cell viability, Western blot analysis, and immunofluorescence are employed. **Results:** Stigmasterol significantly inhibited glutamate-induced neuronal cell death via attenuating ROS production, recovering mitochondrial membrane depolarization, and ameliorating mitophagy aberration by decreasing mitochondria/lysosome fusion and the ratio of LC3-II/LC3-I. In addition, stigmasterol treatment downregulated glutamate-induced Cdk5, p35, and p25 expression via enhancement of Cdk5 degradation and Akt phosphorylation. Although stigmasterol demonstrated neuroprotective effects on inhibiting glutamate-induced neurotoxicity, the efficiency of stigmasterol is limited due to its poor water solubility. We conjugated stigmasterol to soluble soybean polysaccharides with chitosan nanoparticles to overcome the limitations. We found that the encapsulated stigmasterol increased water solubility and enhanced the protective effect on attenuating the Cdk5/p35/p25 signaling pathway compared with free stigmasterol. **Conclusion:** Our findings illustrate the neuroprotective effect and the improved utility of stigmasterol in inhibiting glutamate-induced neurotoxicity.



Neem- Industrial Applications

Effect of the incorporation of Neem (*Azadirachta indica*) wood ash in Kodeck ceramic materials for the manufacture of fired bricks (Far-North Cameroon).

Fadil-Djenabou S, Ndjigui PD, Bukalo N, Ekosse GI.

Heliyon. 2023 Mar 7;9(3):e14335. doi: 10.1016/j.heliyon.2023.e14335. eCollection 2023 Mar.PMID: 36967870

The current study evaluates the suitability of Neem (*Azadirachta Indica)* wood ash as raw material in the production of ceramic bricks for their application in construction. Accordingly, for the fabrication of bricks, compositions were prepared by adding increasing amounts of Neem wood ash (0%, 5%, and 10% in wt.). The specimens were manufactured by mixing clay with a Neem wood ash amendment and subsequently compacted and fired at 850 °C, 950 °C, and 1050 °C. The fired samples were characterized to determine their technological properties. The results indicate that brick formulations containing Neem wood ash decreased the bulk density up to 8%. Water absorption increased up to 10% and porosity also increased up to 20% with wood of ash. These values meet the Turkish TS EN standards for masonry structures. Due to the interesting performances observed, the potential used up to 10 wt% of Neem Wood ash in ceramic formulations of industrial interest was confirmed. Therefore, incorporating ash into a clay material reduces environmental problems and the total cost of raw material disposition. This is very important in the Sahelian zone and it provides a great opportunity for the inhabitants of this zone.

UV-Visible-NIR camouflage textiles with natural plant based natural dyes on natural fibre against woodland combat background for defence protection.

Hossain MA.

Sci Rep. 2023 Mar 28;13(1):5021. doi: 10.1038/s41598-023-31725-2.PMID: 36977725

Woodland combat background (CB) is a common source of natural plant based natural dyes (NPND). Swietenia Macrophylla, Mangifera Indica, Terminalia Arjuna, Corchorus Capsularis, Camellia Sinensis, Azadirachta Indica, Acacia Acuminata, Areca Catechu and Cinnamomum Tamala were dried-grinded-powdered-extracted-polyaziridine encapsulated-dyed-coated-printed with leafy design on cotton fabric and tested against woodland CB under the reflection engineering of ultraviolet (UV)-visible (Vis)-near infrared (NIR) spectrums and photographic versus chromatic techniques of Vis imaging. The reflection properties of NPND treated and untreated cotton fabric were experimented by UV-Vis-NIR spectrophotometer from 220 to 1400 nm. Six segments of field trialling for NPND treated woodland camouflage textiles were also investigated for concealment, detection, recognition and identification of target signature against forest plants/herbs species; common tree of woodland CB such as Shorea Robusta Gaertn, Bamboo Vulgaris, Musa Acuminata; and a wooden bridge made by Eucalyptus Citriodora & Bamboo Vulgaris. The imaging properties such as CIE L*, a*, b* and RGB (red, green, blue) of NPND treated cotton-garments were captured by digital camera from 400 to 700 nm against tree stem/bark, dry leaves, green leaves and dry wood of woodland CB. Therefore, a colorful matching for concealment, detection, recognition and identification of target signature against woodland CB was verified

by Vis camera imaging and UV-Vis-NIR reflection mechanism. UV-protection property of Swietenia Macrophylla treated cotton fabric was also investigated by diffuse reflection for defence clothing. Simultaneous 'camouflage textiles in UV-Vis-NIR' and 'UV-protective' property of Swietenia Macrophylla treated fabric have been investigated for NPND materials-based textiles coloration (dyeing-coating-printing) which is a new concept for camouflage formulation of NPND dyed-NPND mordanted-NPND coated-NPND printed textiles in terms of ecofriendly source of woodland camouflage materials. Therefore, technical properties of NPND materials and methodologies of camouflage textile assessment have been advanced in addition to coloration philosophy of natural dyed-coated-printed textiles.

Neem in Veterinary Science

In vitro antiviral effect of ethanolic extracts from Azadirachta indica and Melia azedarach against goat lentivirus in colostrum and milk.

de Sousa ALM, Rizaldo Pinheiro R, Furtado Araujo J, Mesquita Peixoto R, de Azevedo DAA, Cesar Lima AM, Marques Canuto K, Vasconcelos Ribeiro PR, de Queiroz Souza AS, Rocha Souza SC, de Amorim SL, Paula Amaral G, de Souza V, de Morais SM, Andrioli A, da Silva Teixeira MF.

Sci Rep. 2023 Mar 22;13(1):4677. doi: 10.1038/s41598-023-31455-5.PMID: 36949145

This study aimed to evaluate, in vitro, the use of leaf extracts of Azadirachta indica (A. indica) and Melia azedarach (M. azedarach) as antivirals against caprine lentivirus (CLV) in colostrum and milk of goat nannies. These were collected from eight individuals and infected with the standard strain of CLV. Samples were then subdivided into aliquots and treated with 150 µg/mL of crude extract, and with ethyl acetate and methanol fractions for 30, 60, and 90 min. Next, somatic cells from colostrum and milk were co-cultured with cells from the ovine third eyelid. After this step, viral titers of the supernatants collected from treatments with greater efficacy in co-culture were assessed. The organic ethyl acetate fractions of both plants at 90 min possibly inhibited the viral activity of CLV by up to a thousandfold in colostrum. In milk, this inhibition was up to 800 times for the respective Meliaceae. In conclusion, the ethanolic fraction of ethyl acetate from both plants demonstrated efficacy against CLV in samples from colostrum and milk when subjected to treatment, which was more effective in colostrum.

The Effect of Neem Leaf Supplementation on Growth Performance, Rumen Fermentation, and Ruminal Microbial Population in Goats.

Taethaisong N, Paengkoum S, Kaewwongsa W, Onjai-Uea N, Thongpea S, Paengkoum P. Animals (Basel). 2023 Feb 28;13(5):890. doi: 10.3390/ani13050890.PMID: 36899747

This study aims to investigate the effect of neem leaf supplementation on the feed intake, digestibility, performance, fermentation characteristics, and ruminal microbes in goats. We included 24 Anglo-Nubian Thai native male goats with a body weight of 20 ± 2.0 kg, using 2 × 2 factorial in a completely randomized design for the following four treatments: (1) control, (2) control + 15% PEG in the concentrate, (3) 6% NL in concentrate, and (4) 6% NL + 15%

PEG in concentrate. The results show that supplementation with 6% NL + 15% PEG in the concentrate had a higher ($p < 0.05$) feed intake gDM/d, % BW, g/kgBW$^{0.75}$, nutrient intake, nutrient digestion, weight change, and ADG than did the goats that were fed with 0% NL + 0% PEG, 0% NL + 15% PEG, and 6% NL + 0% PEG in concentrate, respectively. The feeding with 6% NL + 15% PEG had a higher ($p < 0.05$) level of propionic acid at 2 and 4 h post feeding compared to the other treatments. Supplementation with 6% NL + 15% PEG in the concentrate had the lowest ($p < 0.05$) methanogen, protozoa, blood urea nitrogen, ammonia nitrogen, acetic acid, and butyric acid, as well as a lower ratio of acetic acid to propionic acid at 2 and 4 h post feeding than the other treatments. However, supplementation with 6% NL + 15% PEG in concentrate had the highest values of *Butyrivibrio fibrisolvens* and *Streptococcus gallolyticus* at 2 and 4 h post feeding compared to the other treatments ($p < 0.05$). Collectively, this study indicates that neem leaf supplements can increase growth performance and propionic acid and can modulate the abundance of *Butyrivibrio fibrisolvens* and *Streptococcus gallolyticus*. Thus, neem leaf could potentially be a good supplement for goat feed.