International Standard Serial Number (ISSN): 2319-8141

International Journal of Universal Pharmacy and Bio Sciences 8(4): July-August 2019

INTERNATIONAL JOURNAL OF UNIVERSAL PHARMACY AND BIO SCIENCES

*IMPACT FACTOR 4.018****

*ICV 6.16****

Pharmaceutical Sciences

Review Article……!!!

NEEM (*AZADIRACHTA INDICA*): A MIRACULOUS MEDICINAL PLANT FROM INDIA

Arulkumar R [1*], Karthika S [1], Gopalasatheeskumar K [2] and Arulkumaran G [3]

[1] KMCH College of Pharmacy, Coimbatore, Tamil Nadu, India (affiliated to The Tamil Nadu Dr. M.G.R. Medical University).

[2] Department of Pharmacology, KMCH College of Pharmacy, Coimbatore, Tamil Nadu, India (affiliated to The Tamil Nadu Dr. M.G.R. Medical University).

[3] Vice Principal, Head of the Department of Pharmaceutics, KMCH College of Pharmacy, Coimbatore, Tamil Nadu, India (affiliated to The Tamil Nadu Dr. M.G.R. Medical University).

KEYWORDS:

Indigenous plant, Nimbin, Anti viral, Pharmacology, Phytochemistry.

FOR CORRESPONDENCE:

Arulkumar R *

ADDRESS:

KMCH College of Pharmacy, Coimbatore, Tamil Nadu, India (affiliated to The Tamil Nadu Dr. M.G.R. Medical University).

ABSTRACT

Neem (*Azadirachta indica*) has universally been accepted as a wonder tree because of its diverse utility since from the Vedic times and in Ayurveda medicine it has been used for more than 4,000 years due to its medicine properties. So, it has been called by a variety of names like “the village of pharmacy,” “Drug Cabinet of Mother Nature”, ‘arista’ (means a ‘perfect, complete and imperishable’) besides its therapeutic efficacious, Neem has already established its potential as a source of naturally occurring insecticide and pesticide. The stems, roots, leaf and young fruits of the tree are made into capsules, tablets, lotions, creams, soaps, and shampoos, and are used to treat a variety of conditions. The drug interaction of the herb has not been documented scientifically, although Drugs.com states that more than 0.2 ml of neem oil per kilogram of body weight can lead to toxic reactions. The present review highlights a literature on taxonomy, origin and extraction of neem. Various chemical test for phytochemical studies are discussed and it further deals with its pharmacological activity (Neuroprotective and Hepatoprotective Effect, Antitumor, Antidiabetic, Antiviral, Anti-inflammatory, Antipyretic Analgesic, Antimalarial, Antimicrobial activities) and toxicological effect of *Azadirachta indica*.

1. INTRODUCTION:

Human beings have depended on nature for their simple requirements as it acts as a source for many medicines, shelters, foodstuffs, fragrances, clothing, flavors, fertilizers and means of transportation throughout the ages. For the large proportions of the world's population, medicinal plants play a vital role in the healthcare system and this is mainly true in developing countries, where herbal medicine has a continuous history of long- term use[1]. The world health organization(WHO) estimates that the 80% of the population living in the developing countries depends exclusively on traditional medicine for their primary health care and more than half of the world's population still relies entirely on plants for medicines. Here we have taken the divine tree neem (*Azadirachta indica*) which is mainly cultivated in the Indian subcontinent [2, 3]. It is a multipurpose medicinal tree of family Meliaceae. .Various parts of this divine tree such as flowers, leaves, seeds, and bark have been used to treat both acute and chronic human diseases like pyrexia, headache, ulcer, respiratory disorders, cancer, diabetes, leprosy, malaria, dengue, chicken pox, and dermal complications. The tree is popular for its pharmacological attributes like antioxidant, hypolipidemic, microbicidal, antidiabetic, anti-inflammatory, hepatoprotective, antipyretic, hypoglycemic, insecticidal, antifertility, nematicidal, antiulcer, neuroprotective, cardioprotective, andanti-leishmaniasis properties. *A. indica* is also rich in various phytochemicals such as alkaloids, steroids, flavonoids, terpenoids, fatty acids, and carbohydrates. The fungicidal potential of the tree is due to the presence of azadirachtin and Nimbin. Herein, we have compiled a comprehensive review of phytochemical properties, phytoconstituents analysis, biological attributes, and toxicological studies of this multipurpose tree which provides freedom from all diseases [4].

2. Taxonomy of *Azadirachta indica*

Kingdom - Plantae

Division - Magnoliophyta

Class - Dipsacales

Order- Rutales

Sub-order- Rutinae

Genus - Azadirachta

Species – indica

3.Origin and distribution of *Azadirachta indica*

The *Azadirachta indica* tree occurs throughout India. According to an estimate, there are about 20 million trees in the country. The neem tree is noted for its drought-resistant property. Usually, it thrives in the regions with sub-arid to sub-humid conditions, with an annual rainfall of about 400 to 1200 mm. It can grow in the area with an annual rainfall of about <400 mm, but in such cases, it

depends mainly on the under-groundwater levels. Neemcan grow in various types of soil, but it thrives best on well-drained deep and sandy soils (pH 6.2-7.0). It is a typical tropical/subtropical tree and exists at annual mean temperatures at the range of about 21- 32 °C and also it can tolerate high to very high temperatures. It does not tolerate temperature below 4 °C (leaf shedding and death may ensure) [5],[6].

Figure 1: *Azadirachta indica* leaf, stem and fruits

4. Phyto Chemical constituents and properties

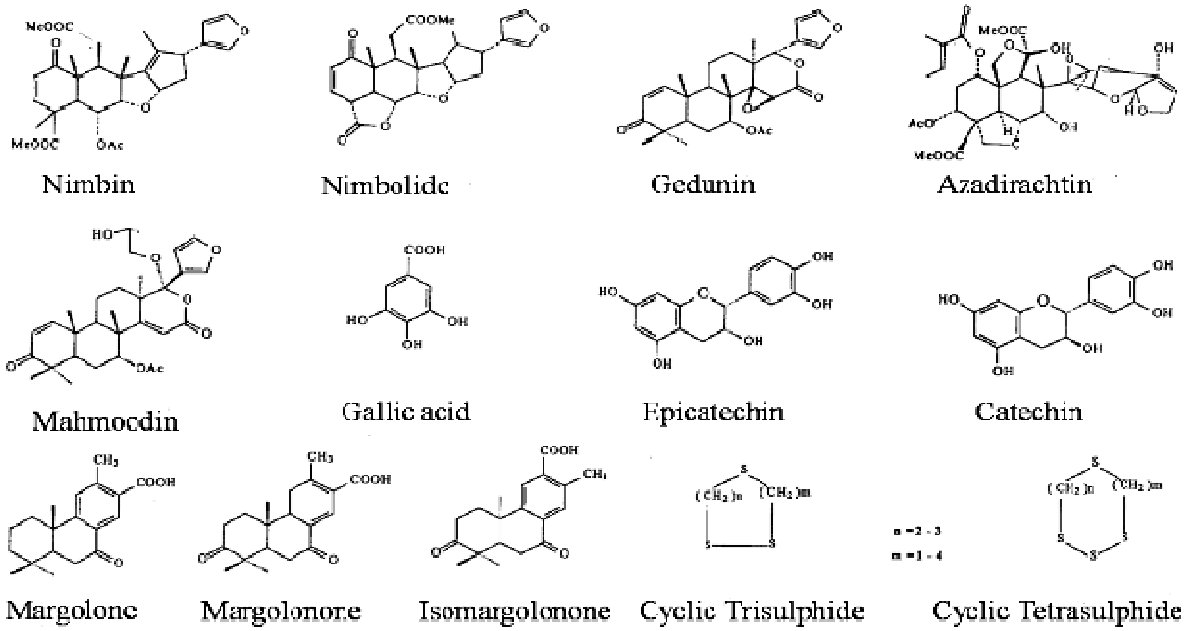

Figure 2: Phytoconstituents of *Azadirachta indica*

Azadirachta indica contains Chemical constituents such as bitter fixed oil, nimbidin, nimbin, nimbinin and nimbidol, tannin and its uses are: Anti-inflammatory activity (nimbidin, sodium nimbidate, gallic acid, catechin, polysachharides). Anti-arthritic, hypoglycemic, antipyretic, hypoglycemic, diuretic, anti-

gastric ulcer activity (nimbidin). Anti-fungal activity (nimbidin, gedunin, cyclic trisulfide). Anti-bacterial activity (nimbidin, nimbolide, mahmoodin, margolone, margolonone, isomargolonone) Spermicidal activity (nimbin, nimbidin)

Anti-malarial activity (nimbolide, gedunin, azadirachtin)

Anti-tumor activity (polysaccharides)

Immunomodulatory activity (NB-II peptoglycan, gallic acid, epicatechin, catechin)

Hepatoprotective activity (neem leaf aqueous extract)

Anti-oxidant activity (neem seed extract).[7],[8]

5. Phytochemical analysis of plant extracts

5.1 Extraction of ethanolic compounds from plant leaves

The fresh leaves were shade dried for 14 days and ground into powder using a pulverizer. The powdered material was weighed using electronic weighing balance and drying of the leaves was continued until a constant weight was obtained. 250 g of the powder was placed in a container and was defatted using petroleum ether, and maceration using 300 ml of 95% (v/v) ethanol in order to obtain the ethanolic plant extract. The mixture was stirred vigorously and kept for 24 hours. The mixture was filtered and further 300 ml of the ethanol was added to the residue and kept for another 24 hours before filtration. This procedure was repeated three times and the combined filtrate was subjected to a rotary evaporator to obtain the crude drug extract. The total weight of crude drug extract obtained was 30.5 g and thus, the percentage yield was 12.2%.[9]

Table 1: Phytochemical test

EXPERIMENT	OBSERVATION	INFERENCE
Test for Terpenoids (Salkowski Test): 0.2 g crude drug extract was mixed with 2ml of chloroform (CHCl3) and 3 ml of concentrated H2SO4 was carefully added to form a layer	Formation of reddish brown color interface.	presence of terpenoids confirmed
Test for Alkaloids: 0.5 g of plant extract was diluted with 10 ml of aqueous hydrochloric acid-1%(w/v), boiled and filtered. Then, 2 ml of dilute NH3 was added to 5 ml of the filtrate. 5 ml Chloroform was added later	The formation of cream with Mayer's reagent	presence of alkaloids

and was shaken vigorously to extract the alkaloidal base. The chloroform layer was extracted with 10 ml acetic acid. Mayer's reagent was added.		
5 ml dilute ammonia was added to the crude extract. Then, 1 ml of concentrated H2SO4 was added.	A yellow coloration disappeared on standing .	presence of flavonoids.
Test for Saponins. 5 ml of distilled water was added to 0.5 g of extract and the solution was shaken vigorously and then observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously.	formation of an emulsion	
Test for Tannins. 0.5 g of extract was boiled with 10 ml of water in a test tube and then filtered. A few drops of 0.1% (w/v) FeCl3 were added and observed.	Formation of brownish-green or a blue-black color.	presence of tannins

6. Therapeutic activity of neem

6.1 Antitumor and Antiviral Activity

Neem seed oil, bark, and leaves contain limonoids and polysaccharides reduced tumors and cancers, and reveal potency against lymphocytic leukemia. Inhibition of Mitotic cell division activity by the neem leaf extract was observed. Numerous research activities have also highlighted the pronounced antiviral effect of aqueous extract of neem leaves against Small Pox, Fowl Pox, Polio and HSV as assessed by virus prevention assay. Aqueous extracts of neem leaf and also some neem oil fraction showed the antiviral activity against HIV and Polio Viruses.[10]

6.2 Antimalarial Activity

Wood scrapings of neem bark were soaked in 5% neem oil (Azadirachtaindica) and then diluted in acetone and in 45 days the breeding of Anopheles stephensi and Aedesaegypti were controlled, when it is placed in water storage tanks.[11] Nimbolide isolated from plantextracts show the antimalarial

activity by inhibiting the *Plasmodium falciparum* growth[12] The aqueous and alcohol extracts of bark and leaves of neem show antimalarial activity, particularly on chloroquine-resistant strains[13].

6.3 Neuroprotective Effects

The neuroprotective effects of neem leaves against cisplatin (-CP-) Induced neurotoxicity and conclude that morphological findings of neem before and after Cisplatin injection implied a well-preserved brain tissue. No changes, in biochemical Parameters were observed with neem treated group.[14]

6.4 Antidiabetic Activity

Hypoglycemic activity of the concethanolic (90%) extract of neem and *Andrographis peniculata* were studied. Experimental results showed that ethanol leaves extract (1 gm/kg) of *Azadirachta indica* and Andrographispeniculata significantly reduced the increased blood glucose level[15].Limonoids from neem are known for their therapeutic potential against pancreatic α-amylase, a known anti-diabetic target. The limonoidsazadiradione and gedunin could bind and inactivate HPA (anti-diabetic target) and may prove to be lead drug candidates to control post-prandial hyperglycemia.[16]Neem serves as an important alternative source in the management of diabetes involved in controlling increased blood glucose level during diabetes mellitus which should be examined further by oral hypoglycemic therapy.[17]

6.5 Hepatoprotective Effect

Medicinal plants play a vital role as a hepatoprotective agent without any adverse effects. The study was performed to investigate the hepatoprotective role of azadirachtin-A in carbon tetrachloride (CCl4) induced hepatotoxicity in rats and histology and ultrastructure results proved that pretreatment with azadirachtin-A dose-dependently reduced hepatocellular necrosis [18]. Effect of neem leaf powder against CCl4 induced hepatic damage. The evaluation markers used were GPT, GOT, glucose, bilirubin, cholesterol, Alkalinephosphate, and total protein. These marker level were significantly changed due to carbon tetrachloride(CCl4) single dose but the treatment of aqueous slurry of neem leaves powder significantly recovers all markers to normal biological levels. In this study, silymarin was taken as the standard for comparison. The observation of these markers, as well as Light and electron microscope photographs, supports the regeneration of liver parenchymal cells. This confirms the overall promising effect of neem against hepatic disorders.[19]

6.6 Anti-inflammatory, Antipyretic and Analgesic Activities

The stem bark of neem was extracted using chloroform which shows significant effectiveness against carrageenin-induced paw edema in rat and mouse ear Inflammation. Inflammatory stomatitis in children is treated by the bark extract. Antipyretic activity has been reported in neem oil. The methanol

extract of neem leaves showed an antipyretic effect when it is administered into male rabbits. Antipyretic and Anti-inflammatory activities in various extracts have been reviewed[20].

6.7 Anti-ulcer activity:

The aqueous and ethanol leaf extract of *Azadirachta indica* (NLEa&NLEe) was investigated for Anti-ulcer effect in pylorus ligation, cold restraint stress and constrain swimming endurance models in Wistar albino rats. The anti-ulcer activity was assessed by determining and comparing the ulcer index in the test drug group with that of the distilled water (-ve) control group and Ranitidine 20mg/kg were taken as a reference standard. NLEa and NLEe 400mg/kg orally produced significantly inhibited the gastric lesions in pyloric ligation, cold restraint stress and forced swimming endurance model of Wistar albino rats. The extract (400 mg/kg) shows remarkable ($P<0.05$) reduction in gastric volume, free acidity, total acidity, combined acidity and ulcer index as compared to control. This present study concludes that aqueous and ethanolic leaf extract of *Azadirachta indica* has a potential anti-ulcer activity which could be either due to cytoprotective action of the drug[21].

6.8 Antimicrobial activity

Antimicrobial study of neem (*Azadirachta indica*) leaf extract against human pathogenic bacteria *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Bacillus pumilus*. Antimicrobial activities of alcoholic neem extracts were used. several concentrations of each extract 200 mg/ml, 150 mg/ml,100 mg/ml,50 mg/ml, 25 mg/ml prepared by using disc diffusion method. When compared to gentamycin10 mg and gentamycin 200 mg, the methanol and ethanol extract show maximum inhibition on *Bacillus pumilus*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* in ascending order[22].

6.9 Antibacterial Activity

The hexane chloroform and methanol extracts of *Azadirachta indica* were screened for antibacterial activity against *E.coli*, *Proteus vulgaris*, *Micrococcus luteus*, *Klebsiella pneumonia*, *Bacillus subtilis*, *Enterococcus faecalis* and *Streptococcus faecalis*. It was reported that methanol extract was the most effective, The chloroform extract was moderately effective and hexane extract showed low antibacterial activity.[23]

6.10 Antifungal activity

The aqueous, ethanol and ethyl acetate extracts of Azadirachtaindica leaves were evaluated for antifungal activity on the growth of some human pathogens (Aspergillusflavus, Aspergillusniger, Aspergillusterreus, *Aspergillus fumigates*, Microsporumgypseum and *Candida albicans*) in vitro. Different concentrations (5, 10, 15 and 20%)were prepared from these extracts which inhibits the test pathogens growth and the effect was gradually increased with concentration. The 20% ethyl acetate

extract shows the strongest inhibition when compared to the activity obtained by the same concentration of the other extracts.[24]To evaluate the in vitro growth inhibition of *Candida albicans*, in the soft-liner material and Shore A hardness from resin-based denture soft lining materials modified by incorporation of neem or garlic Resin discs were prepared with polymethyl methacrylate (PMMA) and soft liners which are incorporated with various concentrations of neem or garlic. For antifungal activity, resin discs were placed on agar plates which are inoculated with *C. albicans* and were evaluated after 2, 4, and 7 days using the streak plate method. The PMMA hardness was evaluated with the use of Shore A at 2, 4, and 7 days. Whether the addition of neem and garlic extracts to the soft liner would inhibit the *C. albicans* growth and also evaluate the hardness of the soft lining material after the incorporation of neem and garlic extract.[25]

6.11 Antioxidant Compounds

The free radical formation is the normal function of the body but the resulting molecules are unstable and can damage other cells. A series of disorders including eye health, cataracts and macular degeneration, cardiovascular disease,age-related neurodegeneration and even cancer can occur due to high levels of free radicals. Neem protects our against chemically induced carcinogens and liver damage by increasing antioxidant levels[26].

6.12 Skin Diseases

Neem has a potent activity against fungi, parasites, and viruses. It has been most helpful in treating a variety of skin problems and diseases like psoriasis, eczema and other persistent conditions. Psoriasis is successfully treated with Neem oil.

6.13 Vitiligo

Vitiligo an autoimmune disorder that causes skin patches to lose its color. The dosage is of 4 g of Neem leaves three times a day, ideally taken before each meal. Neem oil has been applied in the affected areas which could result in the reversal of discoloration[7].Extraction of Azadirachtin from the tree, exhibited no oviposition with traces of ecdysteroids in ovaries of Locusta migratoria female at dose of 10 mg for 15 days. Inhibition of Oogenesis and presence of ecdysteroid was controlled by interference of azadirachtin with the neuroendocrine control of hormone synthesis[27].Controlled amount of *A. indica* and peanut oil was administered through intrauterine route to female Wistar rats for investigation of their comparative efficacy as anti fertility agents. A. indica oil was more efficient in controlling fertility without harming ovaries than peanut oil. Intrauterine administration of *A. indica* oil induced preimplantation blocking of fertility[27].Aqueous extract of neem leaves administered at a dose of 1 ml per male rat for 1 month induced male infertility due to secretion of spermatogenesis and testicular functioning androgenic hormone (Deshpande, Mendulkar, & Sadre, 1980). The above

discussion indicated that various tree extracts had spermicidal activity. It also inhibited the ovum release owing to hormonal changes. As a result, these extracts can be used as birth control pills to delay pregnancy [28].

7. Toxicity study of neem

7.1. The cytogenetic toxicity of the leaf extract of neem was evaluated in murine germ cells

The extract was found to induce structural and numerical changes in the spermatocyte chromosomes as well as synaptic disturbances in them at their first metaphase. A significant increase in the frequency of sperms with abnormal head morphology and the decrease in mean sperm count were also observed. This spermatotoxic effect of the neem extract corroborates its germ cell mutagenicity. The possible role of azadirachtin, the most active principle present in the neem extract, in producing the observed genotoxic effect is discussed[29].

7.2 Oral acute toxicity

In this method, animals were dosed once at a time. If the animal survived, the dose for the next animal was increased and if the animal died, the dose for the next animal was decreased. Six groups of 10 mice (control and test group), each containing an equal number of both male and female, were formed. The first group (control group) received 1% carboxymethyl cellulose (CMC). Groups 2–6 were orally treated with neem oil mixed with 1% CMC at the doses of 18.40, 23.00, 28.80, 36.00 and 45.00 g/kg, respectively. After treating 50min, the mice in top dose group appear to move slowly, chills get together, extreme sensitivity to noise and convulsions. The rest of the dose group of poisoning decrease with decreasing amounts and ease. Death necropsy showed a lot of liquid filling, intestinal swelling in the gut of mice, other tissue and the organ had no obvious abnormalities. By the end of the study (Day 14),the particulars of death of mice[30] .

7.3 The toxicity and behavioural effects of neemlimonoids on Cnaphalocrocismedinalis (Guene´e), the rice leaf folder

Meliaceae plant products have been shown to exert pesticidal properties against a variety of insect species. In agricultural pest control programs, such products may have the potential to be used successfully as botanical insecticides. The effect of the neem (*Azadirachta indica*) limonoidsazadirachtin, salannin, deacetylgedunin, gedunin, 17-hydroxyazadiradione and deacetylnimbin on the biology and mortality of rice leaffolder larvae was investigated. In laboratory experiments, treatment with neemlimonoids suppressed leaf folding behaviour of C. medinalis. Biological parameters (larval duration, pupal duration adult longevity and fecundity) were also affected by the treatment. Azadirachtin, salannin, and deacetylgedunin showed high bioactivity at all doses, while the rest of the neemlimonoids were less active, and were only biologically active at high doses.

Azadirachtin was most potent in all experiments and produced almost 100% larval mortality at 1 ppm concentration. These results indicate neemlimonoids affect the larval behaviour. These effects are most pronounced in early instars[31].

7.4 Toxic encephalopathy

In children, neem oil is reported to cause toxic encephalopathy and Reye's–like syndrome[32]. Neem oil poisoning in a 73-year-old male who presented with vomiting, seizures, metabolic acidosis, and toxic encephalopathy[33].

8. CONCLUSION:

Neem and its ingredients have therapeutics implication and have been traditionally used worldwide especially in Indian Subcontinent since ancient time. In the present review we have highlighted many of the interesting biological activities of ***Azadirachta indica*** like Antibacterial, Antiviral, Anti-inflammatory, antipyretics ,analgesic, Antioxidant, Skin diseases, antitumor, anti malarial, neuro protective, hepato protective, anti diabetic, anti ulcer activitiesand also this article gives information about structure of some bioactive compounds, phyto chemical analysis of plant extract, mechanisam of action and medicinal uses of ***Azadirachta indica*** in human life. Though some herbal products of neem have been prepared but still there is lot of scope for the better utilization of this wonder plant.

9. REFERENCES:

1. Venugopalan, Santhoshkumar, et al: Neem (azadirachtaindica): Prehistory to contemporary medicinal uses to humankind. Asian pac J Biomed 2013; 3(7) :505-514.
2. Sumaira Saleem, Gulzarmuh Ammad, et al: A comprehensive review of phytochemical profile, bioactives for pharmaceuticals, and pharmacological attributes of azadirachtaindica. Phytotherapy Research 2018;32(7):1–32.
3. Gopalasatheeskumar K, Parthiban S, Manimaran T, Boopathi T. Phytochemical screening on various extracts (benzene, ethanolic and aqueous) of stem parts of Zanthoxylum rhetsa (roxb.) Dc. International Journal of Universal Pharmacy and Bio Sciences. 2017; 6(2): 79-91
4. Gupta S.C., Prasad S., et al: Neem (azadirachtaindica): An indian traditional panacea with modern molecular basis. Phytomedicine 2017; 34 : 14-20.
5. Alokmaithani, Vershaparcha et al: Azadirachtaindica(neem) leaf. Journal of Pharmacy Research 2011,4(6),1824-1827
6. Chopra, R. N., Chopra, I. C., et al, Indigenous drugs of india, U.N. Dhur and Sons, Kolkata, 1958;51-595.
7. Debjit Bhowmik et al: Herbal remedies of Azadirachta indica and its medicinal application . J. Chem. Pharm. Res., 2010; 2(1): 62-72.

8. Timothy, S.Y., Goji, S.Y., et al: Antibacterial and phytochemical screening of the ethanolic leaf extract of Azadirachtaindica(neem) (meliaceae). International journal of applied biology and pharmaceutical technology.2011; 2(3): 194-199.
9. Ayoola, G.A., Coker H.A.B., et al: Phytochemical screening and antioxidant activities of some selected medicinal plant used for malaria theraoy in southwestern Nigeria. Tropical Journal of Pharmaceutical Research. 2008; 7(3): 1019-1024.
10. Hassan amer, WafaaA , et al: In vitro antitumor and antiviral activities of seeds and leaves from neem (Azadirachtaindica) extracts; International journal of Academic research. 2010; 2(2): 47
11. Dua V K ,Nagpal B N, Sharma V R , Repellant action of neem cream against mosquitoes. Indian J malarial 1995; 32:47-53
12. Rojanapo W, Suwanno S, Somaree R, Glinsukon T and Thebtaranonth Y: Screening of antioxidants from some thia vegetables and herbs. J. Sci. Thailand. 1985; 11: 177-188.
13. Kabeh JD, Jalingo MGDSS. Mini review exploiting neem for improved life. Int J agribiol.2015;2(5): 1327-1334
14. Abdel Moneim A.E: "Azadirachtaindica attenuates cisplatininduced neurotoxicity in rats," Indian Journal of Pharmacology. 2014;46(3): 316–321.
15. Rashedaakter, M Mahabub-uz-zaman, MD. Saidurrahman: "Comparative studies on antidiabetic effect with phytochemical screening of azadirachta indicia and andrographispaniculata". IOSR Journal of Pharmacy and Biological Sciences. 2013; 5(2): 122–128.
16. Ponnusamy S, Haldar S, Mulani F, Zinjarde S, Thulasiram H, Ravikumar A: Gedunin and Azadiradione: Human Pancreatic Alpha-Amylase Inhibiting Limonoidsfrom Neem (Azadirachtaindica) as Anti-Diabetic Agents. PLoS One 2015;10(10).
17. Shravankumardholi, Ramakrishna raparla, Santhoshkumarmankala, Kannappannagappan: Journal of Applied Pharmaceutical Science. 2011; 01 (04): 100-105.
18. Baligar NS, Aladakatti RH, Ahmed M, Hiremath MB, "Hepatoprotective activity of the neem-based constituent azadirachtin-a in carbon tetrachloride intoxicatedwistar rats," Canadian Journal of Physiology and Pharmacology 2014; 92(4): 267–277
19. Pingaleshirish S: Hepatoprotection study of leaves powder of Azadirachtaindica A. Juss. International journal of pharmaceutical sciences review and research 2010; 3(2): 37-42.
20. Biswaskausik, Chattopadhyayishita, Banerjee K ranjiit and Bandyopadhyayuday: Biological activities and medicinal properties of neem (Azadirachtaindica). Current science, 2002; 82(11): 1336-1345.

21. Bharat Bhusanmohapatra, MangalaCharanDasb, Subas Chandra Dindac: Anti-ulcer activity of aqueous and ethanolic leaf extract of neem(Azadirachtaindica) in albino rats. Journal of Pharmacy Research 2012; 5(3):4060-4062

22. Maragathavalli, S., Brindha, S., Kaviyarasi, N.S: Antimicrobial activity in leaf extract of neem (Azadirachtaindica). I.J.S.N 2012; 3(1): 110-113.

23. Saradhajyothikoona, Subbaraobudida: Antibacterial potential of the extracts of the leaves of Azadirachtaindica. Not SciBiol 2011; 3(1): 65-69.

24. Mahmoud, D.A,Hassanein, N.M., Youssef, K.A., Abouzeid, M.A. Brazilian: Antifungal activity of different neem leaf extracts and the nimonol against some important human pathogens. Brazilian Journal of Microbiology 2011; 42: 1007-1016.

25. Seenivasan Madhankumar, V. Anandkumar, Parathasarthynatarajan, and Gayathrisreenivasan: Antifungal efficacy and the mechanical properties of soft liners against candida albicans after the incorporation of Garlic and Neem: An In vitro study. J IntSoc Prevent Communit Dent 2018; 8(3): 212–217.

26. Bhattiprolu, Subrahmanyam, Thomasmüller: Inhibition of turnover of neurosecretion by azadirachtin in locustamigratoria.Journal of Insect Physiology1989 ;35(6): 493-497, 499-500.

27. Upadhyay, S. N., Kaushic, C., &Talwar, G. P:. Antifertility effects of neem (azadirachtaindica) oil by single intrauterine administration: a novel method for contraception. Proceedings of the Biological Sciences, 1990; 242: 175–179.

28. Saleem s, Muhammad G, Hussain MA, Bukhari SNA: A comprehensive review of phytochemical profile, bioactives for pharmaceuticals, and pharmacological attributes of Azadirachtaindica. Phytotherapy Research 2018; 32(7): 1241-1272.

29. Khan PK, Awasthy KS: “cytogenetic toxicity of neem,” food and chemical toxicology 2003; 41(10): 1325–1328.

30. Litchfield J T and Wilcoxon F A: A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. 1949; 96: 99-113.

31. Sengottayan Senthilnathan, Kandaswamy Kalaivani, Kim Sehoon: The toxicity and behavioural effects of neemlimonoids on cnaphalocrocismedinalis(guenée), the rice leaffolder. Chemosphere. 2006; 62(8): 1381-1387.

32. Sinnaih D, Baskaran G. Margosa oil poisoning as a cause of Reye’s syndrome. Lancet 1981; 317(8218): 487-489.

33. Ajay Mishra, Nikhil Dave: Neem oil poisoning: Case report of an adult with toxic encephalopathy. Indian Journal of Critical Care Medicine 2013; 17 (5):321-322.