THE POTENTIAL PHARMACOLOGICAL AND MEDICINAL PROPERTIES OF NEEM (*AZADIRACHTA INDICA* A.JUSS) IN THE DRUG DEVELOPMENT OF PHYTOMEDICINE

ABSTRACT

The neem plant (*Azadirachta indica* A. Juss) is known worldwide as a medicinal plant of pharmaceutical importance base on its multiple therapeutic and diverse uses. Most part of the neem plant has been reported to show potential medicinal activities and has been commercially exploited in traditional pharmacopoeia in most sub-Saharan countries. Apart from its therapeutic and pharmaceutical potential. *A indica* has been reported to have insecticidal properties with the bioactive compounds already developed in agrochemical industries as bio-pesticides. An understanding of the chemistry of the secondary metabolites, several studies have been done on the biological and pharmacological activities with a considerable progress made with respect to its biological activity and medicinal uses. The neem safety is known from its long communal ethno-pharmacological uses as a category one herbal product. It is readily available with great access to the local population at low cost and also environmentally friendly. This paper attempts to give an insight into the biological activities of some of the compounds isolated, pharmacological actions of the extract, clinical studies and medicinal applications along with their safety evaluations. Issues on the active chemical constituents of various formulations, commercially available neem products, are also mentioned along with their respective application.

Key Words: Neem- *Azadirachta indica*, pharmacological, biological properties, bio-pesticides, agrochemicals, drug development.

INTRODUCTION.

The neem herbal plant (*Azadirachta indica* A. Juss) [1], belongs to the Meliaceae (Mahogany) family, also known as margosa or Indian lilac, has long been recognized for its properties both against insects and in improving human health [1]. The neem tree is a broad leaved evergreen plant with the capacity to grow up to a height of up to 35m in the sudano sahelian climatic zone of Cameroon with extended branches spreading some 12 m across [2]. The flowers and fruits are borne in axillary clusters and at maturity the smooth ellipsoidal drupes are greenish yellow and made up of a sweet pulp that enclose the seed [3]. The seed consist of a shell and

1-3 kernels which contain the active compound azadirachtin and its homologues [2, 3]. The bark and leaves contain biologically active compounds with low levels of azadirachtin. The azadirachtin occurs on amounts of some 4-6 g/kg seeds which varies depending the genotype by environmental interactions [4]. The tree is widely distributed in most tropical and sub-tropical regions of the world with multiple uses for shade, reforestation projects and in alley cropping in plantations, in most cases for the production of bioactive compound for pesticidal, antifeedant and repellent properties against insects [4]. Azadirachtin, isolated as a complex tetranortri-terpenoid limonoid from the neem seeds, is the major active compound implicated in the antifeedant and toxic effects in insects [5]. Other bioactive limonoid and sulphur-containing molecule with repellent, antiseptic, contraceptive, antipyretic and antiparasitic activities has been isolated from leaves, flowers, bark, roots [5, 6]

The first report of neem antifeedant activities was first reported in by Heinrich Schmutterer, in 1952 on the desert locusts (*Schistocerca gregaria* (Forskal)) refusing to feed on neem [2, 7]. Further studies revealed that this species had an unusually high sensitivity to azadirachtin as an antifeedant [2, 7]. Due to the interest of this finding there have been many international conferences on neem and, the first took place in Germany in 1980 [13], leading to a large data base of scientific literature on neem reporting on the antifeedant effects of neem and other pharmacological activities [2, 7]

The medicinal potential of the neem plant *Azadirachta indica* A. Juss has been reported for over a thousand years, most especially in the Asian continent [1]. Within the African sub regions neems is well integrated in the traditional African pharmacopoeia especially in those countries like Ghana, Burkina Faso, Niger [4] where traditional medicine has been well integrated in the primary health care systems [4, 5, 8]. Report by Biswas and collaborators [3, 13], studied the biological and pharmacological activities some of the neem compounds, with some clinical investigations to support the potential medicinal uses and toxicity evaluation of neem [9, 10-13].

Neem has two widely distributed closely related species [13], that are known to be widely distributed. The *A. indica* A. Juss[4] and *M. azedarac* [13], and the former is widely known as Indian neem (margosa tree) or Indian lilac, and the other as the Persian lilac in South East Asia [11, 13]. Neem is also widely useful in ayurveda medicine in India, unani and homoeopathic medicine and the Chinese *material medica* [6, 13]. The neem tree is still considered like a 'village dispensary' for the most communities in India [13]. In the past two decades complimentary alternative medicines in the USA has become very popular and neem

is one of the medicinal plant very recognized by the US National Academy of Sciences, when the report in 1992 entitled 'Neem – a tree for solving global problems' was published The neem tree with fruits and seed has been illustrated in (Figure 1).

.

Figure 1; The neem tree, mature fruits and seeds used for extraction of essential oil [23].

Chemistry, Bioactive Compounds in Neem

The bioactive molecule azadirachtin was isolated from the seeds oil of *A.indica* A. Juss by David Morgan and collaborators [2, 8, 9] and the first structural determination was elucidated in the laboratories of Steven Ley[8-10].

The bioactive compounds have been isolated from neem [10] and there are still more scientists working on more secondary metabolite isolation and characterization [14, 35]. Apart from sodium, potassium, salts, neem also contains chloriphyle, calcium, phosphorus, iron, thiamine, riboflasium, nicocin, vitamin C, carotene, and oxalic acid [10, 15].

The bioactive chemicals are classified as; Nimbin[7]: anti-inflammatory, anti-pyretic, anti-histamine, anti-fungal, Nimbidin: anti-bacterial, anti-ulcer, analgesic, anti-arrhythmic, anti-fungal, Ninbidol: anti-tubercular, anti-protozoan, anti-pyretic, Gedunin: vasodilator, anti-malarial, anti-fungal, Sodium nimbinate: diuretic, spermicide, anti-arthritic, Quercetin: anti-protozoal, Salannin: insect repellent [Azadirachtin[37]: insect repellent, anti-feedant, anti-hormonal [11-15] [Fig 2 a & b]

Other chemicals of neem that form its therapeutic value are:

1. Limonoids [36] 2. Terpenoids and steroids 3. Tetranortarpenoids[36], 4. Fatty acid derivatives like margosinone and margosinolone [36], 5. Coumarins like scopoletin,

dihydrosocoumarins[10] 6.Hydrocarbons like docosane, pentacosane, hetacosane, octacosane etc. 7.Sulphur compounds 8. Phenolics, 9.Flavonoglycosides, 10. Tannins [7].

The highest concentrations of the active ingredients are found in the seed and oil, and to lesser amounts in the bark and the leaves of the trees [7]. In the traditional Ayurveda pharmacopoeia the fresh leaves and barks were used on a locally as a preventive therapy for many conditions, such as periodontal tooth decay, gum disease, and other poverty related diseases like malaria [7, 16]. The neem bark and leaves or strong decoction are used as paste to treat serious condition and for blood purification (in case of bacterial, fungal, or viral infections), malaria, fever, arthritis, rheumatism, and many more [7, 16, 17]. Neem bioactive molecules have also been used as a protective agent from nature's pests both as an insect repellant and insecticide that is both safe and harmless to humans [17]. Neem oil has been reported as effective when applied externally to the hair, scalp, and skin for parasites and as an insect repellant [17]. It is also used in some cases for massage on arthritic joints, after being slightly warmed, to relieve pain and inflammation [3, 19].

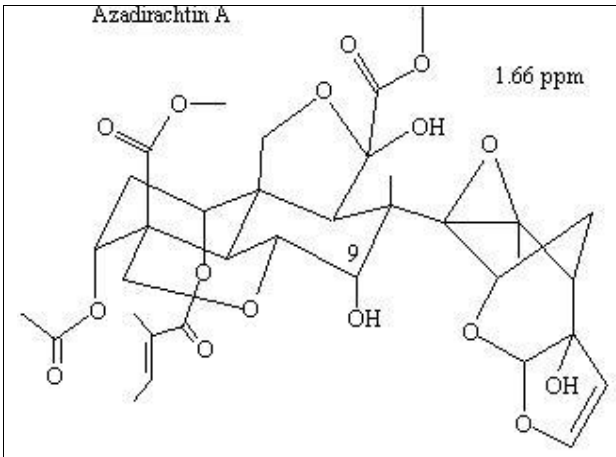

Figure 2a: Chemical structure of the main active ingredient of neem seeds, azadirachtin A.[3]

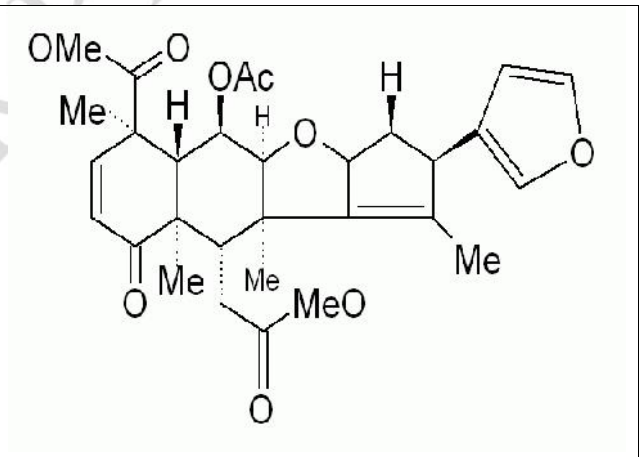

Figure 2b: Chemical structure of Nimbin [3]

A. indica produces many triterpenoids, the biosynthesis of which gives azadirachtin. The biosynthesis of azadirachtin begins with a steroid precursor such as tirucallol, azadirone, azadiradione, and the C-ring opening such as nimbin and salannin, after which can proceed through two main levels of structural complexity: the furan ring formation (e.g. modifications yield azadirachtin [1, 6, 18].

An overview of the antifeedant and toxic properties of azadirachtin with different less structurally complex biosynthetic precursors against the larvae of *Spodoptera littoralis*

(Boisd.), *S. gregaria* and *Oncopeltus fasciatus* Dallas (milkweed bug) showed that toxicity to insects in terms ofsevere growth and moult disruption, was mainly observed with azadirachtin [5, 19]. The less complex, less highly oxygenated molecules were shown to be ineffective in all the larvae stages [19, 20]. The antifeedancy is found in compounds at lower levels of structural complexity, particularly against Lepidoptera e.g. *S. littoralis* that are extremely sensitive to the presence of plant secondary metabolites in their diet [7]. There is no cleart link between antifeedant activity and toxicity of neem triterpenoids along the biosynthetic pathways to azadirachtin. Azadirachtin has a strong antifeedant activity, whereas the toxic insect growth regulatory (IGR) effects are seen in all species. However, antifeedancy varies markedly between insect order and species within orders [4, 22-24]. Neem bio-insecticides, which are extracts of neem seeds, contain many related triterpenoids in addition to azadirachtin including 3-tigloyl-azadirachtin (Azadirachtin B), nimbin and salannin [5, 7]. Their efficacy is closely linked to azadirachtin although many of the other bioactive compounds also have biological activity and give a synergistic effect [13]. The pure azadirachtin has been shown to be effective in the field [7], while the natural mixtures of azadirachtins in neem insecticides may be useful against the development of resistance compared to azadirachtin alone [3, 25].

More than 135 bioactive compounds have been isolated and characterized from the different parts of neem, and many reviews have been published on the chemistry, characterization, structural activity[4] relationship and pharmacological activities of these compounds [27]. The bioactive compounds are divided into two major classes [13]: isoprenoids (like diterpenoids and triterpenoids containing protomeliacins, limonoids, azadirone and its derivatives, gedunin and its derivatives, vilasinin type of compounds and C- secomeliacins such as nimbin, salanin and azadirachtin) and non-isoprenoids, which are proteins (amino acids) and carbohydrates (polysaccharides), sulphurous compounds, polyphenolics such as flavonoids and their glycosides, dihydrochalcone, coumarin and tannins, aliphatic compounds [25-27].

Pharmacological Effects [40]: *A azadirachta* is used for the treatment of dermatologic, gastrointestinal disease, immune dysfunction respiratory disease, inflammatory infection because of the following compounds found in different parts of the neem plant [7]. The highest concentrations of following compounds are found in the leaves and seeds [3, 9, 28].

- Nimbin [7]: anti-inflammatory, anti-pyretic, anti-histamine, anti-fungal

- Nimbidin: anti-bacterial, anti-ulcer, analgesic, anti-arrhythmic, anti-fungal
- Ninbidol: anti-tubercular, anti-protozoan, anti-pyretic
- Gedunin: vasodilator, anti-malarial, anti-fungal
- Sodium nimbinate: diuretic, spermicide, anti-arthritic
- Quercetin: anti-protozoal
- Salannin: insect repellent
- *Azadirachtin* [7]: insect repellent, anti-feedant, anti-hormonal

Adverse Effects: There are no reported side effects in adults using the neem decoction herbal mixture at normal traditional doses [3]. Neem is found to be toxic if consumed in large amounts. In infants, it can produce symptoms like those of Reye syndrome [4, 12].

Biological activity of some neem compounds

Nimbidin, a major crude bitter compound extracted from the oil of seed kernels of *A. indica* have been reported to show multiple biological activities [5, 13]. From the crude extracts some tetranortriterpenes, including nimbin, nimbinin, nimbidinin, nimbolide and nimbidic acid have been isolated. Anti-inflammatory; Antiarthritic; Antipyretic; Hypoglycaemic; Antigastric ulcer; Spermicidal; Antifungal; Antibacterial; Diuretic; Antimalarial; Antitumour; Immunomodulatory [28-32].

Medicinal Uses [22]: Possible medicinal applications of neem

The Neem extract is effective for the treatment of ringworm, eczema and scabies [13]. Lotion derived from neem leaf, when locally applied, can cure some dermatological diseases within 3-4 days in an acute stage or a fortnight in chronic case [4, 19]. A paste prepared from the neem and turmeric has been reported to be effective in the treatment of scabies in nearly 814 people100 [1, 4, 33]. Neem leaf extract has been prescribed for a long time for oral use for the treatment of malaria by Indian ayurvedic practitioners [4, 11]. In the last couple of years clinical trial has been carried out to investigate the efficacy of neem extract to control hyperlipidemia in a group of malarial patients severely infected with *P. falciparum* [4, 17]. The lipid level of cholesterol was found to be lower during therapy when compared to non-malaria patients [10]. Reports are available regarding the use of neem to treat patients suffering from various forms of cancer [10]. One patient with parotid tumour and another with epidermoid carcinoma have responded successfully when treated with neem seed oi1 [5, 13, 34].

Different parts of the neem tree have been used as traditional Ayurvedic medicine in India, material medica in Chinese herbal medicine [13]. In Cameroon and most developing countries

studies have shown that neem oil and the bark and leaf extracts have been therapeutically used as folk medicine to control leprosy, intestinal helminthiasis, antibactierial, anti-diabetic, respiratory disorders, bowel movement and constipation [4, 13]. Its use for the treatment of rheumatism, chronic syphilitic sores and indolent ulcer has also been elucidated my many scientist [4]. Neem oil has also been used to treat various skin infections [13, 17, 35]. The bark, leaf, root, flower and fruit together cure blood morbidity, biliary afflictions, itching, skin ulcers, burning sensations and pthysis (Table 1).

Table 1: Ayurvedic application and traditional uses of neem (*Azadirachta indica*) [4,6].

Plant parts	Medicinal applications	References
Leaf	Leprosy, eye problem, epistaxis, intestinal worms, anorexia, skin ulcers, skin problems, skin ulcers, intestine worms, biliousness	[6, 11]
Fruit	Urinary disorder, diabetes, eye problem, wounds, leprosy, phlegm, piles	[23, 30]
Bark	Curative fever, analgesics	[4, 19]
Twig	Diabetes, asthma, cough, phantom tumour, obstinate urinary disorder	[1, 26]
Flower	Phlegm, intestinal worms, bile suppression	[13, 17]
oil	Leprosy, intestinal worms	[9; 35]
Gum	Wounds, Scabies, skin diseases,	[36]
Seed	Leprosy and intestinal worms	[2, 26]

Traditionally neem has been used in Ayurveda for a number of conditions [7]. It is one of the main compounds used in the preparation of blood purification mixture used in Ayurveda and also in most diabetic formulas in African pharmacopoeia [7, 11]. Other uses have been reported for arthritis, rheumatism, treatment of external and internal parasites, including fevers, as an insect repellent [36, 38]. Neem possesses anti-diabetic, antibacterial and antipyretic (fever reducing) properties [36]. Neem has been used for a wide range of diseases such as flu, fever, sore throat, cold, fungal infections, skin diseases, malaria and many more ailments [36].

THE MAJOR HERBAL USE OF DIFFERENT PARTS OF NEEM IN AYURVEDA [25].

In Ayurveda, neem is useful in treating all sorts of Pitta, Kapha and Vatic disorders [25]. It should be consumed early in morning on empty stomach for 15 days during the end of winter till the starting of summer season [25] and this can possibly prevents most of the diseases throughout the year [25]. Teratogenic studies have shown that neem product can be teratogenic and not advisable for the product in any form to be administered to pregnant women or for treatment of women considering to be pregnant [9, 18, 39].

Chagas' disease [18]

Extracts of neem reportedly affect the kissing bugs that transmit the much-feared Chagas' disease [18]. The extracts do not kill the insect; instead they "immunize" it against parasites that live inside it for part of their life cycle [18]. The neem has a potential for controlling this major health problem in Latin America, although the method of delivering neem materials to these tiny bloodsuckers in the rural communities is very difficult in practice [18, 40-43]. About 20 million Latin Americans are infected with Chagas' disease; many live as helpless cripples, unable to work or enjoy life [18]. A parasite (*Trypanosoma cruzi*) causes this major health problem [18]. It lives and reproduces inside nerve and muscle cells, particularly those of the heart, and drains its victims of all their energy [3, 9, 18].

The parasite - a trypanosome is the main cause of sleeping sickness in Africa. The parasite is transmitted the “kissing bugs." This bug is similar to the large bedbugs, and the insects inhabit crevices in the walls and roofs of huts and houses in many rural areas in the tropics. The insect is active at night as they bite and suck the blood of any sleeping people, pets, or livestock that are exposed [25]. The kissing bugs pick up the parasite, and pass it on through their droppings. The parasite develops and multiplies within the bug's hindgut and, in its infective stage, passes out with the excrement. Kissing bugs when feeding defecate, and when the victim scratches the itchy bump on waking, the excrement, together with the parasite's infective stage, easily rubs into the wound and penetrates the bloodstream [16, 44].

Currently, there is no total control for this dread disease, however some work done by the laboratories in Germany and in Brazil have made some progress in neem research. At the Max Planck Institute for Biochemistry in Martinsried, Germany, Heinz Rembold some species of kissing bug, *Rhodnius prolixus* has been reared, to study the effects of azadirachtin on its hormone system [13]. The studies showed that azadirachtin prevents young kissing bugs from molting prevent maturity and development and reproduction [46].

Neem's as antiviral agents

Antiviral activity.

Investigation in India is very interesting, but anecdotal, information relating antiviral activity to neem. Its efficacy,- particularly against pox viruses. Smallpox, chicken pox, and warts have traditionally been treated with a paste of neem leaves - usually rubbed directly onto the infected skin [3, 47-48].

Experiments with smallpox, chicken pox, and fowl pox indicate that there could be a significant biological basis for the use of neem plant. Crude neem extracts absorbed the viruses, effectively preventing them from entering uninfected cells [[4, 47] unfortunately, no antiviral effects were seen once the infection was established within the cell. Thus neem was effective prevention, but not cures [32, 49].

Recent preclinical preliminary studies have shown that neem leaves possess some antiviral activity [7, 13]. In the United States, studies have shown that aqueous neem-leaf extracts have low to moderate inhibition of the viral DNA polymerase of hepatitis B virus [14, 51] .In addition, in Germany, an ethanolic neem-kernel extract has been reported to show efficacy against herpes virus [30]. In horticultural studies, crude extracts reported to effectively bind some plant viruses, and preventing infection [8, 53]. This result is promising and is being tested with an array of extremely virulent and difficult diseases of people like in wildlife and livestock. Aqueous leaf extract offers antiviral activity against *Vaccinia virus*, Chikungemya and measles virus [2, 54].

There are certain compounds in neem that demonstrate a unique ability to inhibit viruses, and prevent them from causing infection [12]. Neem also inhibits viral multiplication by interacting with the surface of the cells to prevent the cell from becoming infected by the virus. Neem has been observed to be effective against a number of viral pathogens in various clinical studies demonstrating it contains unique properties to inhibit viral disease [19, 28]. Neem is one of just a few known antiviral agents. Chickenpox, shingles, herpes, and hepatitis are viral conditions, which have been successfully treated, in clinical studies by neem's therapeutic compounds [25]. The uncomfortable symptoms of colds and flu's can be relieved during seasonal changes by the regular consumption of neem leaf capsules, extract, or tea [14].

ANTIBACTERIAL ACTIVITIES OF NEEM.

In clinical trials neem oil has inhibited many strains of pathogenic bacteria, such as: *Staphylococcus aureus*. Some common source of food poisoning and many pus-forming disorders such as boils and abscesses. These bacteria can cause secondary infections in peritonitis, cystitis, and meningitis [7]. Many strains are now resistant to penicillin like methicillin resistant *Staphylococcus aureus* and other antibiotics, one of the major reasons for the widespread transmission of staphylococcal infections in hospitals [3, 11, 17]. *Salmonella typhosa* [19, 57] is a very virulen bacterium, which lives in food and water, the causal agent of typhoid, food poisoning, and a variety of infections such as blood poisoning and intestinal inflammation [5]. Antibiotics in the market have low response to address the treatment of this bacterium [7, 58].

The neem so far, has many drawbacks as an antibacterial agent in the sense that in the latter test, neem showed no antibacterial activity against certain strains of the above bacteria, and none was effective against Citrobacter, *Escherichia coli*, Enterobacter, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Proteus morgasi*, *Pseudomonas aeruginosa, Pseudomonas* E01, and *Streptococcus faecalis* [3, 59]

There have been a number of clinical studies showing that neem has significant effects on several bacterial strains. Among some of the more prominent strains studied were *Staphylococcus aureus*, *Streptococcus pyogenes*, *Cornebacterium*, *E. coli*, and *Salmonella typhimurium*[23, 59]. Oil from the leaves, seed and bark possesses a wide spectrum of antibacterial action against Gram-negative and Gram-positive microorganisms, such as *Mycobacterium. tuberculosis* and streptomycin resistant strains [2, 25]. There has been strong evidence of *in vitro* inhibition of *Vibrio cholerae , Klebsiella pneumoniae*, *M. tuberculosis* and *M. pyogenes* [35]. Antimicrobial effects of neem extract have been demonstrated against the strains of *Streptococcus mutans* and *S. faecal* [17], Most of these bacteria can possible cause meningitis, cystitis, sore throats, typhoid, blood poisoning, and food poisoning [27]. Neem's biological and pharmacological activities exert significant effects to address the problem of management and control of bacterial pathogens [18, 60].

NEEM'S EFFECTS AGAINST FUNGI:

The favourable tropical climate in India especially in the coastal regions creates an optimum humid hot atmosphere necessary for fungal growth. Generally, in Ayurveda traditional medicine, smoke from burning dried neem leaves, aqueous extracts of neem leaf, essential oil from neem seed, neem leaf powder, and the neem leaf pastes have been used for the treatment of fungal pathogens to support the primary health care in India [20]. The ringworm, Athlete's

foot, and *Candida albican*, which cause vaginal yeast infections and thrush, are some of the main fungi of dermal infection in humans [17, 62-64]. Neem leaf contains two medicinal compounds, gedunin and nimbidol, which have been clinically tested and proven to control some infective fung [13]i. Jock itch, other fungi that attacks humans, has been treated traditionally in India for thousands of years with neem seed oil and aqueous extracts of neem leaf. Creating medicinal smoke by burning dried neem leaves is an ancient practice in Ayurveda for purifying the atmosphere around a very sick patient. A clinical study investigating the potential efficacy of this ancient practice showed that smoke from burning dried neem leaves inhibited significantly fungal growth, sporulation and germination [15]. With many research of Ayurvedic medicinal plants in South East Asia and India, modern clinical research has shown proven evidence that neem extracts possess some of the most important antifungal bioactive agents for the inhibition of certain fungi. [4, 15, 65]

NEEM'S EFFECT AGAINST SKIN DISEASES:

Dermatological insects

Neem has been report for its promising dermatological properties. Based on its known insecticidal properties neem is commonly used as a traditional remedy against maggots and head lice [30]. In Haiti, for instance, crushed leaves, decoctions, are rubbed into open wounds that have become maggot infested. In is also seen in India and Bangladesh where the villagers apply neem essential oil to the hair to kill head lice and reduction of bald head, with great success [11, 66].

Fungicides

Neem has been shown to have promising efficacy in fungi inhibition against certain fungi that infect the human body [17]. Such fungi are an increasing problem and have been difficult to control by synthetic fungicides. For example, in one laboratory study, neem preparations showed toxicity to cultures of 14 common fungi, including members of the following genera: Trichophyton - an "athlete's foot" the fungi that infects hair follicle, skin surfaces, and nails; Epidermophyton - a "ringworm" that invades both skin and nails of the feet [25, 67];

Microsporum - a ringworm that invades hair, skin, and on very rare occasion the nails; Trichosporon - a fungus of the intestinal tract; Geotrichum - a yeast like fungus that causes infections of the bronchi, lungs, and mucous membranes [3]; and Candida - a yeast-like fungus that is part of the normal mucous flora but can get out of control, leading to lesions in mouth (thrush), vagina, skin, hands, and lungs [24]. Extracts of neem leaf, neem oil seed

kernels are effective against certain fungi including *Trichophyton*, *Epidermophyton*, *Microspor Trichosporon*, *Geotricum* and *Candida* [16, 33, 68].

Eczema:

In the case of eczema clinical investigations have shown that the application of low concentration of neem leaf extracts has an efficacious effect on acute conditions of eczema [11, 13]. Using a Soap or shampoo containing neem oil reduce the itching and redness of eczema [4]. The bark of the neem tree is used in the treatment of eczema. About 25grams each of this bark and the mango bark should be boiled in about 1 liter of water and the vapour allowed fermenting the affected part [3, 69].

Acne: Neem leaf can effectively treat the bacteria that cause acne and studies show that neem can reduce inflammation even when produced by acne [11].

Skin problems: Dry Skin, Wrinkles, Dandruff, Itchy Scalp, Skin Ulcers and Warts are other conditions that can be effectively treated through the use of soaps, lotions, and creams, containing neem leaf extracts and oil. The neem leaves are crushed into powder and mix with turmeric then the paste is applied on the face to remove dark spots [29, 41].

Leprosy:

The sap of the neem tree has been found to be effective in leprosy treatment in some Central African forest zones where the disease is endemic, when administered in daily doses of 60 grams [14]. The patient's body is usually constantly massaged with the sap for many days to observe the effect of treatment success. The treatment is usually continued for 40 days before final assessment of the patient treatment conditions. When the sap is not available or limited, 12 g of neem leaves and 3g pepper can be ground in water and taken [37, 70]. In some situations leprosy and leucoderma treatment requires the application of 10 drops of neem essential oil mixed with 1 teaspoon of sugar powder, acts as a supportive part of treatment used twice a day [12, 70].

Malaria: An infusion or a decoction of the fresh leaves is a bitter vegetable tonic and an alternative, treatment particularly in chronic malarial fevers due to its action on the liver. It should be taken in doses of 15 to 60 grams [9]. Drinking neem teas or chewing a couple of leaves every day reduces the possibility of malaria attack which is mosquito-borne. The water and acetone extracts combination are even more effective than plain neem tea. **N**eem seed and leaf extracts are effective against both choroquin-resistant and sensitive strain malarial parasites [13, 72-73].

Apart from its use against malaria, neem plays a traditional role in the treatment of urinary disorders, skin disease, diabetes, fungi infections and viral diseases. Neem twigs contain antiseptic ingredients which provide dental hygiene and has been used for this purpose by the local community in India and parts of Africa [55]. Neem is also of ecological importance, easily accessible and environmentally friendly: In Africa the tree is used as a shade tree and as a source of fuel wood. In the Sahel countries, neem has been used for halting the spread of the Sahara desert in alley cropping [32]. In many developing countries the wood is used in making fence post, poles for house construction, and furniture [6, 19, 21].

Piles: Three grams of the inner bark of neem with 6 grams of jaggery every morning is very effective in piles treatment [33, 73]. To check bleeding piles, 3 or 4 neem fruits can be administered with water.

Hair Disorder: If there is any hair loss or it has ceased to grow, washing with the decoction of neem leaves may help. This will not only stop hair falling but also help their growth. Frequent application of neem oil also destroys insects and bugs in the hair [30].

Oral Disorder:

The practice of regular cleaning of the teeth with a neem twig prevents gum diseases. It protects loose teeth, relieves toothache, reduces the bad ordour and protects the mouth from various periodontal infections [3, 19]. A neem twig is considered a very effective toothbrush, with its fibers for cleaning, its juice workings both as a mouth freshener, a germ-killing dentifrice and neem twigs also helps to increase the salivary secretion [17, 41, 56]. In most regions of south East Asia and the communities in India the use of neem twigs and leaves to brush their teeth is a common practice, and keep their gums free of periodontal disease and other oral infection. This is a method to solve the problem of limited access to modern dental medications and care, and the problem of poverty alleviation [17]. The ancient Ayurvedic practice of using neem to heal and rejuvenate gum tissue and to prevent cavities and gum disease has been demonstrated in modern clinical studies [36, 55].

NEEM'S EFFECTS ON THE IMMUNE SYSTEM.

Base on the ancient healing system of Ayurveda the bark of the neem tree is known to strengthen an individual's resistance to disease. Modern clinical studies have identified a number of compounds in the neem tree that effectively regulate immune system functions.

There are immunomodulatory polysaccharide compounds, especially present in neem bark, that apparently increase antibody production [3, 15, 31].

Treatment for AIDS: The National Institutes of Health reported positive results from a 1993 study using neem to kill the AIDS virus in in vitro laboratory conditions [9]. The suggested pathway is via neem's immune modulating polysaccharide compounds that may cause increased antibody production [13, 74]. . As far as AIDS is concerned the immunomodulatory properties of neem appear to enhance cell-mediated immune response in people who are HIV positive but who do not have full blown AIDS [14, 59].

TREATMENT FOR SEVERAL DISEASES:

Heart Disease: These include high blood pressure, blood clots, cholesterol, and Arrhythmia/rapid heartbeat [7, 21].

Blood Disorders: Including poor circulation, blood poisoning, and kidney problems [15].

Digestive Disorders: Including heartburn/indigestion, peptic/duodenal ulcers, gastritis, and hemorrhoids [7, 36].

Sexually Transmitted Diseases: Including gonorrhea, syphilis, chlamydia, genital herpes, genital/vaginal warts, candidacies, and urinary tract infections [25]. **Jaundice and Hepatitis:** Drink the diluted juice of the tender neem leaves with a tea spoon of honey to flush out toxins in liver disorders. Neem is anti-pruritic, thermogenic, tonic, stomachic and abdominal movement controller. 2 teaspoon of neem leaves juice with honey is indicated to accelerate the process of cure [56, 75].

Birth Control: Neem is effective for Men and Women, and proposed as a promising solution for birth control in men in resource poor countries, as experiments have shown it can make sperm infertile without affecting sex drive or impeding the sperm count [9, 17, 30].

Respiratory disorder: Decoction of neem bark is used as an anti-tussive for dry cough. Dried neem leaves as reported if powder given daily in 1 g dose twice a day with honey could reduce cough. Neem oil 5-10 drops given with 2 tablespoon of sugar once a day for 15 days helps in tropical Eosinophilia [12].

DIABETES AND HYPOGLYCAEMIC ACTIVITY.

Neem is a biter plant, stomachic, anti-pruritic [25], and plays a revitalizing role in this disease [55]. One table spoon of neem leaves juice approximately 5 ml taken early in the morning on empty stomach for 3 months acts as antidiabetics [55]. Neem leaves chewed or powder taken daily in the morning is useful in diabetes management [25]. The neem leaf has been shown to reduce insulin in the blood and this has motivated the Indian Government to allow the sale of

neem for this purpose [25]. Administration of low doses as small as one tablespoon a day can reduce insulin levels by 50% [21, 36-38].

Aqueous extract of neem leaves significantly decreases blood sugar level and prevents adrenaline as well as glucose-induced hyperglycaemia [3, 9]. Recently, hypoglycaemic effect was observed with leaf extract and seed oil, in normal as well as alloxan-induced diabetic rabbits [45, 57].

ANTICANCER ACTIVITY.

Neem leaf aqueous extract effectively suppresses oral squamous cell carcinoma induced by 7, 12-dimethylbenz[a] anthracene (DMBA) [41], as demonstrated by reduced incidence of neoplasm [4]. Neem may exert its chemopreventive effect in the oral mucosa by modulation of glutathione and its metabolizing enzymes [55]. neem being depurative purifies the blood and being an astringent decreases the body heat. Chewing of 10 to 20 neem leaves early morning with warm water is helpful [36].

Allergy: 8 to 10 fresh Neem leaves are to be eaten early morning on an empty stomach. This helps in purifying blood and controls the allergic condition. It is used also in Tuberculosis, Bronchitis, Conjunctivitis, Allergies, Bad Breath, Hangover, Stress, Insomnia, Smoking, and on and on [19, 44].

Neem's Effects against Parasites: Ayurveda has recommended the use of neem to rid the body of all forms of parasites. Simple aqueous extracts of neem leaves have been the standard treatment for external parasitic infestation without any side effects throughout India [7]. Neem's effectiveness against parasites is due to compounds that mimic hormones [7, 11, 32, 75]. This activity interrupts the life cycle of parasites by inhibiting the ability of the parasites to feed, and preventing parasite eggs from hatching. Neem has demonstrated these effects against lice, and against itch mites which cause scabies [7]. Intestinal worms are treated and prevented throughout the tropics by regular consumption of neem teas [7]. Neem has both curative and preventative effects on the malaria parasite the methods for effective therapy is are as varied as the active ingredients in neem. There are two active compounds in neem [10], which have been clinically proven to be as effective against the malarial parasite compared to both quinine and chloroquine[7]; these are gedunin, a limonoid and quercetin, a flavonoid [7, 30]. The leaves can be chewed and consumed on a daily basis as a preventative like quinine and neem leaf extracts are clinically proven to be as effective as chloroquine against the malarial parasite [10]. Other studies have shown that neem leaf extracts prevent the normal development of the malarial plasmodia by increasing the state of oxidation in the red blood

cells [7, 9]. One of the most important therapeutic use neem is the antiparasitic role and its effectiveness against encephalitism[70-24].

Antifertility effect

Intra-vaginal application of neem oil, before coitus, can successfully prevent pregnancy [13]. This application could be a novel method of contraception [6, 44]. Neem is very useful at the time of childbirth [61]. Administration of the juice extract of neem leaves to a woman undergoing labour before child birth produces normal contraction in the uterus and prevents possible inflammation [5, 44, 49] .It corrects bowls movements and checks onset of fevers thereby aids or facilitate the normal delivery [5]. The use of decoction of neem leaves as a vaginal cleanser heals any wounds caused during delivery and disinfects the vaginal passage [4, 44]. Neem is also a powerful insecticide capable of killing soil nematodes and other plant parasites and also useful as a mosquito repellant [27]. Neem juice is used in toothpastes and contraceptives [44]. Neem leaves eaten every morning on empty stomach has also shown to be beneficial in combating diabetes [19, 56].

Hepatoprotective activity: The aqueous extract of neem leaf has been reported to offer protection against paracetamol induced liver necrosis in rats [24, 57]. The elevated levels of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma glutamyl transpeptidase (GGT) indicative of liver damage were found to be significantly reduced on administration of the neem leaf aqueous extract [57].

Antioxidant activity: The antioxidant activity of neem seed extract has been demonstrated *in vivo* during horse grain germination, which is associated with low levels of lipooxygenase activity and lipid peroxides [58].

Effect on central nervous system: Varying degrees of central nervous system (CNS) depressant activity in mice was observed with the leaf extract [59]. Fractions of acetone extract of leaf showed significant CNS depressant activity [60]. Leaf extract up to a dose of 200 mg/kg body weight produces significant anxiolytic activity in rats [24, 61]. The crude ethanolic extracts of stem bark and root bark showed hypotensive, spasmolytic and diuretic activities [63].

PESTICIDAL APPLICATION

Effects on Feeding

Insects from different Orders significantly differ from their behaviour responses to azadirachtin. Lepidoptera for example show extreme sensitivity to azadirachtin and antifeedancies from <1-50 ppm, species dependent [14, 35]. Hemiptera, Coleoptera, and Homoptera show lesser sensitivity to azadirachtin in terms of behaviour, with up to 100% antifeedancy that is achieved at 100-600 ppm, with some aphid species also showing behavioral sensitivity such as the. strawberry aphid. The Orthoptera are reported to show a significant variation in sensitivity from *S. gregaria* (a polyphagous species which has their chemoreceptors well-conditioned to many plant bioactive compounds) to *Locusta migratoria* (L.) (a graminaceous species which does not have chemoreceptors conditioned to feeding deterrents) , to the extreme insensitivity of *Melanoplus sanguinipes* (Fab.), the North American Plains grasshopper which based on its evolutionary history has never been tested on *A. indica* and has no known information on chemoreceptors response to azadirachtin [6, 11]. Such 'primary' (or gustatory) antifeedancy which is the inability to ingest resulting from the perception of the antifeedant at a sensory level'[44, 76] is responsible for crop protection in several species of Lepidoptera and S. *gregaria.* Desert locusts (*S. gregaria*) are very sensitive to azadirachtin and fail to feed on sugar impregnated discs when the compound is present at the concentrations of ≥ 0.01 ppm [21, 45, 76]. Azadirachtin sprayed on barley seedlings infested with *S. gregaria* nymphs have been shown to protect plants at low doses (2 ppm) . *S. littoralis* (African cotton leafworm), *Spodoptera frugiperda* (J.E. Smith) (fall armyworm), *Heliothis virescens* (F.) (tobacco budworm) and *Helicoverpa armigera* (Hüb.) (old world bollworm) also show some behavioural response to low concentrations of azadirachtin and are bioactive compound at 0.1 - 10 ppm prevent feeding which is dose dependent[30-33, 67].

Table 2: Insect behavioural sensitivity response to azadirachtin showing the effective dose (ED50) which causes 50 % inhibition in feeding [13, 40, 72].

Insect order	ED50 (ppm)
Lipidoptera	<0.001-50
Coleoptera	100-500
Hemiptera	100-500
Hymenoptera	100-500
Orthoptera	0.001->1000

The antifeedant effects observed in these species are highly correlated with the sensory response of chemoreceptors on the insect mouthparts [25, 30, 47]. Feeding behaviour is

dependent upon both neural input from the insects' chemical senses (taste receptor on tarsi, mouthparts and oral cavity) and the central nervous integration of this 'sensory code [2, 9]. Azadirachtin stimulates specific 'deterrent' cells in chemoreceptors and also blocks the firing of 'sugar' receptor cells, which normally stimulate feeding [9, 72].

In most other species of phytophagous insect crop protection results from a combination of antifeedancy and physiological effects resulting from ingestion of azadirachtin [2]. These physiological effects include 'secundary'antifeedancy where the feeding is reduced post-ingestively. These "secondary" antifeedant effects involve a reduction in food consumption and digestive efficiency as a consequence of ingestion, application or injection of the antifeedant [19, 31].

Such secondary antifeedant effects result from the disturbance of hormonal and/or other physiological system such as. movement of food through the gut, inhibitions of digestive enzyme production, effects on the stomatogastric nervous system etc [3, 11, 68]. For example locusts injected with azadirachtin, which by-passes the taste receptors, show a reduced ingestion of food as seen by faecal pellet production [2, 21]. Hemipteran insects feeding on tobacco seedlings that had been systemically treated with 500 ppm azadirachtin, were shown initially to feed normally but, after termination of the initial feed, the interval prior to the next subsequent feed was significantly increased and feeding activity thereafter was suppressed [5]. Also aphids which had fed on artificial diets containing much lower concentration of azadirachtin (25 ppm) exhibited no signs of primary antifeedant effects during an initial 24h period of access to the diets however, their feeding rate fell significantly in the subsequent 24h period [29, 73].

A consequence of interrupted feeding activity can be an effect on the ability of insects to transmit pathogens. Aphids require extended feeding periods to acquire persistently-transmitted luteoviruses (e.g. potato leafroll virus, PLRV) from plants. Treatment of PLRV-infected tobacco plants with azadirachtin reduced sustained feeding by *Myzus persicae* (Sulzer) (peach-potato aphid) and reduced the ability of aphids to acquire and transmit PLRV. However, azadirachtin does not always reduce the spread of plant virus diseases by aphids [19, 75]. Treatment of uninfected seedlings with the same concentrations of azadirachtin (500 ppm) failed to prevent them from becoming infected when viruliferous aphids fed on them [46, 75]. The successful infection of a plant with luteoviruses is dependent upon the transfer of aphid saliva to the plant, a process which may be brief by comparison with the time required for virus acquisition by the aphid, and is not overcome by the presence of the

antifeedant [2, 10]. Similarly, azadirachtin failed to protect seedlings from infections with a non-persistently transmitted potyvirus (potato virus y) from viruliferous aphids [15, 76-78].

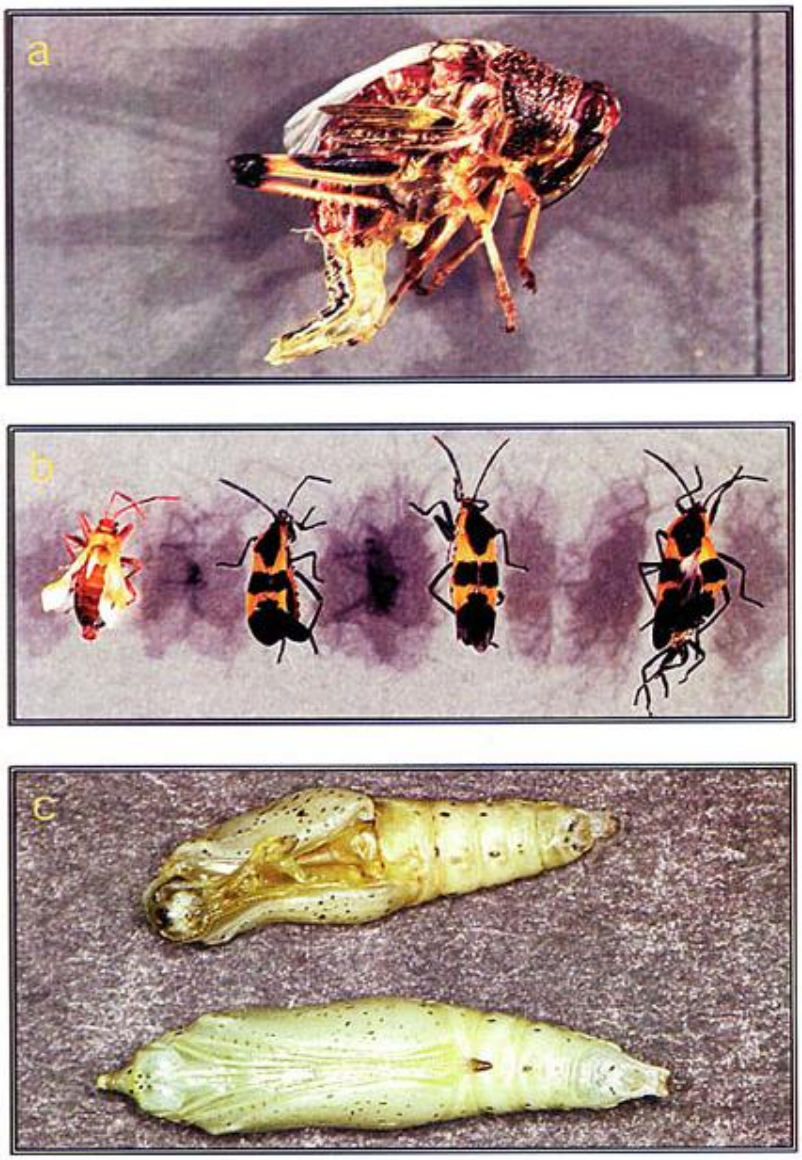

Plate 1. Moulting abnormalities caused by exposure to Azadirachtin treatment of (a)-desert locus (Schistocerca gragaria) showing death at ecdysis (x1.5); (b)-milkweed bug (*O.fasciatus*) showing moulting defects (x 1.5); (c)-cabbage white butterfly (*P. brassicae*) showing abnormal (top) versus normal pupal (bottom) (x3) [14].

Neem pesticides may also have a useful role to play in resistance management. It has been demonstrated that the effects of neem in reducing levels of detoxification enzymes (due to its blockage of protein synthesis) may make insecticides more effective in resistant strains of insect [1, 13, 17]. It has also been shown in *Bacilus thuringiensis* Bt resistant strains of *Leptinotarsa decemlineata* Say, the Colorado potato beetle, that 0.25% Neemix combined with *Bacillus thuringiensis* can act as a resistance breaking compound [16, 55, 67]. In this instance depending upon the resistance mechanism, the neem effects may be due also to blockage of enzyme production, or to the reduced midgut cell turnover rate [14]

Table 3: The insecticidal properties of azadirachtin active compound on insect pests [4, 55, 73]

Effects	Target	Mode of action
Primary antifeedants	Mouthpart and other chemoreceptors	Deterrent cell simulation Sugar inhibition
Secondary antifeedant	Gut	Peristalsis inhibition Enzyme production reduced
Insect growth regulation	Cuticle	Alterations to ecdysteriods and moulting defects
Sterility	Reproductive organs	Alterations to ecdysteroids, reduction of production of viable egg and life progeny
Cellular processes	Dividing cells, muscles and nervous systems	Blockage of cell division, loss of muscle tone, inhibit protein synthesis, blockage of digestive enzymes in gut

Toxicity testing on neem products.

Oral administration of neem essential oil at 200 mg/rat is known to cause severe hypoglycaemic effect [4, 21]. Neem seed oil showed acute toxicity in rats and rabbits with LD_{50} of 14 ml/kg and 24 ml/kg respectively, with the possible target organs for toxic effects being the CNS and the lungs [50]. Neem seed oil produces toxic effect in humans in several isolated cases. Neem essential oil intoxication by humans produces vomiting, acidosis, diarrhea, nausea, and encephalopathy [4, 70]. These toxic effects might be due to the presence

of aflatoxin and other toxic compounds found in neem oil [81]. Mechanistic investigations indicate that neem oil uncouples mitochondrial oxidative phosphorylation, thus inhibiting the respiratory chain [9, 13]. It also decreases intramitochondrial levels of acetyl CoA and acid soluble CoA esters and reduces the mitochondrial ATP content. [63, 78-80].
Various preclinical studies have been reported on the safety evaluation of different parts of neem as well as its various biologically active products [5, 13, 81].

Nimbidin studies in rat models are shown to produce sub-acute toxicity in adult rats after daily administration of 25, 50 or 100 mg/kg for six weeks [4, 79]. A significant hypoglycaemic and spermicidal activity[35] was observed by feeding nimbidin to fasting rabbits. Nimbolide, a major chemical compound of neem seed oil, and nimbic acid were reported to be toxic to mice when administered intravenously or intraperitoneally [4] but less toxic to rats and hamster [4, 19]. Nimbolide and nimbic acid at a lethal dose cause death in most animals by dysfunction of kidney, small intestine and liver followed by significant sudden drop of arterial blood pressure [30, 41, 72].
Neem appears to be safe for most adults when taken by mouth for a short period of time but if taken in large doses or for long periods of time might be unsafe and with potential to cause damage to the kidneys and liver [81].

CONCLUSION.

Neem, *A azadirachta* as a traditional herbal plant has been widely used therapeutically and industrially for the preparation of extracts and isolation of bioactive compounds, which has generated a lot of interest by scientists in exploring and research to provide vital proof of concept information about this medicinal plant. There are more preclinical studies on the bioactive molecules to understand the mechanism of action on receptors for the treatment of identified pathologies. With the changing landscape and the adherence to environmentally friendly approach has encouraged a shift in paradigm as the global scenario is now changing towards the use of nontoxic plant products having traditional medicinal use. The development of phytomedicine and bio-pesticides from neem is very promising for the control various diseases and crop pests. In Cameroon where this plant is already in high use at the northern region, there is the need to promote its use in the primary health care where access to modern drugs can be limited and also in the promotion of Cameroon traditional pharmacopoeia. The WHO has put in place the strategic plan for the integration of medicinal plants in the health system of most African countries, and therefore there is the need to exploit the rich

biodiversity of medicinal flora. For the last few years, there has been an increasing trend and awareness in neem research. Quite a significant amount of research has already been carried out during the past few decades in exploring the chemistry of different parts of neem. There is an opportunity here for developing countries, with their rich sources of bioactive compounds, to play a more important role in integrated pest management (IPM).

.

REFERENCES

1. Tuchinda P, Pohmakou M, Korsalkulkam. The dicpetalin triterpenoid and lignans from aerial parts of Phyllanthus acutissima. J. Nat. Prod. 2008, 71 (4):655-663.

2. Yamazaki M, Nakamura A, Hanai R, Hirota, H. Chemical constituents and the diversity of Ligularia lankongensis in Yunnan Province of China. J. Nat Prod 2008.71 (4) 520-524.

3. Rupani R, Chavez A. Medicinal plants with traditional use: Ethnobotany in the Indian subcontinent. Clin Dermatology. 2018, 36 (3):306-309. Doi;10.1016.
4. Allan, E.J., P. Eeswara, S. Johnson, A.J. Mordue (Luntz), E.D. Morgan & T. Stuchbury. The production of azadirachtin *in-vitro* tissue culture of neem, A*zadirachta indica*. Pestic. Sci. 1994, 42: 147-152.
5. Allan, E.J., T. Stuckbury & A.J. Mordue (Luntz). II *Azadirachta indica* A. Juss. (Neem tree): In vitro culture, micropropagation, and the production of azadirachtin and other secondary metabolites. Biotec. Agric. For. 1999, .43: 11-41.
6. Kanth MR, Prakash AR, Sreenath G, Reddy VS, Huldah S. Efficacy of specific plant products on microorganisms causing dental caries. J Clin Diagn Res. 2016 Dec;10(12):ZM01-ZM03. doi: 10.7860/JCDR/2016/19772.9025. Epub 2016 Dec 1.

7.
8. Saleem S, Muhammad G, Hussain MA, Bukhari SNA. A comprehensive review of phytochemical profile, bioactive for pharmaceuticals and pharmacological attributes of *Azadirachta indica*. Phytother.Res, 2018, 19.doi:10.1002/ptr.6076.

9. Chutulo EC, Chalannavar RK. Endophytic Mycoflora and Their Bioactive Compounds from Azadirachta Indica: A Comprehensive Review. J Fungi (Basel). 2018 Mar 24;4(2). E42. doi: 10.3390/jof4020042.
10. Mandhwani R, Bhardwaz A, Kumar S, Shivhare M, Aich R Insights into bovine endometritis with special reference to phytotherapy. Vet World. 2017 Dec;10(12):1529-1532. doi: 10.14202/vetworld.2017.1529-1532. Epub 2017 Dec 28.

11. Graz H, D'Souza VK, Alderson DEC, Graz M.Diabetes-related amputations create considerable public health burden in the UK.. Diabetes Res Clin Pract. 2018 Jan;135:158-165. doi: 10.1016/j.diabres.2017.10.030. Epub 2017 Nov 11. Review.

12. Gupta SC, Prasad S, Tyagi AK, Kunnumakkara AB, Aggarwal BB. Neem (Azadirachta indica): An indian traditional panacea with modern molecular

basis. Phytomedicine. 2017 Oct 15;34:14-20. doi: 10.1016/j.phymed.2017.07.001. Epub 2017 Jul 3

13. Benelli G, Caselli A, Di Giuseppe G, Canale A. Control of biting lice, Mallophaga - a review.
Acta Trop. 2018 Jan;177:211-219. doi: 10.1016/j.actatropica.2017.05.031. Epub 2017 Jun 3.

14. Benelli G, Buttazzoni L, Canale A, D'Andrea A, Del Serrone P, Delrio G, Foxi C, Mariani S, Savini G, Vadivalagan C, Murugan K, Toniolo C, Nicoletti M, Serafini M Bluetongue utbreaks: Looking for effective control strategies against Culicoides vectors.Res Vet Sci. 2017 Dec;115:263-270. doi: 10.1016/j.rvsc.2017.05.023. Epub 2017 May 20.
15. Aerts, R.J. & A.J. Mordue (Luntz).. Feeding deterrence and toxicity of neem triterpenoids. J. Chem Ecol. 1997, 23: 2117 -2133.
16. Linton, Y.M., A.J. Nisbet & A.J. Mordue (Luntz). The effects of azadirachtin on the tests of the desert locust, *Schistocerca gragaria*. J. Ins. Physiol. 1997, .43: 1077-1084.
17. Lowery, D.T. & M.J Smirle. Toxicity of insecticides to oblique banded leaf roller, *Choristoneura rosaceana*, larvae and adults exposed previously to neem seed oil. Entomol. Exp. Appl. 2000, 95: 201-207.
18. Morales-Covarrubias MS, García-Aguilar N, Bolan-Mejía MD, Puello-Cruz AC.Evaluation of medicinal plants and colloidal silver efficacy against Vibrio parahaemolyticus infection in Lipopenaeus vannamei cultured at low salinity. Dis Aquat Organ. 2016 Nov 22; 122 (1):57-65.
19. Martinez, S.S. & H.F. van Emden.. Sublethal concentrations of azadirachtin affect food intake, conversion efficiency and feeding behaviour of *Spodoptera littoralis* (Lepidoptera: Noctuidae). Bull. Entomol. Res. 1999, 89: 65-71.
20. Mordue (Luntz), A.J., G. Davidson, R.G. McKinlay & J. Hughes.. Observations on azadirachtin for the management of cabbage caterpillar infestation in the field. BCPC symp. Proceed. 1995, 63: 371-378.
21. Mordue (Luntz), A.J., A. Zounos, I.R. Wickramananda & E.J. Allan. Neem tissue culture and the production of insect antifeedant and growth regulatory compounds. BCPC Symp. Proceed. 1995, .63: 187-194.
22. Jiang ZH, Yang QX, Tanaka T, Kouno I.. Bicyclic polyketide lactones from Chinese medicinal ants, Polyrhacis lamellidens. J Nat Prod. 2008 Apr;71(4):724-7.

23. Chen JJ, Fei DQ, Chen SG, Gao K. Antimicrobial triterpenoids from Vladimiria muliensis.
J Nat Prod. 2008 Apr;71(4):547-50.

24. Dwivedi VD, Tripathi IP, Mishra SKzadirachta indica against therapeutic targets of dengue virus, NS2B-NS3 protease. J Vector Borne Dis. 2016 Apr-Jun; 53(2):156-61.

25. Sujarwo W, Keim AP, Caneva G, Toniolo C, Nicoletti M.Ethnobotanical uses of neem (Azadirachta indica A. Juss., Meliaceae) leaves in Vali (Indonesia) and the India subcontinent in relation with historical background and phytochemical properties. J Ethnopharmacol. 2016 Aug 2;189:186-93. doi: 10.1016/j.jep.2016.05.014. Epub 2016 May 10.

26. Saklani A, Kutty SK. Plant-derived compounds in clinical trials. Drug Discov Today. 2008 Feb; 13(3-4):161-71. Epub 2007 Nov 26.
27. Lin LG, Tang CP, Ke CQ, Ye Y. Terpenoids from the stems of Cipadessa baccifera. J. Nat Prod .2008. Apr; 71 (4):628-632.

28. Mordue (Luntz), A.J., A.J. Nisbet, M. Nasiruddin & E. Walker.. Differential thresholds of azadirachtin for feeding deterrence and toxicity in locusts and an aphid. Entomol. Exp. Appl. 1996, 80: 69-72.
29. Mordue (Luntz), A.J., M.S.J. Simmonds, S.V. Ley, W.M. Blaney, W. mordue, M. Nasiruddin & A.J. Nisbet. 1998. Actions of azadirachtin, a plant allelochemical, against insects. Pestic. Sci. 54: 277-284.
30. Nasiruddin, M. & A.J. Mordue (Luntz). The effect azadirachtin on the midgut histology of the locusts, *Schistocerca gregaria* and *Locusta migratoria*. Tissue Cell 1993, 25: 875-884.
31. Nisbet, A.J., J.A.T. Woodford, R.H.C. Strang. The effects of azadirachtin-treated diets on the feeding behaviour and fecundity of the peach-potato aphid, *Myzus persicae.* Entomol. Exp. Appl. 1994, 71: 65-72.
32. Nisbet, A.J., A.J. Mordue (Luntz) & W. Mordue. Detection of [22, 23-$^{3}H_2$] dihydroazadirachtin binding sites on membranes from *Schistocerca gregaria* (Forskål) testes. Ins. Biochem. Molec. Biol. 1995, .25: 551-558.
33. Nisbet, A.J., A.J. Mordue (Luntz), R.B. Grossman, L. Jennens, S.V. Ley & W. Mordue. Characterisation of azadirachtin binding to Sf9 nuclei in vitro. Arch. Inst. Biochem. Physiol. 1997. 34: 461-473.
34. Perera, D.R., G. Armb & N. Senanayake. Effect of antifeedants on the diamondback moth (*Plutella xylostella*) and its parasitoid *Cotesia plutellae*. Pest Manage. Sci. 2000, .56: 486-490.
35. Raguraman, S. & R.P. Singh. Biological effects of neem (*Azadirachta indica*) seed oil on an egg parasitoid, *Trichogramma chilonia*. J. Econ. Entomol, 2000.. 92: 1274-1280.
36. Reed, E. & S.K. Majumdar. Differential cytotoxic effects of azadirachtin on *Spodoptera frugiperda* and mouse cultured cells. Entomol. Exp. Appl. 1998., 89: 215-221.
37. Simmonds, M.S.J., W.M. Blaney, S.V. Ley, J.C. Anderson & P.L. Toogood.. Azadirachtin: Structural requirements for reducing growth and increasing mortality in lepidopterous larvae. Entomol. Exp. Appl 1990. 55: 169-181.
38. Simmonds, M.S.J., W.M. Blaney, S.V. Ley, J.C. Anderson, R. Banteli, A.A. Denholm, P.C.W. Green, R.B. Grossmam, C. Gutteridge, L. Jennens, S.C. Smith, P.L. Toogood & A. Wood. Behavioral and neurophy-siological responses of *Spodoptera littoralis* to azadirachtin and a range of synthetic analogues. Entomol. Exp. Appl. 1995, .77: 69-80.
39. Simmonds, M.S.J., J.D. Manlove, W.M. Blaney & B.P.S. Khambay. Effect of botanical insecticides on the foraging and feeding behaviour of the coccinelid predator *Cryptolaemus montrouzieri*. Phytoparasitica 2000, 28: 99-107.
40. Trisyono, A. & M.E. Whalon. Toxicity of neem applied alone and in combination with *Bacillus thuringiensis* to Colorado potato beetle (Coleoptera: Chysomelidae). J. Econ. Entomol. 2000., 92: 1281-1288.
41. Trumm, P & A. Dorn. Effects of azadirachtin on the regulation of midgut peristalsis by the stomatogastric nervous system in *Locusta migratoria*. Phytoparasitica 2000, .28: 7-26. Bibliography

42. Biswas, Kausik, Ishita Chattopadhyay, Ranajit K.Banerjee and Uday Bandyopadhyay.. Biological activities and medicinal properties of Neem (*Azadirachta indica*). *Current Science* 2002, 82(11): 1336-1345.
43. Chianese G, Yerbanga SR, Lucantoni L, Habluetzel A, Basilico N, Taramelli D, Fattorusso E, Taglialatela-Scafati O –Antiplasmodial triterpenoids from the fruits of neem, Azadirachta indica..J Nat Prod 2010. 27; 73(8):1448-52.i. 2009., 71 (5):562-563.
44. Choudhury MK. Toxicity of neem seed oil against the larvae of Boophilus decoloratus, A one host tick in cattle. Indian J. Phar S
45. Piyadarsini RV, Murugan RS, Sripriya P, Karunagaran D, Nagini S. The neem limonoids azadirachtin and nimbolide induce cell cycle arrest and mitochondria mediated apoptosis in human cervical cancer (HeLa) cells. Free Radic Res; 2010, 44 (6):624-634.
46. Maciel MV, Morais SM, Bevilaqua CM, Silva RA, Barros RS, Sousa RN, Sousa LC, Machado LK, Brito ES, Souza-Neto MA. *In vitro* inseticidal activity of seed neem oil on Lutzomyia longipalpis (Diptera : Psychodidae).Rev. Bras Parasitol Vet. 2010, 19 (1):7-11.
47. Zhang YQ, Xu J, Yin ZQ, Jia RY, Lu Y, Yang F, Du YH, Zou P, Lv C, Hu TX, Liu SL, Shu G, Yi G. Isolation and identification of the antibacterial active compound from petroleum ether extract of neem oil. Fitoterapia; 2010, 81 (7):747-750
48. Tripathi A, Chandrasekaran N, Raichur AM, Mukherjee A. Antibacterial applications of silver nanoparticles synthesised by aqueous extract of *A indica* (Neem) leaves. J. Biomed Nanotechnol., 2009, 5 (1):93-98.
49. Singh B, Sharma DK, Kumar R, Gupta. A development of a new controlled pesticide delivery system based on neem leaf powder. J. Hazard Mater, 2010, 15; 177 (1-3):209-299.
50. Thoh M, Kumar P, Nagarajaram HA, Manna SK, Broglio-Micheletti SM, Valente EC, de Souza LA, Dias Nda S, de Araújo AM. Plant extracts in control of Rhipicephalus (Boophilus) microplus (Canestrini, 1887)
51. Goswami S, Bose A, Sarkar K, Roy S, Chakraborty T, Sanyal U, Baral R. Azadirachtin interacts with the tumour necrosis factor (TNF) binding domain of its receptors and inhibits TNF-induced biological responses. J.Biol Chem. 2010, 19 (8):5888-5895.
52. Moslem MA, El-Kholie EM. Effect of neem (*Azadirachta indica* A. Juss) seed and leaves extract on some plant pathogenic fungi. Pak J. Biol Sci. 2009, 15; 12 (140:1045-1048.
53. Gunasekaran K, Vijayakumar T, Kalyanasundaram M. Larvicidal and emergence inhibitory activities of neem against vectors of malaria. Indian J.Med Res. 2009 130 (2):138-145.
54. Batabyal L, Sharma P, Mohan L, Maurya P, Srivastava CN. Relative toxicity of neem fruit, bitter gourd and castor seed extracts against the larvae of filarial vector. Parasitol. Res. 2009; 105 (5):1205-1210.
55. Dua VK, Pandey AC, Raghavendra K, Gupta A, Sharma T, Dash AP. Larvicidal activity of neem oil formulation against mosquitoes Malar.2009, 8:89-124.

56. Takeda Y, Tomonari M, Arimoto S, Masuda T Honda G. A new phenolic glucoside from an Uzbek medicinal plant, Origanum tytanthum. Nat Med Tokyo, 62 (1):71-74.

57. Ohtsuki T, Miyagawa T, Koyano T, Kawahara N, Goda Y. Acylated triterpenoid saponins from Schima noronhae and their cell growth inhibitory. J Nat Prod.2008 May; 71 (5):918-21.

58. Liu S, Zhu H, Zhang Abietane diterpenoids from Clerodendrum bungei.J Nat Prod. 2008 May; 71(5):755-9. Epub 2008 Mar 19. Apr; 17 (4):678-683.

59. Yang MC, Choi SU, Kim SY, Lee KR. Guaiane sesquiterpene lactones and amino acid-sesquiterpene lactone conjugates from the aerial parts of Saussurea pulchella. J. Nat. Prod. 2008

60. .Zhang Y, Gan M, Lin S, Song W, Zi J, Li S. Glycosides from the bark of Adina polycephala. J. Nat Prod 2008. May;71 (5):905-909.

61. SaiRam M, Ilavazhagan G, Sharma SK, Dhanraj SA, Suresh B, Parida MM, Jana AM, Devendra K, Selvamurthy W. Anti-microbial activity of a new viginal contraceptive NIM-76 from neem oil (Azadirachta indica). J Ethnopharmacol. 2000, 71 (30:377-382.

62. Bhanwra S, Singh J, Khosla P. Effect of *Azadirachta indica* Neem leaf aqueous extracts on paracetamol-induced liver damage in rats. Indian J.Physiol Pharmacol. 2000; 44 (1):64-68.

63. Subapriya R, Nagini S. Medicinal properties of neem leaves: A review. Curr. Med.Chem Anticancer Agents. 2005, 5(2):149-156.

64. Chaudhary S, Kanwar RK, Sehgal A, Cahill DM, Barrow CJ. Azadirachta based biopesticides in replacing synthetic toxic pesticides front. Plant. Sci.2017. may 8;8:610.doi:10.3389/fpls.2017.00610 e-collection.

65. Farahnik B, Sharma D, Alban J, Sivamani R. Oral systemic botanical agents for the treatment of Psoriasis. A review J. Altern. Complement .Med.2017. Jun; 23(6):418-425.doi:10.1089/acm.2016.0324.

66. Mickenautsch S. High viscocity glass-ionomer cements for direct posterior tooth restorations in permanent teeth. The evidence in brief. J.Dent. 2016. Dec; 55:121-123.doi; 10.1016.10.007. Epub.2016 oct 17.

67. Vijayakumar C, Ramesh M, Murugesan A, Panneerselvam N, Subramaniam D, Bharathiraja M Biodisel from plant seed oils as an alternate fuel for compression ignition engines. A review. Environ. Sci. Pollut. Res. Int. 2016. Dec; 23(24): 24711-24730. doi: 10.1007/s11356-016-7754-2. Epub 2016 Oct 15.Review.Benelli G, Canale A, Toniol C, Pavela R, Nicoletti M. Neem (Azadirachta indica): towards the ideal insecticide? Nat. Prod. Res. 2017.Feb; 31(4):369-386.doi:10.1080/14786419.2016.1214834.Epub 2016.Aug 12.
68. Roy S, Handique G, Dashora K, Mukhopadhyay A. Use of plants extracts for tea pest management in India. Appl. Microbiol. Biotechnol. 2016. Jun; 100(11):4831-4844.doi:10.1007/s00253-016-7522-8.Epub Apr 22.
69. Alzohairy MA. Therapeutics role of Azadirachta indica (Neem) and their active constituents in diseases prevention and treatment. Evid based Complement Alterna. Med. 2016; 2016:7382506.doi:10.1155/2016/7382506.Epub.2016. mar.1.
70. Patel SM, Nagulapalli KC, Sethi G, Bishayee A. Potential of neem (Azadirachta indica) for prevention and treatment of oncologic diseases. Semin. Cancer. Biol. 2016 Oct; 40-41:100-116. Doi: 10.1016/j.semcancer.2016.03.002.Epub 2016. Mar 24. Review.

71. Dhingra K, Vandana KL. Effectiveness of Azadirachta indica (neem) mouth rinse in plaque and gingivitis control: a systematic review. Int. J. Dent; Hgy. 2017. Feb; 15(1):4-15. Doi:10.1111/idh.12191.Epub. 2016. Feb 15.

72. Mickenautsch S. Are high-viscosity glass-ionomer cements inferior to silver amalgam as restorative materials for permanent posterior teeth? A Bayesian analysis BMC. Oral Health. 2015 Oct 8; 15(1):118.doi:10.1186/s12903-015-0108-5.

73. Lakshmi T, Krishnan V, Rajendran R, Madhusudhanan N.Azadirachta indica. A herbal panacea in dentistry. An update. Pharmacog. Rev. 2015.Jan-Jun; 9(17):41-4 doi: 10.4103/0973-7847.156337.

74. Talbot W, Duffy N. Complementary and alternative medicine for psoriasis: what the dermatologist needs to know. Am J.Clin. Dermatol. 2015. Jun; 16(3):147-165. Doi:10.1007/s40257-015-0128-6.

75. Elumalai P, Arunakaran J. Review on molecular and chemopreventive potential of nimbolide in cancer. Genomics Inform. 2014. Dec; 12(40:156-164.doi 10.5808/GI.2014.12.4.156. Epub 2014 Dec 31.Review.

76. Chan PK, Zhao M, Che CT, Mak E. Cytotoxic acylated triterpene saponins from the husks of *Xanthoceras sorbifolia*. J. nat. Prod. 2008. Jul: 71(7):1247-1250. Epub. 2008. Jun 13.

77. Zhu Y, Miao Z, Ding J, Zhao W. Cytotoxic dihyroagafuranoid sesquiterpenes from the seed of *Celastrus orbiculatus*. J. nat. Prod. 2008. Jun; 71(6):1005-1010.Epub. 2008. May 29.

78. Souccar C, Cysneiros RM, Tanae MM, Torres LM, Lima-Landman, MT, Lapa AJ. Inhibition of gastric acid secretion by a standardized aqueous extracts of cecropia glaziovii. Sneth and underlying mechanism. Phytomedicine. 2008 Jun; 15(6-7):462-9.

79. Mistry KS, Sanghvi Z, Parmar G, Shah S, Pushpalatha K. Antibacterial efficacy of Azadirachta indica. Mimusops elengi and 2% CHX on multispecies dentinal biofilm. J. conserve.Dent. 2015. Nov-Dec; 18(6):461-6.doi: 10.4103/0972-0707.168810.

80. Azmi MA, Khatoon N, Ghaffar RA. Ultra structural study of the rat cheek epithelium treated with neem extract. Pak J Pharm Sci. 2015 Nov;28(6):1991-6.

81. Chaturvedi I, Dutta TK, Singh PK, Sharma A Effect of combined herbal feed additives on methane, total gas production and rumen fermentation. Boinformation. 2015 May 28;11(5):261-6. doi: 10.6026/97320630011261. eCollection 2015.