Review paper

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

ABSTRACT

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An understanding of the chemistry of the secondary metabolites of neem plant (*Azadirachta indica* A. Juss) is essential and important due to its medicinal properties. 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 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, is well known and established for its insecticidal and antimicrobial activities. [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 produce within the axillary clusters that during maturity the drupes produced are greenish yellow which constitutes a sweet pulp enclosing 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 bioactive metabolite azadirachtin yield can range between 4-6 g/kg seeds which may vary with the genotype by environmental interactions [4]. The neem tree has a wide distribution in the tropics most especially in the

sub-tropical regions of the world, use mostly for providing shades, alley cropping and other agroforestry activities, and in most cases to produce bioactive molecules pesticidal, antifeedant and insect repellent potentials to combat insects [4]. Azadirachtin, has been identified and isolated as a complex tetranortriterpenoid limonoid derived from the neem seeds, as the main active metabolite shown in the antifeedant and contributing to the toxic effects on the insects [5]. Other bioactive molecules related limonoid and sulphur-containing biomolecules with antiseptic, antipyretic and antiparasitic properties has also been successfully isolated from the leaves and barks of the tree [5, 6]

The antifeedant properties of neem were first reported by Heinrich Schmutterer, in 1952 studying the desert locusts (*Schistocerca gregaria* (Forskal)) that were not able to feed on the neem product [2, 7]. More studies have been done to show that the desert locust has a high sensitivity to azadirachtin compound as an antifeedant [2, 7]. Many international conferences have been organized on neem since 1980 and the first took place in Germany. [13]. This has led to creation of a huge data base of scientific literature for neem information and reporting on the antifeedant properties of the neem plant and other pharmacological and biological properties elucidated [2, 7]

The medicinal importance of neem plant *A. indica,* has been shown for the last five decades, particularly in Asia where most of the studies have been done [1]. In sub Saharan Africa neem is well integrated in the traditional African Pharmacopoeia in countries like Ghana, Burkina Faso, Niger [4, 6, 8] Studies by Biswas et al [3, 13], elucidated the biological and pharmacological activities of neem compounds, with some clinical trials investigations to support the medicinal uses and safety margins [9, 10-13].

Neem has two closely related species that is ubiquitous [13]. The *A. indica* [4] and *Melia. azedarac* [13], the former widely known as the Indian neem (Margosa tree) or Indian lilac, while the other is known popularly as the Persian lilac in South East Asia [11, 13]. Neem is very popular in Ayurveda medicine in Asian countries especially in India, *unani* and homoeopathic medicine, and in the Chinese Materia Medica [6, 13]. The neem tree is traditionally considered to be a 'village dispensary' for a wider population of the communities in India [13]. In the last three decades complementary and alternative medicines has become very popular and neem in particular is one of the medicinal plants of economic importance in the USA. The neem tree with fruits and seed has been illustrated in (Figure 1).

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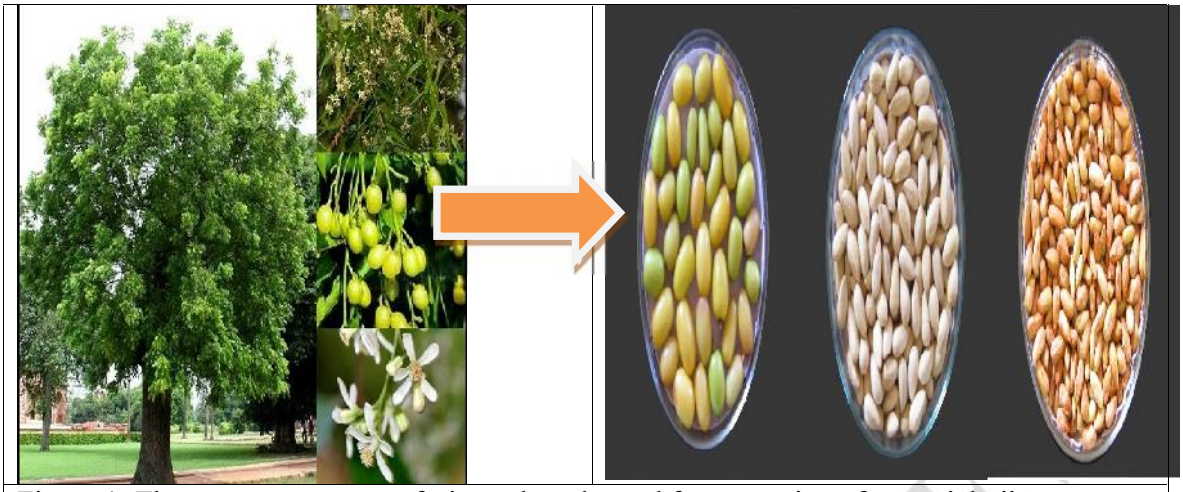

Figure 1; The neem tree, mature fruits and seeds used for extraction of essential oil [Source:Personal Photos from field survey]

Chemistry: Bioactive Molecules in Neem

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

The bioactive molecules have been isolated from the neem plant [10] and there are more scientist interested in the research on secondary metabolite isolation and characterization [14, 35]. Studies show that the neem plant contains in varied ratios in the plant parts microelements of importance such as chlorophyll, calcium, phosphorus, iron, thiamine, riboflavin, niacin, vitamin C, carotene, and oxalic acid, sodium, potassium, salts, [10, 15].

The bioactive molecules are classified as; Nimbin [7]: antibacterial, antiulcer, antipyretic, antifungal, antihistamine, Nimbidin: anti-ulcer, antibacterial, antiarrhythmic, analgesic, antifungal, Ninbidol: antiprotozoal, antitubercular, antipyretic, Gedunin: vasodilator, antifungal, antimalarial, Sodium nimbinate: spermicide, diuretic, anti-arthritic, Quercetin: antiprotozoal, Salannin: insect repellent [37]: insect repellent, antifeedant, antihormonal [11-15] [Fig 2 a & b]

Other chemicals of neem that constituting its therapeutic value:

1. Limonoids [36] 2. Terpenoids and steroids 3. Tetranorterpenoids [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 in the neem plant can be found in the seed and oil, and to a lesser extend in the bark and the leaves. [7]. In the India traditional Ayurveda Pharmacopoeia, the fresh leaves and barks are mostly locally as a preventive therapy for many illnesses , such as periodontal tooth decay, gum disease, and other poverty related diseases like malaria [7, 16]. The neem bark and leaves prepared as strong decoction are mostly applied as paste to treat skin and other dermatological condition and in the purification of blood (bllod tonics. They are effectively use in the treatment of fungal, bacterial, and in a lesser extend viral infections. Uses as anti-malaria, treatment for fever, arthritis, rheumatism, and many more is also common in India [7, 16, 17]. Neem bioactive molecules have also been used as a good protective substance for pests, as insect repellant and insecticide environmentally friendly, safe and harmless to humans [17]. Neem oil is effective when externally applied to the hair, scalp, and skin as anti-parasites and as an insect repellant [17]. It is also used in most situation in Africa for aromatherapy, massage on rheumatoid arthritis pain relieve and other inflammations [3, 19].

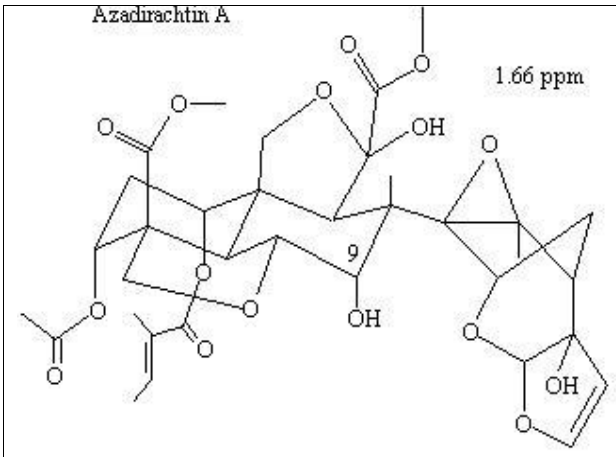

Figure 2a: Chemical structure azadirachtin A.[3]

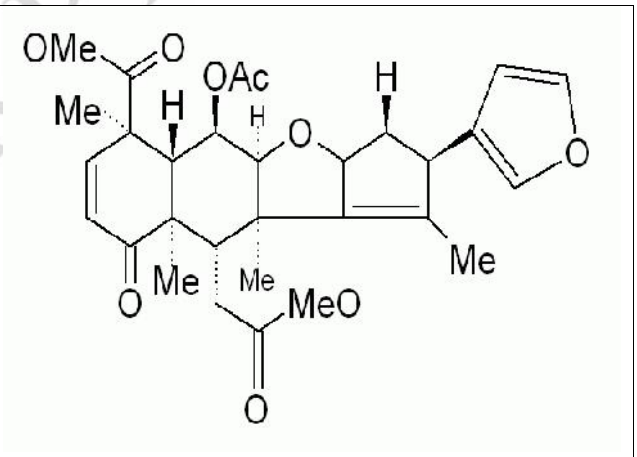

Figure 2b: Chemical structure of Nimbin [3]

A. indica produces many triterpenoids, the biosynthesis of which gives azadirachtin. The biosynthesis of azadirachtin starts from a steroid precursor called tirucallol, azadirone, azadiradione, with the C-ring opening such as nimbin and salannin, then this proceed through two main stages of structural complexity: the furan ring formation whose modifications yield azadirachtin [1, 6, 18].

Antefeedant activity

Studies on the 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) revealed that the toxicity to insects in terms of severe growth and moult disruption, was attributed to azadirachtin [5, 19]. The less complex and less highly oxygenated bio-molecules were shown to be ineffective in all the larvae stages [19, 20]. Anti-feedancy is found in compounds at lower levels of structural complexity, specifically against Lepidoptera such as. *S. littoralis* extremely sensitive to the presence of plant secondary metabolites found in their diet [7]. There is no correlation between antifeedant activity and toxicity of neem triterpenoids within the biosynthetic pathways to azadirachtin. Azadirachtin has a strong antifeedant activity, whereas the toxic insect growth regulatory (IGR) effects are seen in most insect species. However, antifeedancy varies significantly between insect order and inter-species, and also within orders [4, 22-24]. Neem bio-insecticides, extracts derived from neem seeds, contain many related triterpenoids in addition to azadirachtin, including 3-tigloyl-azadirachtin (Azadirachtin B), nimbin and salannin [5, 7]. Neem efficacy is closely linked to azadirachtin although many of the other bioactive molecules also show promissing biological activities with a synergistic effect [13]. The pure azadirachtin compound 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 insect resistance compared to azadirachtin alone [3, 25].

Bioactive compounds of neem

Over 150 biomolecules have been elucidated and characterized derived from neem parts and structural activity, pharmacological activities well studied [4, 27]. The bioactive molecules are classed into two main groups [13]: isoprenoids such as diterpenoids and triterpenoids which contains protomeliacins, limonoids, azadirone and its derivatives, gedunin and its derivatives, vilasinin type of compounds and C- secomeliacins such as nimbin, salanin and azadirachtin. The non-isoprenoids are proteins and carbohydrates sulphurous containing compounds, polyphenolics such as flavonoids and their glycosides, dihydrochalcone, coumarin and tannins, aliphatic compounds [25-27].

Pharmacological Activities [40]: *A azadirachta* extracts are well documented for the treatment of skin, gastric ulcer, respiratory disorder, inflammatory infection due to the

presence of the bioactive molecules present in the different parts of the neem plant [7]. The main active molecules present in the leaves and seeds include the following compounds.

- Nimbin [7]: anti-histamine, anti-fungal, antiinflammatory, antipyretic, [7]
- Nimbidin: antiulcer, analgesic, antibacterial, , antiarrhythmic, antifungal [15, 27]
- Ninbidol: antiprotozoan, antitubercular, antipyretic [21, 38]
- Gedunin: antifungal, vasodilator, antimalarial, [8]
- Sodium nimbinate: spermicide, antiarthritic diuretic [12, 30]
- Quercetin: antiprotozoal [8, 11]
- Salannin: insect repellent [32]
- *Azadirachtin* [7]: antifeedant, antihormonal, insect repellent

Adverse Effects: No known side effects have been reported so far on the use of neem decoction extracts in the traditional pharmacopoeia in most communities in developing countries [3, 35]. Neem poses a potential risk of safety and organ toxicity when consumed in large amounts on a regular basis. In children symptoms associated to Reye syndrome has been reported in India [4, 12].

Biological activity of some neem compounds

Nimbidin, is a major crude bitter compound that is extracted from the oil of seed kernels and have been reported to show many pharmacologic activities [5, 13]. From the crude extracts some tetranortriterpenes, including nimbin, nimbinin, nimbidinin, nimbolide and nimbidic acid have been isolated with anti-inflammatory; antiarthritic; antipyretic; hypoglycaemic; anti-gastric ulcer; spermicidal; antifungal; antibacterial; diuretic; antimalarial; antitumour; Immunomodulatory activities [28-32].

Medicinal Potentials

The neem extract is known to be effective for the treatment of skin diseases such as ringworm, eczema and scabies [13, 22]. Lotion derived from neem leaf, on local application is promising for curing some dermatological diseases within 3-4 days in an acute stage or in a chronic situation [4, 19]. Paste preparationsd from the neem and turmeric has been shown to be effective in the treatment of skin conditions in nearly 814 patients [1, 4, 33]. Neem leaf extract is reported for a long term oral use for the treatment of malaria in India [4, 11]. Clinical trials are in progress to investigate the efficacy of neem extract in the control of hyperlipidemia in a cohort of malarial patients severely infected with *P. falciparum* [4, 17]. The cholesterol level in this study was found to be lower during therapy when compared to

non-malaria patients [10]. More reports have shown the use of neem to treat patients suffering from different forms of cancer [5, 10, 13, 34].

Four over a century 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 in traditional medicine to treat leprosy, anti-diabetic, respiratory disorders, intestinal helminthiasis, antibactierial, bowel movement and constipation [4, 13]. New reports demonstrate its use in the treatment and management of rheumatism, chronic syphilitic sores and ulcer [4]. Neem oil is widely used to treat various skin infections [13, 17, 35]. The bark, leaf, root, flower and fruit in combination can treat biliary infections, blood morbidity, itching, ulcers, and burning sensations (Table 1).

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

Plant parts	Medicinal applications	References
Leaf	Leprosy, skin ulcers, skin, eye problem, epistaxis, intestinal worms, anorexia, 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 several conditions [7]. It is one of the main compounds used in the preparation of blood purification mixture and also in most diabetic management in African Pharmacopoeia [7, 11]. Other uses have been reported for rheumatoid arthritis, treatment of external and internal parasites, malaria, as an insect repellent [36, 38]. Neem possesses antidiabetic, antibacterial and antipyretic potentials [36].

and has been effectively used for a wide spectrum of diseases such as fever, flu, sore throat, cold, fungal infections, skin diseases, malaria and many more ailments [36].

THE MAJOR TRADITIONAL USE OF NEEM IN AYURVEDA [25]

In Ayurveda, neem plays a vital role in the treatment and management of a wide range of ailments in India such as *Pitta, Kapha and Vatic* disorders [25]. The concoction is usually consumed early in morning before 9 am on an empty stomach for the period of 15 days [25] and this can possibly prevent most of the diseases throughout the year [25]. Teratogenic studies have shown that neem product can cause fetal malformations and not advisable to be administered to pregnant women or for treatment of women considering to be pregnant [9, 18, 39].

Chagas' disease.]

Studies on the aqueous extracts of neem showed control on the *kissing bugs* that transmit the Chagas disease reported in Asian countries [18]. The extracts do not kill the insect directly; however they cause an immune inbalance against parasites that live inside it during the part of their life cycle [18]. The neem has a promising 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 Latinos are infected with Chagas' disease; making life very difficult for the affected population to work and enjoy life [18]. A parasite (*Trypanosoma cruzi*) is responsible for chagas as a major health problem [18]. It lives, survives and reproduces inside nerve and muscle cells, in particular those of the heart, and saps energy from those infected [3, 9, 18].

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 faeces. 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].

So far, there is no total control for this chagas disease, however some work done by the laboratories in Germany and in Brazil have made some progress in neem research for control and mangement. At the Max Planck Institute for Biochemistry in Martinsried, Germany, Heinz Rembold studies have been done on some species of kissing bug, *Rhodnius prolixus* on the effects of azadirachtin on its hormone system [13]. The studies revealed that azadirachtin

has promising insecticidal properties on young kissing bugs and can affect molting, prevent metamorphosis process, sexual reproduction, development and maturation [46].

Neem Antiviral activity.

Many scientists are interested in conducting studies in this domain in India, and very interesting findings has been reported relating antiviral activity. Neem efficacy particularly against pox viruses, Smallpox, chicken pox, and warts have traditionally been treated with a paste of neem leaves – in most cases are usually rubbed directly onto the infected skin [3, 47-48].

Studies with smallpox, chicken pox, and fowl pox have shown promising results from the use of neem plant. Crude neem extracts absorbed the viruses, effectively preventing them from entering uninfected cells [[4, 47] however, no antiviral effects have been shown once the infection was established within the cell [32, 49].

Recent preclinical preliminary studies have shown that neem leaves possess some antiviral activity [7, 13]. Studies have shown that aqueous neem-leaf extracts have low to moderate inhibition of the viral DNA polymerase of hepatitis B virus [14, 51]. Furthermore, in Germany, studies conducted on the ethanolic neem-kernel extract were effective against herpes virus [30]. Neem has been effective in horticultural studies, as the crude extracts have been demonstrated to effectively bind some plant viruses, and preventing infection [8, 53]. This result is promising and further testing has been done using an array of extremely virulent and difficult diseases of patient population, some wildlife and livestocks. Aqueous leaf extract shows antiviral activity against *Vaccinia virus*, chikungunya and measles virus [2, 54].

There are certain bioactive compounds in neem that possess an antiviral ability, and prevent them from causing infection [12]. Neem also inhibits viral transmission by interacting with the surface of the cells to prevent the cell from becoming infected by the virus. Neem has been shown to be effective against several viral diseases in different clinical studies that has demonstrated the antiviral potential [19, 28]. Neem extracts has been established as a phyto-antiviral agents tested for management of chickenpox, shingles, herpes, hepatitis in clinical studies with some progress established by neem's therapeutic bioactive compounds [25]. The colds and flu symptoms 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 been shown to inhibit many strains of pathogenic bacteria, such as *Staphylococcus aureus*. Bacteria of some common source of food poisoning and many pus-forming disorders. These bacteria can cause secondary infections in peritonitis, cystitis, and meningitis [7]. Many bacterial strains are now resistant to penicillin like methicillin resistant *S. aureus* and other antibiotics, one of the major reasons for the widespread transmission of staphylococcal infections in hospitals a public health concern for nosocomial infections [3, 11, 17]. *Salmonella typhosa* [19, 57] is a virulent bacteria, common in food and water sources, which is the causal agent of typhoid, food poisoning, and a variety of infections such as blood poisoning and intestinal inflammation [5]. There have been several clinical studies showing that neem has significant effects on several bacterial strains. Among some of the more prominent strains studied are *S. aureus*, *S. pyogenes*, *Cornebacterium*, *E. coli*, and *S. 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 possibly 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].

Antibiotics in the market have low response to address the treatment of this bacterium [7, 58]. The neem however has demonstrated many drawbacks as an antibacterial agent in the sense that in the other preclinical test, neem showed no antibacterial activity against certain strains of the 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].

ANTIFUNGAL EFFECTS OF NEEM

The favourable warm humid tropical climate creates an optimum growth condition for fungi. In the Ayurveda traditional medicine for example, smoke produce from burning dried neem leaves, aqueous extracts of neem leaf, essential oil from neem seed, neem leaf powder, and

the neem leaf pastes are reported for use in treatment and management of fungal pathogens in India [20]. The ringworm, Athlete's foot, and *Candida albican*, causal agent of cause vaginal yeast infections and thrush, are some of the major skin infection fungi in humans [17, 62-64]. Neem leaf contains two medicinal bioactive compounds called nimbidol and gedunin that has been clinically tested to be efficacious to inhibit some infective fungi [13]. Most fungi of human infection have been treated traditionally in India with neem seed oil application and leaf aqueous extracts [5, 31]. Further modern clinical research of Ayurvedic medicinal plants in South East Asia and India, has shown evidence that neem extracts possess some of the most important antifungal bioactive agents for the inhibition of certain fungi. [4, 15, 65]

Dermatological effects of neem.

Dermatological insects

Neem has been reported for its promising dermatological properties. Based on the insecticidal property's neem is widely used in most part of Asia as a traditional remedy against maggots and head lice [30]. In Haiti, studies have shown that, crushed leaves, decoctions, are rubbed into open wounds and injured cuts [11, 66].

Fungicides

Neem has been shown to have promising antifungal properties against some fungi that infect the human body [17]. Such fungi pose more problems of public health concern and have been difficult to control by synthetic fungicides. In a study, neem preparations showed toxicity to cultures of 14 common fungi, including members of the following genera:

Trichophyton – causing athlete's foot, the fungi that infects hair follicle, skin surfaces, and nails;

Epidermophyton – causing ringworm that infect both the skin and nails of the feet [25, 67];

Microsporum that is a ringworm that infect hair, skin, and to some extend the nails; *Trichosporon* - a fungus of the intestinal tract; *Geotrichum* - a yeast like fungus that causes infections of the bronchi, lungs, and the mucous membranes [3]; and then Candida which is 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]. The extracts of neem leaf, neem oil, seed kernels are effective against certain fungi such as *Trichophyton*, *Epidermophyton*, *Microspor Trichosporon*, *Geotricum* and *Candida* [16, 33, 68].

Eczema:

Clinical studies have shown that the application of low doses of neem leaf extracts has an efficacious effect on acute conditions of eczema [11, 13]. The use of Soap or shampoo containing neem oil reduces the itching and redness of eczema [4]. The bark of the neem tree is used in the treatment of eczema. Reports have shown that combination of about 25 grams each of this bark and the mango bark boiled in about 1 liter of water produce vapour that is effective in treating affected part [3, 69].

Acne.

Neem leaf can effectively treat the bacteria that cause acne and studies have shown that neem can reduce inflammation produced by acne [11].

Dry skin, wrinkles, dandruff, itchy scalp, skin ulcers and warts are other conditions that can be effectively treated using neem 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.

Sap derived from the neem tree has been report to be effective in leprosy treatment in some Central African forest zones where the disease is endemic, and when administered in daily doses of about 60 g [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 in reduced dose a combination of about 12 g of neem leaves and 3 g pepper can be ground mixed in one litre water and taken daily for two weeks [37, 70]. In some cases leprosy and leucoderma treatment requires the application of 10 drops of neem essential oil mixed with 1 teaspoon of sugar powder that acts as a supportive part of treatment and applied twice a day [12, 70].

Malaria: Infusion or decoction extracts of the fresh leaves of neem is a bitter tonic preparation that serve as an alternative treatment of chronic malarial fevers due to curative action on the liver [23]. Recommendation is to take about 15 to 60 g [9]. Consumption of neem tea from leaves every day can potentially reduce the risk of malaria attack from mosquito bites [7, 11]. Water and acetone extracts preparations are more effective than the normal herbal neem tea preparations. **N**eem seed and leaf extracts show efficacy against both choroquin-resistant and sensitive strain malarial parasites [13, 72-73].

Apart from the use of neem against malaria, its traditional role in the treatment of urinary disorders, skin disease, diabetes, fungi infections and viral diseases has been demonstrated [14]. Neem twigs contain antiseptic properties that make its use in dental hygiene very effective, in the local community in India and parts of Africa [55]. In the Sahel countries, neem has been used for wind shields and prevents the spread of the Sahara Desert when applied in alley cropping [32]. In many developing countries the wood is used for building of fence post, poles for house construction, and furniture [6, 19, 21].

Piles: The consumption of 3-6g of the bark 3 or 4 neem fruits administered with water every morning for one week is very effective in piles treatment [33, 73].

Hair Disorder: Washing of hair with decoction neem leaves have been shown to prevent any hair loss. Neem does not only stop hair falling but also help their growth and therefore frequent application of neem oil also destroys insects and bugs in the hair [3, 19, and 30].

Oral Disorder:

Traditional practice of regular brushing of the teeth with neem twig potentially reduce gum diseases, by protected loose teeth, relieves toothache, reduces the bad ordour and protects the mouth from various periodontal infections [3, 19]. A neem twig is highly recommended locally as a very effective toothbrush, with fiber contents for cleaning, its sap content useful as a mouth freshener, a germ-killing dentifrice and increases 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 the teeth is a common practice and keep the gums free of periodontal disease and other oral infection. 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.

In India the healing system of Ayurveda uses the bark of the neem tree to strengthen an individual's resistance to disease [8, 12]. A number of active compounds from neem base on clinical studies have identified several compounds in the neem tree that the effective control and regulation of the immune system functions [11, 17]. There are immunomodulatory

polysaccharide compounds, commonly found on the neem bark that can increase antibody production [3, 15, and 31].

Treatment for AIDS: The National Institutes of Health (NIH) have reported positive results on the use of neem bioactive compounds in the control of AIDS virus in an *in vitro* laboratory condition [9]. The suggested pathway is suggested to be through the neem's immune modulating polysaccharide compounds that may cause increased antibody production [13, 74]. 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: Disease reported in this category includes high blood pressure, blood clotting, high cholesterol, and arrhythmia/rapid heartbeat [7, 21].

Blood Disorders: Including poor circulation, blood poisoning, and kidney problems are known to be managed with neem products [15].

Digestive Disorders: Neem can be used to treat heartburn/indigestion, peptic/duodenal ulcers, gastritis, and hemorrhoids in most communities in India[7, 36].

Sexually Transmitted Diseases: Neems leaves and other parts like the bark can successfully treat gonorrhea, syphilis, chlamydia, genital herpes, genital/vaginal warts, candidacies, and urinary tract infections [3, 25].

Jaundice and Hepatitis: The use of diluted juice of the tender neem leaves mixed with a tea spoon of honey has been used to detoxify toxins in liver disorders [11, 27]. With 2 teaspoon of neem leaves mixed with honey and used regularly can be antipruritic, thermogenic, tonic, stomachic and abdominal movement controller [56, 75].

Birth Control: Neem traditional preparations in India have been shown to be effective for men and women and provide a promising efficacy for birth control in resource poor countries [32, 40]. Studies indicate that neem products can make sperm infertile without affecting sex drive or impeding the sperm count [9, 17, 30].

Respiratory disorder: Decoction of neem bark is effective as an antitussive for dry cough. Dried neem leaves powder when administered 1 g with honey, twice daily, could reduce cough [41, 54]. Neem oil of 5-10 drops given with 2 tablespoon of sugar once a day for 15 days can be effective in the control of tropical eosinophilia [12].

DIABETES AND HYPOGLYCAEMIC ACTIVITY.

Neem is bitter in nature with stomachic and antipruritic properties [25], and very useful in revitalizing patients suffering from metabolic diseases like diabetes [55]. The administration of a table spoon (5 ml) leaves of juice of neem without food, early in the morning for 3 months can control diabetes in patients [55]. Neem leaves chewed or taken daily in powdered form is also effective in diabetes management [25]. The neem leaf in most studies significantly reduce insulin in the blood and this result has led to the authorization of neem sale in India for the treatment of diabetes, and the administration small doses of 1 teaspoon daily for one month can effectively lower insulin levels by 50% in diabetic patients[21, 25, 36, 38].

Other studies on aqueous extract of neem leaves have shown a significant reduction in blood sugar level and the prevention of adrenaline and glucose-induced hyperglycaemia [3, 9]. Hypoglycaemic effect has also been observed with the use of leaf extract and seed oil, in normal and alloxan-induced diabetic rabbit models [45, 57].

ANTICANCER ACTIVITY.

Neem leaf aqueous extract can effectively reduce oral squamous cell carcinoma induced by 7, 12-dimethylbenz[a] anthracene (DMBA) [41], as shown by the reduced incidence of neoplasm [4]. Neem can express its chemopreventive effect in the oral mucosa through the regulation of glutathione and its metabolic enzymes [55]. Neem with its depurative effects can purify the blood and reduces the body heat. This can be achieved by consuming about 10g of freshly harvested neem leaves mix with warm water early in the morning for about one week [36].

Allergy: application of 8 to 10 fresh neem leaves are recommendation to be eaten early morning without food for a month. This can significantly purify the blood and controls the allergic condition [38]. Neem extract can also be used in the management of respiratory conditions like tuberculosis, bronchitis, eye infection like conjunctivitis. Reports on the treatment of allergic conditions, bad breath, hangover, stress, insomnia, smoking has been reported [19, 44].

Antiparasitic properties of neem: Neems extracts show antiparasitic effect on most body parasites [55]. Neem leaves aqueous extract is a recommended standard treatment for external parasitic infestation without any less side as reported in India [7]. The antiparasitic effect of neem is due to the presence of bioactive compounds that mimic hormones and interrupts the reproductive cycle of the parasites by inhibition of the ability of the parasites to feed, and slow down of the hatching process [7, 11, 32, 75]. Antiparasitic effect of neem has shown to

be effective against lice, mites causing scabies [7]. Intestinal worms can be treated and managed in most parts of sub Saharan Africa through regular consumption of neem teas [7]. Neem has both curative and preventative properties on the malaria parasite and the methods for better treatment are as varied as the active ingredients in neem. There are two known bioactive compounds in neem that have been clinically proven to be as effective against the malarial parasite compared to both quinine and chloroquine [7]; these are the gedunin, a limonoid and quercetin, a flavonoid [7, 30]. The leaves are generally consumed by chewing on a daily basis as a preventative like quinine, while neem leaf extracts have been clinically proven to be as effective as chloroquine for the control of the malarial parasite [10]. More studies have shown that neem leaf extracts inhibit 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 curative applications of neem is the antiparasitic role and its effectiveness against encephalitis [70-24].

Antifertility effect

Intra-vaginal application of neem oil, before coitus, can successfully prevent pregnancy [13]. This application has become popular locally in East Asia a promising method of traditional birth control [6, 44, 61]. Report of the administration of the juice extract of neem leaves to a woman in labour produces normal contraction in the uterus and reduces potential inflammation [5, 44, 49]. Adminstration of neem juice controls bowls movements and prevent the onset of fevers and facilitate a normal birth delivery [5]. The use of decoction of neem leaves as a vaginal cleanser treats residual wounds resulting from delivery and can disinfects the vaginal passage [4, 44]. Neem is a very useful phytoinsecticide, and insect repellant, with ability to kill soil nematodes and other plant parasites [27]. Neem juice extract is used locally as toothpastes and contraceptives [44]. Neem leaves consumed on a regular basis every morning on empty stomach for a month has been shown to be beneficial in the control and management of diabetes [19, 56].

Hepatoprotective activity: The aqueous extract of neem leaf is known to be hepatoprotective 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) indicators 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 neem grain germination, which is associated with low levels of lipooxygenase activity and lipid peroxides [58].

Effect of neem on the central nervous system: Studies on fractions of acetone neem leaf extracts *in vivo* has shown significant varying degrees of central nervous system (CNS) depressant activity in mice [59, 60]. Leaf extract of 200 mg/kg body weight has been reported to show significant anxiolytic activity in Wistar rats [24, 61]. The crude ethanolic extracts of stem bark and root bark showed hypotensive, spasmolytic and diuretic activities from in vivo studies in rats [63].

PESTICIDAL APPLICATION

Effects of neem products on insect feeding

Generally insects from different Orders have different behavioural patterns and behavioural responses when exposed to azadirachtin. Studies have shown that Lepidoptera are very sensitive to azadirachtin with antifeedancies ranging from <1-50 ppm, that is species dependent [14, 35]. Hemiptera, Coleoptera, and Homoptera on the other hand show lesser sensitivity to azadirachtin with respect to their behaviour responses, with up to 100% antifeedancy that can be achieved within the range of 100-600 ppm. Some aphid species such as the strawberry aphid also show high behavioral sensitivity with total antifeedancy [60].. The Orthoptera are reported to show a high variation in sensitivity with the *S. gregaria* (a polyphagous species which has their chemoreceptors well-conditioned to many plant bioactive compounds), the *Locusta migratoria* (L.) (a graminaceous species which does not have chemoreceptors conditioned to feeding deterrents), the extreme insensitivity of *Melanoplus sanguinipes* (Fab.), which belong to the North American Plains grasshopper that based on their evolutionary history has never been tested on *A. indica* and no known information has been reported on the chemoreceptors response to azadirachtin [6, 11]. The exhibition of gustatory antifeedancy that relates to the inability to ingest resulting from the perception of the antifeedant at a sensory level has been reported'[44, 76]. Antifeedancy is responsible for crop protection in several species of Lepidoptera and S. *gregaria.* More research have shown that the desert locusts (*S. gregaria*) show high sensitivity to azadirachtin

and show low response feeding 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 generally to protect plants at low doses (2 ppm) . *S. littoralis* (African cotton leafworm), *Spodoptera frugiperda* (fall armyworm), *Heliothis virescens* (F.) (tobacco budworm) and *Helicoverpa armigera* (Hüb.), show some behavioural response to low concentrations of azadirachtin bioactive compound at 0.1 - 10 ppm effectively preventing 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 the five orders indicated above 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 has the potential to blocks the firing of 'sugar' receptor cells, which are link to the normal stimulation of feeding [9, 72].

In most 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 [43]. These "secondary" antifeedant effects causes a reduction in food consumption and digestive efficiency because of ingestion, application or injection of the

antifeedant [19, 31]. The secondary antifeedant effects result from the disturbance of hormonal and other physiological system such as. movement of food through the gut, inhibitions of the production of digestive enzymes, and effects on the stomatogastric nervous system [3, 11, 68]. For example locusts that are injected with azadirachtin, which by-passes the taste receptors, have shown a reduced ingestion of food as shown by faecal pellet production [2, 21]. Hemipteran insects feeding on tobacco seedlings that had been systemically treated with 500 ppm azadirachtin, showed 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, 27]. Also, aphids which had fed on artificial diets containing a reduced dose 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].

The effect of interrupted feeding activity by insects 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 [5, 13, 35]. 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) was not effective to prevent them from becoming infected when viruliferous aphids fed on the plant [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 was not effective to protect seedlings from infections with a non-persistently transmitted potyvirus (potato virus y) from viruliferous aphids [15, 76-78].

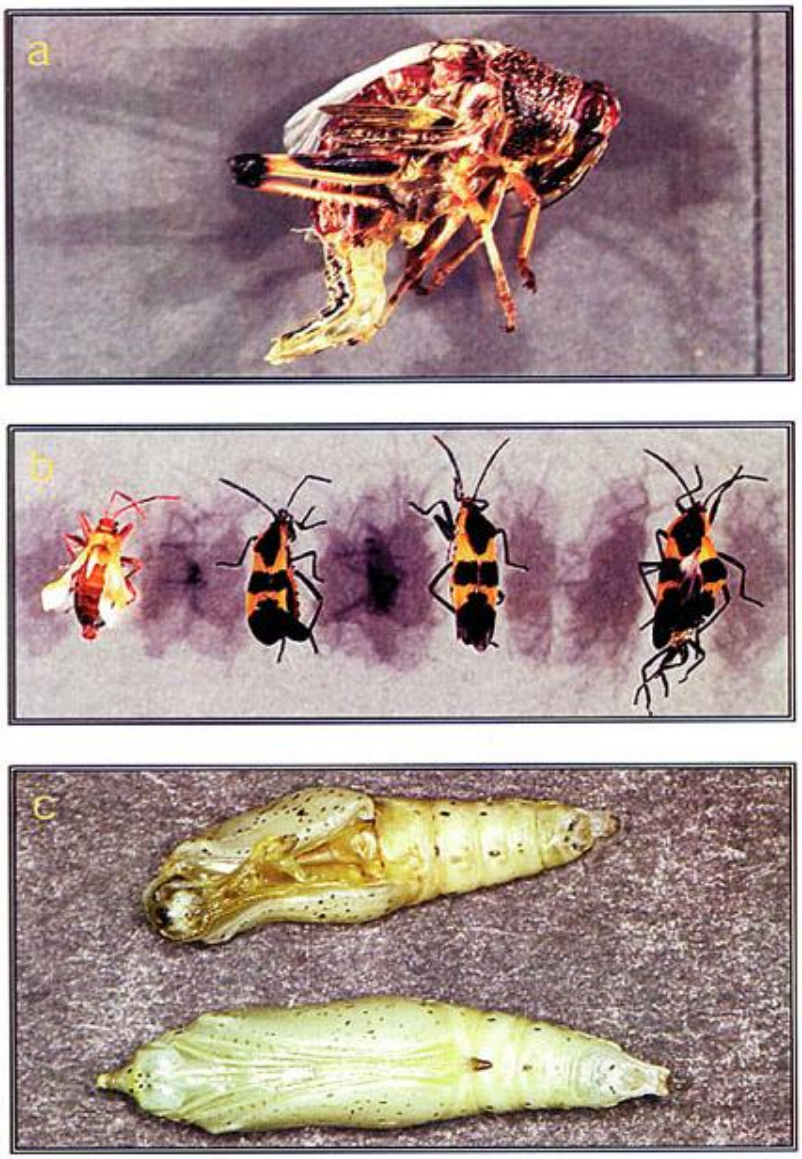

Plate 1. Moulting abnormalities caused by exposure to azadirachtin treatment of (a)-desert locus (*Schistocerca gragaria*) showing death at ecdysis (x1.5); (b)-milkweed bug (*O. fasciatus*) showing moulting defects (x 1.5); (c)-cabbage white butterfly (*P. brassicae*) showing abnormal (top) versus normal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

Neem application as 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 *B. thuringiensis* Bt resistant strains of

Leptinotarsa decemlineata The Colorado potato beetle, with 0.25% Neemix combined with *Bacillus thuringiensis* can act as a resistance breaking compound [16, 55, 67]. In this case depending on the resistance mechanism, the neem effects may be due to the blockage of enzyme production, or due 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 central nervous system (CNS) and the lungs [50]. Neem seed oil produces toxic effect in humans in several isolated cases and this 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 was observed by feeding nimbidin to fasting rabbits. Nimbolide, a major bioactive molecule 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 kidney dysfunction, small intestine and liver followed by significant sudden drop of arterial blood pressure [30, 41, 72].

Neem is a promising, safe and environmental friendly 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 (nephrotoxic and hepatotoxic [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 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).

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