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## Applications and formulations of *Azadirachta indica* A. juss (Neem)-A versatile Indian origin plant

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### ABSTRACT

*Azadirachta indica* A. juss, commonly known as known as Neem, has proven to be a miracle herb since the Vedic era in India. Neem is beneficial for both short term and long term use and its effectiveness has been validated from its utility in the traditional folk medicine. Extensive studies have focused on its biological, pharmacogenetic, pharmacokinetic and cosmetic aspects but complete description of its benefits, usage, safety measures and clinical toxicity still remains to be identified. Neem has been a powerful antimicrobial agent but its other inherent features makes it important as a diuretic, hypoglycemic and hepatologic agent. Its efficacy for cancer treatment is still unexplored. This review highlights the structural features of this wonder plant along with its history, ecology, compositional aspects, its formulations and major applications in therapy and medicine. © 2015 Trade Science Inc. - INDIA

### KEYWORDS

*Azadirachta indica*;  
Neem;  
Azadirachtin;  
Nimbidin;  
Pharmacuetics;  
Cosmocueticals.

### INTRODUCTION

Medicinal plants have been exploited for their valuable properties and pharmacologically important elements for the welfare of mankind. It has almost been an era since Neem has been explicated as one of the most marvelous trees in the world. *Azadirachta indica*, commonly called as Neem, or Indian Lilac belongs to the family Meliaceae. It is autochthonous to India and the Indian subcontinent and is distinctively found in the tropical and semi-tropical zones. One of the two species of the genus *Azadirachta*, is also seen to be growing in southern

Iran<sup>[1]</sup>. The extensive studies carried out with this tree has substantiated its exceptional properties and this makes neem a prime component of cosmoceutical and pharmaceutical industries.

*Azadirachta indica* has now been immensely used for its valuable medicinal attributes for over many decades. This tree and its parts are not only employed as medicaments but also considerably utilized in traditional Hindu vedic medicine system viz., Unani and in the homeopathic system of alternative medicine. Neem substantially has a beneficial effect on the respiratory, cardiovascular, gastrointestinal as well as the reproductive systems and serves

to act as a protective barrier against some major diseases related to these systems<sup>[2]</sup>. Neem tree and its parts -leaf, stem, seed, fruit, flower, bark and root are known for their assemblage of pharmacologically active compounds such as alkaloids, peptides, phenols, flavonoids, tannins, sterols, etc. which have a composite structure and are diverse in their chemistry<sup>[3,4]</sup>. Neem oil has been seen to have potent antibacterial property because of the presence of glycosides that confer protection against antibacterial infections. It participates in the process of wound healing and permits the skin to retain its litheness during this time. Neem oil has the capacity to improve natural immunity as it acts as a crude purifier of various channels in the body and helps in tissue regeneration<sup>[5]</sup>. Varying from golden yellow to a tinge of reddish brown in color, neem oil comprises of mainly triglycerides and abundance of terpenoids that confer an acrid taste.

Azadirachtin, a highly oxidized tetra nortriterpenoid, extensively studied and characterized in neem oil, possesses considerable amount of acetal and hemiacetal groups, tetra substituted ethylene oxide, esters derived from carboxylic acids and a variety of alkenes with an alkoxy substituent<sup>[4]</sup>. Azadirachtin, the structurally complex amalgam, is a member of the limonoid group which is present in the seeds of neem as a secondary metabolite. The molecular structure of this tetranortriterpenoid comprises of a tetrahydrofuran and both secondary and tertiary hydroxyl groups, across sixteen stereogenic centres, of which seven are tetrasubstituted. In addition to this, neem also contains certain steroids such as campesterol, beta-sitosterol, etc. The content of this highly complex compound differs from 300ppm to almost 2600ppm and depends upon the technique of its extraction from the seeds<sup>[6-8]</sup>.

Neem oil is rarely used for cooking and is considered as non-edible, but its substantial use in cosmoceuticals (creams, soaps, hair products) has accentuated its usage in the pharma industry to a great extent<sup>[9]</sup>. The oil has been employed to treat skin diseases, inflammations, rheumatic disorders, pyogenic infections, fever, malaria, ophthalmitis, tuberculosis and also as a larvicide and insect repellent<sup>[10]</sup>. Biswas *et al* has elaborated on the bioactivity of

neem compounds, the pharmacology of neem extracts and its usage in medicine along with assessment of its safety<sup>[11]</sup>.

### DESCRIPTION, ECOLOGY AND HISTORY OF NEEM

Neem encompasses two close associated species *viz.*, *Melia azedarach* and *Azadirachta indica* A.Juss., otherwise known as Indian neem, a tree of the mahogany family Meliaceae. It grows at a rapid rate and can extend up to 60 ft and seldom upto 130 ft in height and is resistant to drought. It thrives best in humid conditions with considerable amount of rainfall and soil, with about 85-90% of sand content. This evergreen tree can sustain high temperatures and usually grows with ease in sub-tropical regions. Neem has been immensely used in Ayurvedic treatment and traditional folklore medication and every part of the tree has been utilized in the diagnosis and treatment against many disorders from ancient times. Neem derives its name from the word 'Arishtha' and is considered as the 'Friend and protector of the villager' in India. Vedic literature contemplates neem as "Sarvaroganirvarini" - that restores the normal functioning of the body curing all disorders. Neem is a powerful antibacterial, antifungal, anti-parasitic agent and is favorable for the treatment of many infections<sup>[10,12]</sup>.

### COMPOSITION

Extensive research and examination has depicted much about the chemical composition and structural diversification of neem. Nimbin was the first crude compound segregated from neem oil and reported. Since 1942, innumerable compounds have been identified and reported<sup>[12,13]</sup>. Studies have unveiled that the compounds belong to the major classes of di and tri-terpenoids including limonoids, protomeliacins and its derivatives, C-secomeliacins such as salanin, nimbin, azaridirectin and nonisoprenoid derivatives such as polysaccharides, macromolecules with amino acid residues, polyhydroxyphenols like flavonoids, benzopyrone, tannins, phenethyl phenyl ketone, straight chain hydrocarbons, organosulphur

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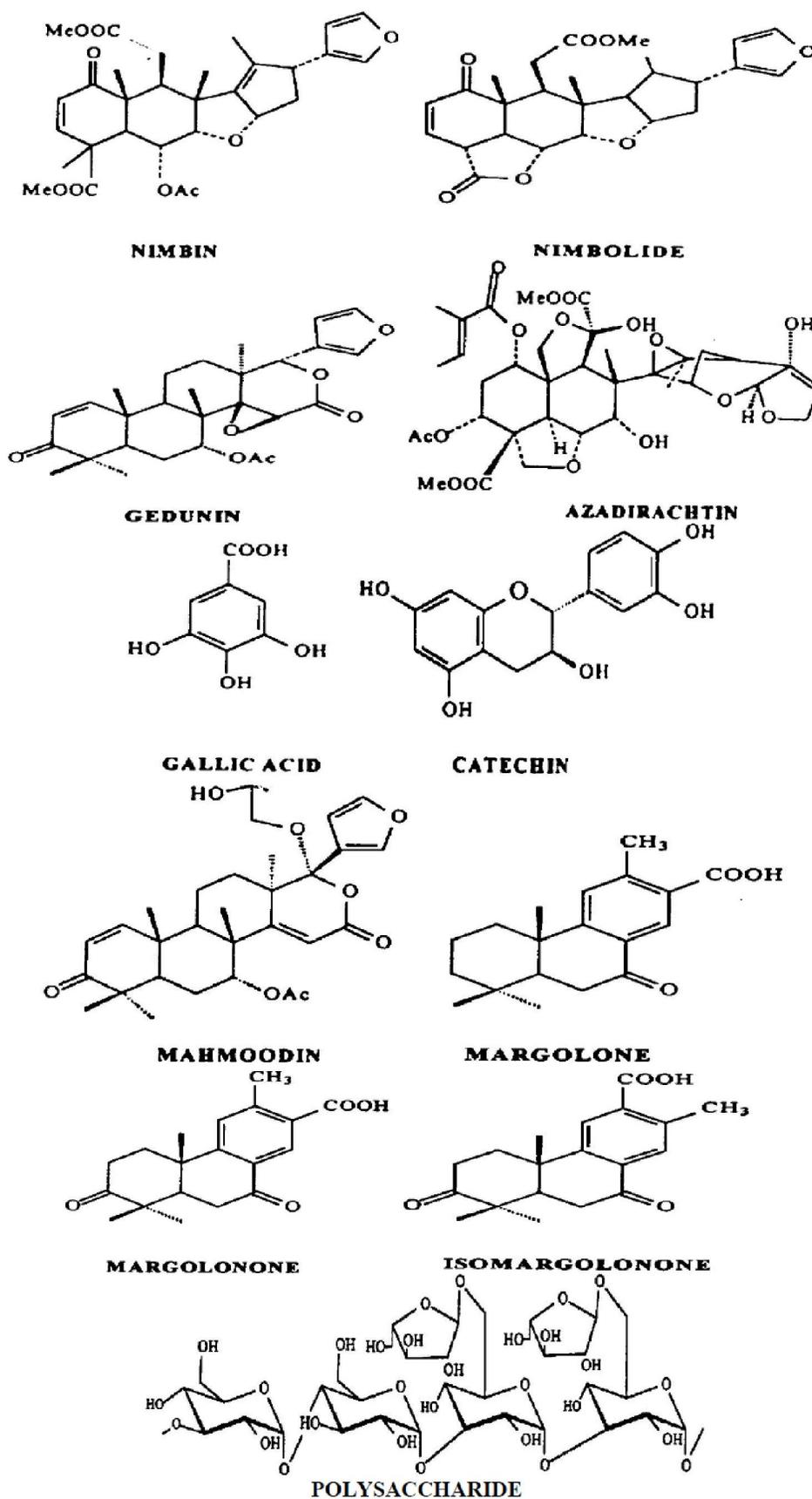


Figure 1 : Structure of various bioactive compounds in neem<sup>[11]</sup>

compounds, etc. There are previous reports that describe the details of these compounds and their biological activities<sup>[13-17]</sup>. The structure of the compounds have been depicted in Figure 1.

Neem oil also comprises some of the extremely essential biologically active components, nimbidin, nimbin, nimbolide, sodium nimbidate, gedunin<sup>[18-23]</sup> and a variety of polysaccharides which exhibit strong antibacterial, antifungal, antimalarial, spermicidal, anti-inflammatory, antipyretic, hypoglycaemic and diuretic properties. Gallic acid and catechins<sup>[24-28]</sup>, which have potent immunomodulatory functions, are obtained from the bark of the tree. Margolone, its derivatives and cyclic sulphides, also a part of neem, is readily utilized in bacterial and fungal infections and inflammations<sup>[29,30]</sup>. In addition, neem oil has ample amount of fatty acids including oleic, linoleic, stearic, palmitic, arachidic behenic, lignoceric and palmiticoleic acids, that provide effective treatment for impaired skin and hair and hence emerged as a primary component of cosmetics<sup>[31,32]</sup>.

### BIOACTIVITY OF VARIOUS COMPOUNDS OF NEEM

Among the components discovered, a few of them have been substantially studied and many reviews have manifested their effect on the living systems. As discussed earlier, one of the chief constituent, *viz.*, nimbidin, and its tetraterpenoids (nimbin, nimbinin, nimbolide, etc.) have shown remarkable concentration dependent anti-inflammatory activity in rats that developed paw edema induced by carrageenan, kaolin and bradykinin and arthritis induced by formalin<sup>[18,19]</sup>. Nimbidin, administered through oral route, showed abnormally diminished levels of glucose in blood of rabbits abstained with food. Antipyretic<sup>[23]</sup> and antiulcer effects have been noted and validated for nimbidin. It has been observed that this compound can prevent the formation of histamine induced ulcers in duodenum, and also salicylate, 5-hydroxytryptamine or indomethacin induced lesions in the gastrointestinal tract<sup>[22,23]</sup>. Nimbidin obstructs the histamine H<sub>2</sub> receptors and tends to repress the basilar as well as carbamylcholine triggered gastric acid output (GAO)<sup>[33]</sup>. The *in-vitro*

studies carried out have shown to entirely retard the growth of *Trichophyton rubrum* and *Mycobacterium tuberculosis*<sup>[24]</sup>.

Nimbolide can be manifested as an antimalarial agent for its effective inhibition of the malarial parasite *Plasmodium falciparum* in the erythrocytic stage of malarial infection and also restrains the growth of *Staphylococcal* species primarily *S. coagulase*<sup>[26-28]</sup>. Recent reports by Gupta *et al.* has illustrated that this limonoid triterpene has a constant effect and impedes the abnormal multiplication. It also instigates programmed cell death and prevents modulation and activation of Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) protein complex, thus contributing to tumorigenesis in cancer cells of colon or rectum by the chronological repression of IκB kinase (IKK) initiation followed by addition of phosphate group to IκBα (nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha). IκBα are cellular peptides which block the NFκB transcription factor by concealing certain amino acid signals called the NLS (nuclear localization signals) (which tags a protein for the entry into the cell nucleus) that remain in the cytosol, inactivated. This finally leads to the displacement of NFκB p65 subunit proteins<sup>[34]</sup>. Nimbolide, upon administration into the peritoneal cavity of mice that are inoculated with tumor, notably minimizes the size of xenotransplants of the cancer cells of colon. These xenografts also show a decrease in the manifestation of antiapoptotic proteins (Bcl-2, Bcl-xL) and myeloid cell differentiation protein (Mcl-1), metastatic and angiogenic factors *in vivo*. Considerable amounts of the triterpenoids have been isolated from the plasma and tumor of the mice treated with the compound<sup>[11,32,34]</sup>.

Neem oil also has large amounts of other triterpenoids - gedunine, nimbin, deacetylnimbin, azadirachtin and salanin, which are highly oxygen enriched components, and deoxygedunine, 17-hydroxyazadiradione, azadirachtol, azadirone, etc that have powerful anti-bacterial, antifungal, feedant and malarial action. Gedunin is effective against *Culex quinquefasciatus*, causal parasite for dengue, filariasis and *Ostrinia nubilalis*<sup>[15,29]</sup>. Mahmoodin or deoxygedunin, a comparatively new

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tetranotriterpenoid, isolated from neem seed oil, is actively effective against human pathogens and readily hinders the growth of many gram-positive and gram-negative microbes<sup>[16]</sup>. Trihydroxybenzoic acid, gallo catechol and catechol, extracted from the bark of the tree, chiefly retards the rapid release of reactive oxygen species such as superoxide radicals and hydrogen peroxide during the process of inflammation of certain granulocytes, thus stopping the chemiluminescent behavior of these granulocytes<sup>[35]</sup>. Furthermore, the species of *Klebsiella* and *Serratia* are affected by two diterpenoids, viz., margolone and margolonone, present in the stems of neem, and the growth of *Trichophyton* species, causative agent for zoonotic diseases in humans and animals, was hindered by cyclic sulphides extracted from the leaves. Certain polysaccharides are identified to have potential antiulcer activity during stress and are capable of reducing the complement activation<sup>[31]</sup>. Neem oil acts as a hydrophobe and thus requires a suitable surfactant for its emulsification and dispersion in water.

### APPLICATIONS OF NEEM

#### Traditional aspects

The advantages of Ayurveda and its extensive usage over a period of over one hundred years has now become a crucial element in allopathic system of medicine. This ancient vedic medicine has demonstrated its own modus operandi and these methodologies form a part of the modern medicine. Ayurveda, Unani and indigenous medicinal practices have highlighted the benefits of neem to a considerable extent. Neem seed oil as well as other parts of the tree are effective in therapeutics but most reports have focused on medicinal properties of leaves and bark<sup>[36]</sup>. The oil, along with leaf extricates has been beneficial in treating Hansen's disease, helminth infection, Chronic Obstructive Pulmonary Disease (COPD), dyschezia, blood infections, bile disorders and various skin ailments like lesions, ulcers blisters, rashes, scabies, skin irritation, mycobacterial cervical lymphadenitis and non-chronic infections by *Streptococcus* sp. The traditional manifes-

tations also explain the use of neem as an herb that stimulates the blood flow in pelvis area and uterus. Neem sometimes may be termed as an "emmenagog" that prevents oligomenorrhea and it also acts as a barrier for fertility control. Thus neem extricates have been suggested for oral, genital and topical usage. Neem is also a potent insecticide, antifebrile agent, pediculocide and antiseptic agent<sup>[37-39]</sup>. A mixture of various oils along with neem can be used to cut down number of pests during farming and in the control of must and rust fungi, anthracnose and other fungal diseases in plants.

#### Pharmacological and medicinal utilities

##### Nature's NSAID

The powerful action of neem to reduce and inhibit inflammation, swelling, febrifuge and pain precisely fits it into the role of commercially available non steroidal anti-inflammatory drug(NSAID) without any undesirable side effects. Neem bark extract is observed to cure inflammation of mucous membrane of mouth and lips in infants<sup>[40]</sup>. Substantial decrease of fever in male rabbits has been observed when treated with extract of neem leaves prepared in methyl alcohol<sup>[42]</sup>. Acute paw edema in rat and swelling of ear in mice is reduced to a considerable extent on application of bark extract of neem in trichloromethane. Studies of neem being a prime analgesic in laboratory mice has been evident<sup>[24,40-42]</sup>.

##### As stimulators of immune system

Recent reports have stated that a water based preparation of neem interferes with the complement system stimulation and mobilization in the blood of humans<sup>[35]</sup> and intensifies the response of the albino (laboratory-bred strain of mouse to SRBC (sheep erythrocytes)<sup>[44,45]</sup>. Neem affects both antibody-mediated beta cellular immune system and cellular immunity, thus demonstrating its stimulating effect on the immune system and increased response to mitogens and antigens. Neem, on oral dispersion, could possibly increase the levels of immunoglobulins G and M<sup>[43,46,47]</sup>.

##### Diminish excess glucose level in blood

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Neem in water formulations are efficacious in diminishing the abnormal sugar level in blood by arresting the degree of production of epinephrine and rise in quantity of dextrose in blood<sup>[48]</sup>. The formulation indicates decreased blood sugar in rats that were induced with diabetes mellitus *in vivo* but maintenance as a normal level of glucose in blood of normal rats fed with the leaf extract, depicting that neem keeps a check on the blood glucose level in the body which is mainly due to the presence of certain flavonoids and polysaccharides<sup>[49]</sup>.

### Intercepts ulceration

The presence some of phenolic glycosides, which is a part of neem, averts the degradation of mucus membrane and release of cytotoxic granules from the labrocytes, thus arresting ulceration in rats<sup>[50]</sup>. This bioactive component is responsible for minimizing the acid secretion and development of abscesses in ulcer induced mice.

### Fecundity control

*In vitro* studies have determined appreciable reduction in the sperm count in *Rhesus macaque* and humans. Administration of neem oil prior to copulation on the inner perineum can arrest or delay the gestation period. Much of the investigations on animal models have delineated that neem seed oil, given through the oral route, could induce antifertility in mice and repeal gestation in *Macaca radiata* and *Papio* species<sup>[51-54]</sup>. Neem oil has lesser side effects as compared to steroids due to its initiation of cellular immunity, rather than directly altering the hormonal balance. The spermicidal effect of neem oil is dose dependent and it can restrain contraception in mammals of the order Rodentia, upto a high concentration<sup>[55]</sup>.

### Microbiostatic agents

The development of resistant protozoan parasite *Plasmodium falciparum* is arrested during its non-dividing and dividing stages, on application of a mixture of neem oil and alcohol, evincing the anti-parasitic activity of the plant. Their potent effect on malarial parasites have also been reviewed<sup>[56,57]</sup>. Pathogenic fungi, including species of *Trichophyton*, *Epidermophyton*, *Microsporium*, *Trichosporon*,

*Geotricum* and *Candida* are susceptible to neem extricates from leaves, roots and kernels. Neem oil prevents mycoses by targeting the fungi<sup>[43,58]</sup>.

Oil from the plant is a dominant bactericidal agent as growth of most of the gram positive and negative bacteria, viz., *Mycobacterium tuberculosis*, *Klebsiella pneumoniae* and *Streptococcus* species including *S. pyrogenes*, *S. faecalis* and *S. mutans*, and *Vibrio cholera* are retarded by it<sup>[12,14,17]</sup>. It not only has bacteriostatic and fungistatic action, but formulations of neem restrict the growth of viruses as well. Antiviral activity of leaves against Vaccinia virus, Chikungunya and Rubeola virus has been reported<sup>[59]</sup>. Neem with ethyl alcohol hinders plaque formation of Coxsackie virus by attacking its preliminary replication stages<sup>[60]</sup>.

### Carcinopreventive agent

Neem is validated to have a diminished effect on squamous cell cancer incited by 1,4-Dimethyl-2,3-benzophenanthrene, by reducing the occurrence of tumors. Neem oil is seen to regulate and modify  $\gamma$ -L-Glutamyl-L-cysteinylglycine and various other selective catalytic protein molecules exerting a chemopreventive action. Not to overlook the antioxidant activity neem possesses, its extracts have been seen to reduce abnormal distress in the body and conserve a balance between the production of reactive oxygen species (free radicals) and antioxidant defenses that is obstructed by carcinogenic chemicals like 1-methyl-3-nitro-1-nitrosoguanidine. The mechanism includes the abstinence of toxic enzymes from the gut and 'hepar' which is the vital target for this cancer causing chemical<sup>[61,62]</sup>. Antioxidant capacity of neem is mainly due suppression in the rate of activity of the enzymes that catalyse the dioxygenation of polyunsaturated fatty acids in lipids.

### Denouement on brain and spinal cord

Neurotransmission levels are greatly lowered by acetone-neem mixtures that is used to diminish the arousal in different parts of the brain. Neem shows effects like dystaxia, anxiolysis, sedation, memory impairment, drowsiness, decreased muscle tone and hypertension or heart pulse, thus relaxing the neural

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networks of brain and spinal cord. In inclusion, neem root and stem relieves spasm of smooth muscles and induces water pills<sup>[63-65]</sup>.

### Consequence on epidermis

Cosmetological aspects are extremely important in the present era as recent trends are focusing more on the utility of natural products as cosmetics without substantial harmful effects. This limonoid terpene not only shows outstanding applications in therapeutics but also has lot of other applications in the global cosmetic market. Systematic implementation of neem in the form of oil or extracts, assists in clearing acne, pimples, skin pigmentation, blackheads and whiteheads. Neem helps to maintain a balance of moisture and manages excess of oil thus preventing drying of skin. It is a vital anti-ageing ingredient of the commercially available skin creams. Neem has some stimulatory constituents, that have a direct effect on the immune system and regenerates the skin and makes it supple by detoxifying the toxins from blood and deprecating periorbital dark blemishes. The renewal properties of neem is responsible for its beneficiary effects on reduction of hairfall, upregulation of blood circulation in scalp, stimulation of hair growth and naturally conditioning dry and frizzy hair follicles<sup>[66]</sup>.

### CONCLUSION

Neem, with its extensive medicinal, therapeutic, pharmacocosmetic and industrial applications is a very versatile herbal plant. Novel formulations and preparations with neem are an area of intensive research nationally and internationally. For the last decade, the focus has entirely been on the discovery of its new elements, chemical nature, structural multiplexicity and its applications Contemporary perspective of drug discovery, using this herb, has created a buzz globally. However, more intensive studies on its bioactivity and pharmacological aspects is warranted and hence initiation of focused research in this area would go a long way to exploit this herb much more fruitfully. More versatile products and applications would be a natural consequence of this research.

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