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MULTI-PHARMACOLOGICAL ACTIVITIES OF NEEM FLOWER

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ABSTRACT

Neem is a tree in the mahogany family Meliaceae. It is one of two species in the genus Azadirachta, and is native to India and Burma, growing in tropical and semi-tropical regions. Neem flower is considered as an antiseptic that can cleanse your system. It is also known to cure skin impurities when applied on the skin. Young flowers of neem plant are commonly consumed as a bitter tonic vegetable. The flowers are also used for treatment of fever. This review mainly focuses on the various pharmacological activities associated with neem flower. The potential of neem flower is explored through this article.

INTRODUCTION

Neem is scientifically named as *Azadirachta indica*, the Indian lilac. It belongs to Meliaceae family.[1] Neem is belived to cure sickness and it is a rapid growing tree which can reach to a height of 15-20 mt. and rarely to 35-40 mt. It was evergreen, but in severe drought it may shed most or nearly all of its leaves. The branches of the tree are wide and spreading.The fairly dense crown is roundish and may reach a diameter of 20–25 meters (66–82 ft). [2] The neem tree has very similarities with the Chinaberry (*Melia azedarach*). The (white and fragrant) flowers are arranged in more-or-less drooping axillary panicles which are up to 25 centimeters (9.8 in) long. The neem branch having up to 250 to 300 flowers. The flowers are 5–6 millimeters (0.20–0.24 in) long and 8–11 millimeters (0.31–0.43 in) wide.[3] Bisexual flowers and male flowers are existing on the same individual tree. Every part of neem tree has bitter taste and a medicinal value either directly or indirectly.For e.g. the fruits which are bitter in taste have oval to roundish shape and the size varies from 1.4-2.8 cm.[4-6]The fruit skin (exocarp) was thin and the bitter-sweet pulp (mesocarp) are yellowish-white and very fibrous. The mesocarp is 0.3–0.5 centimeters (0.12–0.20 in) thick. The endocarp i.e inner shell encloses one/two/three seeds which are brown in color. [7-9] The neem tree was often confused with a similar looking tree called bakain. Bakain is also having toothed leaflets and similar looking fruit. Only one differencethat neem leaves are pinnate but bakain leaves are twice- and thrice-pinnate. The neem trees are noted for its drought resistance.[10-12] Normally it thrives in areas with sub-arid to sub-humid conditions, with an annual rainfall of 400–1,200 millimeters (16–47 in). It can grow in regions with an annual

rainfall below 400 mm, but in such cases it depends largely on ground water levels. Neem trees can grow in different types of soil, but it thrives best on well drained deep and sandy soils. It was typical tropical to subtropical tree and exists at annual mean temperatures of 21–32 °C (70–90 °F). It can tolerate high to very high temperatures and does not tolerate temperature below 4 °C (39 °F). [13]Dried neem leaves have a specific use in India that, they are used to prevent worms in rice and insects in clothes. Neem flowers are used along with other natural ingredients like tamarind, jaggery to give protection against the forthcoming 7seasonal changes in Indian festivals like Ugadi. In ayurvedic herb, neem was also used in baths. Neem products areusedby Siddha and Ayurvedic practitioners tobe anthelmintic, antifungal, antidiabetic, anti bacterial, contraceptive, and sedative.[14, 15] It is considered a major component in Siddha medicine and Ayurvedic and Unani medicine and is particularly prescribed for skin diseases. Neem oil was also used for healthy hair, to improve liver function, detoxify the blood, and balance blood sugar levels.Neem leaves used to treat skin diseases like eczema, psoriasis, etc.[16, 17]In adults, short-term use of neem was safe, while long-term use may harm the kidneys or liver; in small children, neem oil is toxic and can lead to death. Neem can also cause side effects like miscarriages, infertility, and low blood sugar.[18]

EXTRACTION METHODS OF NEEM FLOWERS

Maceration: To macerate the Siamese neem flowers, their powder was taken with 50% ethanol (1:20, w/v) at room temperature and kept for four days, then filtered through whatman (No.1) filter paper. Other portions of the solvent are added to the marc and the extraction is repeated until the last extract was colorless. The extracts are combined and concentrated under decreased pressure at 45^{0}C using a rotary vacuum evaporator. The crude extract is then evaporated on a boiling water bath until a constant weight is obtained to afford the maceration extract.

Percolation: The powder of Siamese neem young flowers is percolated with 50% ethanol (1:20, w/v) at room temperature (flow rate 1 ml/min). Other portions of the solvent are added and the extraction is repeated until the last extract is colorless. The combined extract was filtered and the filtrate was concentrated and evaporated under the same condition as described before to afford the percolation extract.

Soxhlet extraction: The powder of Siamese neem young flowers is extracted with 50% ethanol using a Soxhlet apparatus (60- 80^{0}C; 1:50, w/v) until the last extract is colorless. The combined extract was filtered and the filtrate was concentrated and evaporated under the same condition as described before to afford the Soxhlet extract.

Ultrasonic extraction (UE): The powder of Siamese neem young flowers is separately extracted by sonication with 50% ethanol and distilled water (1:20, w/v) for 30 min each and then filtered. Other portions of the solvent are added to the marc at the same portion and the extraction is repeated until the last extract is colorless. The combined extract was filtered and the filtrate was concentrated and evaporated under the same condition as described before to afford the 50% and aqueous ultrasonic extracts.

Decoction: The powder of Siamese neem young flower is extracted by boiling with distilled water (1:20, w/v) for 6 hrs and then filtered. Other portions of the distilled water are added to the marc and the extraction is repeated until the last extract is colorless. The combined extract was filtered and the filtrate was evaporated on a boiling water bath until constant weight was obtained to afford the decoction extract.

Microwave assisted extraction (MA): A commercial household microwave oven (Panasonic Model NN-MX21WF) was used for extraction. The microwave oven was operated at 2450 Hz single-phase output of 800 W. A portion of the sample (10.0 g) is placed in a 600 ml flask, followed by the addition of 200 ml of distilled water. The irradiation cycle was as following: 3 min preheated (temperature 70^{0}C), 1 min power-on, followed by 2 min power-off to hold the temperature at 70-85^{0}C,

the extraction process was then repeated for 8 cycles. After finishing the extraction, the flask is allowed to cool to room temperature, then the mixture is filtered through a whatman no. 1 filter paper. Other portions of the solvent are added to the marc and the extraction is repeated until the last extract is colorless. The combined extract was then evaporated on a boiling water bath until a constant weight was obtained to afford the microwave extract.

Thin-layer chromatographic fingerprints: The extract of Siamese neem flower prepared by each extraction method was analyzed using thin-layer chromatography (TLC). The TLC is performed on TLC precoated silica gel G60 F254 plate 10×10 cm (Merck, Germany) using a homogenous solvent system comprising ethyl acetate: dichloromethane: formic acid: acetic acid: water at 100: 25: 10:10:11 as the mobile phase. The TLC plate is detected by using a CAMAG viewing box UV detector (CAMAG, Muttenz, Switzerland) and a natural product polyethylene glycol (NP-PEG) spraying reagent under UV 366 nm is used for detecting the flavonoids.[19]

BIOLOGICAL ACTIVITIES OF NEEM FLOWER: Suppresses cholesterol absorption by interfering NPC1L1 and intestinal Caco-2 cells. The flower extract of *A. indica* decreases intestinal cholesterol absorption, meddles with micellar cholesterol formation and thus weakens cholesterol synthesis. There is a reported reduction in HMG-CoA reductase activity, hence accumulation of intracellular cholesterol is low, and this leads to the ultimate increase in cytosolic NPC1L1 protein expression and decrease in PPARδ gene expression.*In vitro* cholesterol analysis is carried out by cholesterol micelle formation. This formation also used for the evaluation of micelle size and solubility, and transport of cholesterol into the Caco-2 cells. western blot and real time polymerase chain reaction are used for the determination of expression of niemann-pick C1 like 1 (NPC1L1), and its major regulator, peroxisome proliferator-activated receptor δ (PPARδ), respectively. *A. Indica* flower extract reduces pancreatic cholesterol esterase activity and increased cholesterol micelles size. Uptake of cholesterol into Caco-2 cells is inhibited by *A. indica* flower extract in a dose-dependent manner. As such, *A. indica* flower extract have potential for developing into nutraceutical product for prevention of hypocholesterolemia. Siamese neem (*Azadirachta indica A. Juss var.)* (*A. indica*) leaf extract, is also a traditional ayurvedic medicine, used as antipyretic, antibacterial, antidyslipidemic, and antihyperglycemia effects. [20]

Antioxidant activity and different compositions in Neem flower powder: Neem (*Azadirachta indica*) Flower Powder (NFP) is evaluated for composition, fatty acid profile, volatile oil composition and antioxidant activity. NFP is rich in protein (17.34%), fiber (12.32%) and ash (9.16%). The volatile oil (0.07%) are contains 85% of caryophyllene. The lipid yield of NFP was 12%, which was composed of equal quantities (45 ± 1%) of saturated (SFA) and poly unsaturated fatty acids (PUFA). In SFA, the palmitic (31.76%) is major, in PUFA, linoleic (18.57%), linolenic (12.64%) and oleic (9.74%) are higher. Arachidonic (20:4) and docosatrienoic acids (22:3) are also present to an extent of 7.38% and 5.7%, respectively in the total lipid. NFP hasshowed 25.31% radical inhibition with 0.6 mg and 58.50% at 2 mg comparable iron reducing power. The results reveal that neem flower volatile fraction and lipid can also be used for pharmaceutical or food applications.[21] Among the hydro distilled extracts of leaf, heart wood and flowers of *Azadirachta indica Juss*., the highest free radical scavenging activity of 70.66±0.78 % was obtained with the leaf hydro distilled extract at 500 µg/ml. The Neem heart wood has the highest amount of total phenol content (160 µg/ml), which was responsible for highest percentage of inhibition of DPPH radical. Neem leaf, flower, and heart wood have potential for use in human health which is used as food by common people and in diabetes. Different extracts of Neem were widely used for variety of diseases and also antioxidant potential for use in different pharmaceutical industries. [22]

Figure. 1: Neem tree (Adapted from Ferry Zievinger)

Figure. 2 Neem fruits, leaves, and flowers (Esterlingsindia.com)

Figure. 3Neem flower (pinterest.com)

Taxonomy		Vernacular Names	
Kingdom	Plantae	English	Margosa
Division	Magnoliophyte	Hindi	Neem
Class	Magnolipsida	Sanskrit	Arishta, Nimbah
Order	Sapindales	Telugu	Vaepa, Vemu
Family	Meliaceae	Kannada	Baalanthi Baevu
Genus	*Azadirachta*	Malayalam	Aria-Bepou
Species	*Indica*	Marathi	Balantanimba
Scientific Name	*Azadirachta Indica*	Tamil	Acutakimaram

Table. 1 Neem taxonomy

Toxicity Testing of neem flowers: Flowers of neem (*Azadirachta indica A. Juss*) possess a strong cancer chemo preventive potential in rats as well as anti-micronucleus formation in mice. Acute toxicity was conducted by feeding methanol extract of neem flowers (MENF) suspending in 20% propylene glycol at 6, 9 and 12 g/kg body weight(bw) in rats. For 90 days, continuously the subacute toxicity testing is also carried out by feeding MENF suspending in 0.5% tragacanth at 150, 750 and 1,500 mg/kg bw. Hematology, blood chemistry and histopathology were evaluated at the end of the experiment. The results shown that MENF had LD50 value higher than 12 g/kg bw. In subacute toxicity study, there is a marked increase in the relativeliver weights of those receiving MENF at 750 and 1,500 mg/kg bw, but MENFhas caused a substantial decrease in the growth of male, but not female rats. Blood chemistry values of most rats are within normal ranges. However, ALP (alkaline phosphatase), creatinine and potassium values were significantly higher in female group receiving MENF at 750 mg/kg bw while in male rats, the levels of AST (aspartate aminotransferase) and

BUN (blood urea nitrogen) were lower, but that of creatinine was higher than those of the control groups. Histopathological examination of visceral organs showed no significant change. In conclusion, LD50 value of MENF in rats was greater than 12 g/kg bw (~ 800 times of human use). Subacute toxicity testing at 750 and 1,500 mg/kg bw (~ 50 and 100 times of human use) showed the effects on some biochemical parameters.[23]

High free radical scavenging activity of Siamese neem flowers: Siamese neem tree (*Azadirachta indica A. Juss.)* is a medicinal plant found in Thailand. Young leaves and young flowers of this plant are commonly consumed as a bitter vegetable tonic. The flowersused to treat the fever. The flower extract has reported to exhibit *in vitro* free radical scavenging activity and can reduce lipid peroxidation of bronchogenic cancer cell line. Active compounds in the flowersare flavonoids such as rutin and quercetin. The content of these compounds in the crude extract depends on the method of extraction. Therefore, the appropriate extraction method promoting high yield of total flavonoids and high free radical scavenging activity was investigated in this study. The methods of extraction of young flowers are of many types and few of them were used here like, percolation, Soxhlet extraction, maceration, microwave assisted extraction (MA), decoction and ultrasonic extraction (UE) The solvent used for maceration, percolation, and Soxhlet extraction is 50% ethanol, while distilled water is used for decoction and MA, and both solvents are used for UE. The content of crude extract, free radical scavenging activity, and total flavonoids content of each extract are investigated and compared. Through the decoction extraction method, an extract witha good number of total flavanoids (17.54 mgRE/g extract) was obtained and also maximum scavenging activity produced at EC50 11.36 μg/ml. Decoction is also simple, cheap, and convenient and could be used in developing countries. These extraction methods of Siamese neem tree flowersare used in development of antioxidant pharmaceutical preparations.[19]

***A.Indica* flowers on specific and non-specific immune response:** Neem flower is traditionally used as tonic and stomachic. Neem leaf, stem and seed oil have been proved to have immunomodulatory activity along with the adverse effect on sperm count. The extract is also examined for the ability to induce secretory and cellular responses in murine peritoneal macrophages *in vitro*. The extract is increasing the production of super oxide ($O2^-$) and nitric oxide (NO) in murine peritoneal macrophages. When macrophages are treated with the extract, the results showed that reactive oxygen intermediates (ROI), reactive nitrogen intermediates (RNI) are likely major mediators of cytotoxic activity. The extractincreases the phagocytic index which indicates activation of reticuloendothelial system through release of the mediators. The extract has other additional benefits of protection from the cyclophosphamide induced myelosuppression in dose dependent manner and also it showed a rise in antibody titer value against ovalbumin. Increase in cell mediated immunity by increasing paw edema against ovalbumin showed significant effect of the extract on stimulation of T cell at the dose 400 mg/kg. It shows that the extract holds promise as immunomodulatory agent, which acts by stimulating both specific (humoral and cell mediated immunity) and non-specific immune response (cytotoxic and phagocytic activity of macrophages).[24]

Neem bark and flower effect on streptozotocin – induced diabetes in mice: 3-6 hours after the administration of extracts of neem bark or flower, the blood glucose levels of normal and streptozotocin-induced diabetic mice dropped. However, neem flower possesses efficient hypoglycemic activity than the bark. Hypoglycemic action of these extracts may be due to their metabolic effect on tissue and or due to increase in insulin secretion.[25]

Phytochemical screening and Antibacterial activity of Neem flowerand Production of Homemade Soap: The different parts of neem tree contain various active compounds which are rich in antibacterial activity. Various bioactive compounds like alkaloids, flavonoids, coumarin, leucoanthocyanin etc., were present in aqueous and acetone extract. A true soap needs to cleanse the body properly without disturbing the pH level of skin. The preparation

of homemade neem flower soap, neem flowers are rich in cholesterol so the soap prepared by these neem flower contains sufficient fat content when compared to the soaps prepared by other neem components (leaf, flower, unripened and ripened fruit and seed). Flowers of neem plant contain antibacterial activity, so preparing the soap using neem components destroys the microorganism which keeps our skin safe and healthy. So, the homemade neem soap can be replaced with other synthetic soaps for better results.[26]

Quality assessment of Siamese neem flower extract: Flowers of Siamese neem tree (*Azadirachta Indica A. Juss*.) is commonly consumed as a bitter tonic vegetable. Active antioxidant components in the flowers are rutin and quercetin flavonoids. The quantitative assessment of rutin and quercetin was carried out by using high-performance liquid chromatography. For this purpose, young neem floweres were collected from 14 different places in Thailand. They are determined for the loss on drying, heavy metals and pesticide residues, microbial contamination, solubility, chromatographic fingerprints and acute toxicity. The extracts having rutin and quercetin in the ranges from 388 to 1178 mg% dry weight (average 772 mg%), and 1 to 10 mg% dry weight (average 5 mg%), respectively. EC50 of the scavenging activity of all extracts is found in the range of 27–133 mg /mL. Loss on drying of the extracts was less than 7% w/w and no sign of toxicity y (LD_{50}> 5 g kg^{-1}) was found. **[27]**

Effects of Neem Flowers &*In Vitro* Metabolic Activation of Chemical Carcinogens in Rats: The effect of neem flowers in combination was also studied in Thailand. In this study among the commonly consumed varieties of Thai, four types were taken i.e flowers of neem tree (*Azadirachta indica* var. siamensis), one of Thai fruits and the Chinese bitter gourd (*Momordica charantia Linn*.) and leaves of sweet basil (*Ocimum basilicum Linn*) on the levelphase I include cytochrome P450 (P450), aniline hydroxylase (ANH) and aminopyrine-N-demethylase (AMD) as well as the capacity to activate the mutagenicities of aflatoxin B1 (AFB1) and benzo[a]pyrene (BaP), and to induce the phase II enzymes [i.e. glutathione S-transferase (GST)] in rat liver. GST (*Glutathione S-Transferase)* activity was enhanced when diet containing only 12.5% neem flowers and Thai bitter gourd fruits was given for two weeks. The GST activity got improved as 2.7- and 1.6-fold of the pair-fed control values, respectively, when resulting in a marked reduction of the levels of most phase I reactions. Fruits of the Chinese bitter gourd, which was in the same species as Thai bitter gourd, had no effect on GST activity but decreased AMD (aminopyrine-N-demethylase) activity and the *in vitro* metabolic activation of AFB1 and BaP. Dietary sweet basil leaves increase the levels of both GST and all phase I enzymes. The neem flowers and Thai bitter gourd fruits having the monofunctional phase II enzyme inducers and compounds capable of repressing some monooxygenases, they especially involved in the metabolic activation of chemical carcinogens, while sweet basil leaves having compounds, probably bifunctional inducers, capable of inducing both phase I and phase II enzymes and Chinese bitter gourd fruits having only compounds capable of repressing some monooxygenases. They suggest that neem flowers and Thai bitter gourd fruits may possess chemo preventive potential, while those of Chinese bitter gourd fruits and sweet basil leaves are uncertain. [28]

Neem Flowers on Liver Carcinogenesis: The dietary neem flowers (*Azadirachta indica A. Juss*) caused a marked increase in glutathione S-transferase (GST) activity in the liver, while resulting in a significant reduction in the activities of some hepatic P450-dependent monooxygenases. These results strongly indicate that neem flowers may have chemo preventive potential. The inhibitory effects of neem flowers on 9,10-dimethyl-1,2-benzanthracene (DMBA)-generates mammary gland carcinogenesis in female Sprague Dawley rats and on aflatoxin B1 (AFB1)-generates hepatocarcinogenesis in male Wistar rats. Freeze dried neem flowers (10-25%) were combined with AIN-76 purified diets and were administered to young animals as follows; i.e one week prior to, during and after the administration of each carcinogen.Interestingly, it was found that neem flowers resulted in a marked reduction of the incidence of mammary

gland (about 35.2%) and liver tumors (61.7% and 80.1% for benign and malignant tumors, respectively). Furthermore, the multiplicity of tumors per rat are also lower in the neem flower groups, i.e. those for mammary gland tumors and benign and malignant liver tumors were reduced to 44.0%, 87.9% and 88.9%, respectively. WhileAFB1 and DMBA are found to be the cause for carcinogenesis in liver and mammary glands in rats, the neem flowers proved to contain some chemo preventive agents that can inhibit AFB1 and DMBA. [29]

Antifertility potential of Neem flower extract: Adult Sprague-Dawley rats are used for the detection of alcoholic extract of Neem flowers effect on the estrous cycle, ovulation, fertility and foetal morphology. Adult female Sprague-Dawley rats, weighing between 140-180g were taken and separated as three main experimental groups. Group 1 rats treated with 1 g/kg of alcoholic extract of neem flower by gavage for 3 weeks and the effect on estrous cycle studied. Group 2 rats are administered 1 g/kg of neem flower alcoholic extract at 9 a.m. and at 6 p.m. Group 3 Rats are administered with 1 g/kg of alcoholic extract of neem flower on days 1 to 5 postcoitum. The anti-implantation / abortifacient effects and possible teratogenic effects on the foetuses are observed. All the groups are control-matched. The estrous cycle of 80% of the rats are altered with a marked prolongation of the diestrus phase. Neem flower creates a statistically significant ($p < 0.05$) reduction in the number of ova shed in the morning of estrus in rats fed with the extract at 9 a.m. on proestrus.Alcoholic extract of neem flower was developed as a female contraceptive, as it was able to block ovulation partially in Sprague-dawley rats by disrupting the estrous cycle.[30]

Clastogenic and Anticlastogenic Potential of Neem Flower extract: Clastogenic and anticlastogenic effects of neem flower (*Azadirachta indica A. Juss*) extract is determined using a rat liver micronucleus assay. Methanol extracts of neem flowers (MENF) in 15% Tween 80 at 100 and 500 mg/kg body weight were orally given to male Sprague Dawley rats daily for 6 weeks [2 weeks prior to and 4 weeks during repeated administration of diethylnitrosamine (DEN)]. All rats were anesthetized and their livers were collected. Hepatocytes were isolated from the livers using a collagenase solution without performing liver perfusion, then stained with 4', 6-diamidino-2-phenylindole dihydrochloride (DAPI). Incidence of the micronucleated hepatocytes was evaluated by fluorescence microscopy. these results showed that MENF had no clastogenic effects on rat hepatocytes. On the contrary, MENF at high doses significantly reduced micronucleus formation in the rat livers compared to the control group. MENF at 100 and 500 mg/kg body weight had no clastogenic effects. It possesses anticlastogenic potential in the rat liver, particularly at high doses. [31]

CONCLUSION

Plants are one of the most important sources of medicines. Neem belongs to use pharmacological potential as panacea. From the literature survey it is found that neem is a potential source of anticancer, anti-diabetic, anti-inflammatory, antimicrobial drugs as well as it also used as cardio protective, radio protective, recognition of memory and many others. In recent studies shown that neem flowers are also having the biological activities. They are hypocholesterolemia, antioxidant activity, Immunostimulatory activity etc.

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