Review Article

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PHYTOCHEMICAL AND PHARMACOLOGICAL ASPECTS OF *STROBILANTHES CILIATUS* Nees (Bremek.): A REVIEW

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ABSTRACT

In recent years the use of medicinal plant has increased as a low cost alternative to the expensive modern drugs. *Strobilanthes ciliatus* Nees (Bremek.) Known as "Sahachara" is widely used today in indigenous Indian systems of medicine has been shown to possess a range of folk and proven biological activities such as antiinflammatory, analgesic, anticancer, antimicrobial, antidiabetic and hepatoprotective. The roots and leaves of the plant are used as major ingredient in many of the Ayurvedic preparations especially meant to relieve pain and inflammation Compounds such as lupeol, stigmasterol, betulin, stigmasterol glycosides and 4- acetyl-2, 7-hydroxy-1, 4, 8,-triphenyl-octane-3, 5-dione were reported from the acetone extract of the stem. Lupeol is the major compound reports from the various parts of the plant and is well reported for its broad pharmacological potential. The reported biological activities and the phytoconstituents together, however, contribute to the medicinal importance of the plant. The present review covers the available information on the studies carried out on the biological and chemical aspects of *S.ciliatus* Nees.

Keywords: *Strobilanthes ciliatus*, Pharmacology, Phytochemistry, Lupeol

INTRODUCTION

Natural sources such as plants and herbs have received considerable attention for the discovery and development of leads as new drug able molecules, because of its diversity[1]. The chemical novelty associated with natural products is higher than that of any other source. 40% of the chemical scaffolds in a published database of natural products (Dictionary of Natural Products, Chapman & Hall) are absent from synthetic chemistry. Despite the commonly held assumptions, natural products can be a more economical source of chemical diversity than the synthesis of equivalent numbers of diverse chemicals[2].

Strobilanthes ciliatus Nees (Bremek.) is a traditionally known and medicinally potent plant that belongs to the genus 'Strobilanthes'. The plant has received greater attention recently due to the presence of a wide range of secondary metabolites and various pharmacological activities. Strobilanthes is the genus of perennial flowering herbs and shrubs with 350 species, usually seen in the hills of tropical Asia of which 150 species are available in the Indian subcontinent. The flowering plants of this genus belong to the family Acanthaceae. It is observed throughout the evergreen forests of the Western Ghats up to 1200 meters, comprising Kerala and Karnataka[3,4]. Fairly common in semi evergreen forest, to 1meter high, sometimes in partial shade with terete or sub quadrangular stems, diffusely branched, sulcate on two sides when young, glabrous, lenticulate,dark green or purple in colour with white dots, often winged at nodes. Nodes are jointed, prominent, and often fimbriate. Leaves are simple, opposite, lanceolate, serrate, almost glabrous, attenuate at base, acuminate at apex. Flowers are 4-seriate, white or pale purple in dense spikes. Capsules are oblong and apically ciliate. Calyx 5-6.5 mm long, divided to 2/3rd of its length; unequal segments, linear to lanceolate, acute at apex, almost glabrous with few glandular hairs. It is the annual flowering and fruiting plant and is observed during the months of December to March[5, 6].

Taxonomical Classification

Kingdom: Plantae
Phylum: Tracheophyta
Class: Mangoliopsida
Order: Lamiales
Family: Acanthaceae
Genus: Strobilanthes
Species: *Strobilanthes ciliatus*[13]

S.ciliatus is a strong aromatic plant and is widely used in ayurveda in the drug 'Sahachara' and it is also believed to be used in other Indian systems of medicine such as Unani and Sidha[7, 8] . The plant is a major ingredient in many Ayurvedic preparations such as Sahacharadi thailam, Sahacharadi kashayam, Varanadi kashayam, bhonagathailam, Ashtavargam kashayam, maharasnadi kashayam, Sathavaryadi kashayam, Balasahacharadi kashayam, Balaaireyakadi kashayam, Balakulathyadi kashayam and Aragwadharishtam[9].. This medicine is well recommended by 'Sahasrayogam' for relieving pain especially low back pain, lumbar spondilytis and sciatic [10] .The plant has also been mentioned for use in neurological disorders. Based on this an Ayurvedic preparation is available in the market as neurotonic for neuritis and motor neuron disease (neuton capsule, 90 mg of *S.ciliatus* (manufactured by K.S Warrier's Ashtanga Ayurvedics) [4, 11]. The roots found to be bitter, sweet, thermogenic, emollient, diuretic, febrifuge, diaphoretic, depurative, expectorant and tonic. They also traditionally used in various conditions such as inflammations,

rheumatalgia, lumbago, sciatica, limping, chest congestion, fever, leucoderma, skin diseases, cough, bronchitis, odontalgia and general debility. The leaves and bark are diaphoretic, expectorant, depurative and febrifuge, and are also useful in whooping cough, fever, dropsy, leucoderma, leprosy, pruritis, inflammations and fever. The leaves applied externally in gout, lumbago and pain in joints and are also used in the treatment of jaundice, dropsy, rheumatism and disease of urino-genital tract. The extracts of leaves and bark are suggested for itching, leprosy, diabetics, tooth ache and urinal disorders. In folk medicine drinking of the leaf decoction and applying of leaf paste over affected area for relieving rheumatic pain has been practiced[4, 12, 13]. Kurinjhi kuzhambu is another medicinal preparation given for woman after delivery for good health[37]. Seeds are used in the treatment of jaundice, dropsy, rheumatism, disease of genitourinal tract and against gonorrhoea and spermatorrhea[12].

The essential oil present in the plant is traditionally used in neurological disorders but no research attempts have been carried out so far to isolate and evaluate the same[4, 14]. *S.Ciliatus* has been reported for several pharmacological activities such as antioxidant, antimicrobial, antiinflammatory, analgesic, antidiabetic, hepatoprotectivity, and anticancer activities using suitable in vitro and in vivo methods. The plant has also been reported for its DNA protective effect on cultured lymphocytes. The main chemical constituent isolated from the plant is lupeol and it has been reported for its various biological activities[12]. Although the plant has been reported for its strong ethnopharmacology, very few studies have been conducted to investigate the chemical moieties present in the plant. The plant is rich in different types of phytoconstituents such as terpenoids, phytosterols, flavanoids, carbohydrates and tannins[15, 16, 17, 18]. In this article, an attempt has been made to review the available literatures information on the plant to highlight its traditional, ethno-pharmacological and phytochemical importance.

Phytochemical Aspects

Reports on the phytochemical investigations carried out so far by standard chemical tests in various literatures give an idea about the presence of different chemical moieties in the plant. The reports indicate the presence of mainly terpenoids, phytosterols, flavonoids, carbohydrates and tannins[17,18]. Some of these phytoconstituents such as flavanol constituents (8.86%), flavonoids (1.635%), flavanols (4.57%), tannins (106.75%), and lipids (2.20%) were quantified. Physicochemical parameters such as ash values, loss on drying and extractive values have also been studied; total ash 15%, acid insoluble ash 7.5%, water insoluble ash 6.1%, loss on drying 5.03, ether soluble extract 1.24%, ethanol soluble extract 2.12%, water soluble extract 4.36% and the crude fibre content 17.61%[18].

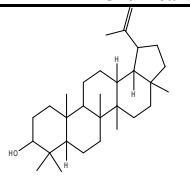
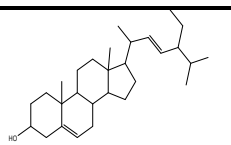
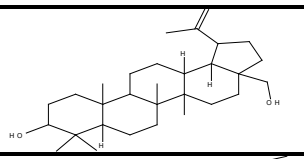
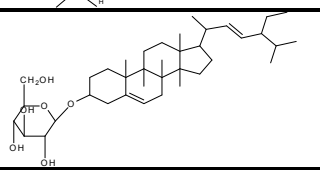
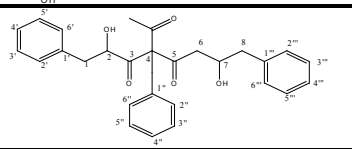
GC-MS analysis was carried out on the ethanolic leaf extract of the plant. The result reveal the presence of compounds such as 3-octyne,2,2,7-trimethyl ($C_{11}H_{20}$), 3,7,11,15- tetramethyl-2-hexadecen-1-ol ($C_{20}H_{40}O$), dibutyl phthalate ($C_{16}H_{22}O_4$), (R)-(-)-(Z)-14-methyl-8-hexadecane-1-ol ($C_{17}H_{34}O$), hexadecanoic acid ethyl ester ($C_{18}H_{36}O_2$), 1-dodecanol,3,7,11-trimethyl ($C_{15}H_{32}O$), phytol ($C_{20}H_{40}O$), 2-n-heptylcyclopentanone ($C_{12}H_{22}O$), 9,2,15-octadecatrienoic acid methyl ester ($C_{19}H_{32}O_2$), 2-propenoic acid, 2-(dimethylamino) ethyl ester ($C_7H_{13}NO_2$), squalene ($C_{30}H_{50}$), β-tocopherol ($C_{28}H_{48}O_2$), vitamin E ($C_{29}H_{50}O_2$), campesterol ($C_{28}H_{48}O$), stigmasterol ($C_{29}H_{48}O$), β-amyrin ($C_{30}H_{50}O$) [20].

Terpenoids and steroids such as lupeol, stigmasterol, betulin and stigmasterol glycosides were also reported from the acetone extract of the stem [14]. Although the plant part was first extracted with petroleum ether, the terpenoids and steroid molecules were separated from the acetone extract by column chromatography. Acetone extract eluted with 100% petroleum ether yielded lupeol, 49:1 petroleum ether: ethyl acetate yielded stigmasterol, 19:1 petroleum ether: ethyl acetate yielded betulin, 1:1 petroleum ether: ethyl acetate yielded stigmasterol glycoside. 4-Acetyl-2, 7-hydroxy-1, 4, 8,-triphenyl-octane-3, 5-dione is another compound isolated from the acetone extract of the plant with 4:1 ratio of petroleum ether: ethyl acetate[21].

In another study, lupeol was isolated from the petroleum ether extract over column chromatography with 80:20 of petroleum ether: ethyl acetate. The presence of lupeol was confirmed by HPTLC using the standard. Lupeol appeared as a pink band at an R_f value of 0.67 in the finger print where the standard lupeol was also in pink colour. The lupeol content was quantified using TLC densitometric methods as 0.16±0.02% w/w[16]. Chemical structure and the various physical characteristics of the compounds isolated are shown in Table 1.

Lupeol, the major constituent found in the plant, exhibits a broad spectrum of biological activities such as antiinflammatory, antitumor, antiprotozoal and antimalarial. Biological tests aiming for antiplasmodial and antimalarial mode of action of lupeol has revealed that the presence of C_{28}hydrogen donor groups may be responsible for the incorporation of erythrocyte membrane leading to an irreversible change in the membrane shape. Lupeol is also reported for its ability to decrease IL-4 production by T helper cell type-2 in addition to evidence for significant reduction of eosinophil infiltration. Studies state that the anticancer potential of lupeol may be due to its ability to inhibit Topoisomerase-II and also it exhibits lyase activity on DNA polymerase –β, which sensitize cancer cells to DNA damaging agents. Betulin, a related analogue has also been reported for its wide range of biological properties[22, 23, 24]. The other steroid constituents reported from the plant such as stigmasterol and stigmasterol glycoside are known for their antiinflammatory potential.

Table: 1 Chemical structure and physical characteristics of the isolated compounds[15, 21]

Isolated compound	Chemical structure	Physical characteristics
Lupeol		White crystals MP-206^{0}C
Stigmasterol		White powder MP-169^{0}C
Betulin		White powder MP-245^{0}C
Stigmasterol glycoside		White powder MP-270^{0}C
4-Acetyl-2,7-dihydroxy-1,4,8-triphenyl-octane-3,5-dione		White powder MP-170^{0}C

Pharmacological Aspects

Investigations made on the plant supports the same and also prove its pharmacological potential as antioxidant, antimicrobial, antiinflammatory, analgesic, antidiabetic, hepatoprotective and anticancer using suitable in vitro and in vivo methods [15-19, 25-29].

Antimicrobial

Several reports prove the antimicrobial activity of the different extracts of the plant against various strains of bacteria and fungi. In the first report, a study was performed to evaluate acetone and ethanolic extracts of the plant for their antimicrobial potential using three strains of bacteria such as *Staphylococcus aureus, klebsiella pneumonia, Pseudomonas aeruginosa* and *Aspergillus* as fungal strain by disc diffusion method. In this study, both the stem and root extracts showed moderate activity against all the strains[9].

Petroleum ether, chloroform, ethanolic and aqueous extracts on the leaves of *S. ciliatus* were evaluated against different bacterial (*S.aureus, B.subtilis, E.coli, P.aeruginosa*) and fungal (*A.niger, C.albicans*) strains.. *Trichophyton rubrum, Microsporum gupseum, Monascuspurpureus* were used as fungal strains. The antimicrobial activity was assessed by disc diffusion and determination of MIC by serial dilution methods using Ciprofloxacin (5mg) and clotrimazole (10mg) as standards. Petroleum ether extract showed maximum activity against *E.coli, Klebsiella* and *Cornybacterium* with minimum inhibitory concentration of 125µg/ml whereas the maximum antifungal activity against *M.purpureus* was seen with 250 µg/ml. Methanolic extract also showed good activity with MIC values ranges from 250-500 µg/ml[17].

Petroleum ether, chloroform, ethanolic and aqueous extracts on the leaves of *S. ciliatus* have been evaluated against different bacterial (*S.aureus, B.subtilis, E.coli, P.aeruginosa)* and fungal (*A.niger, C.albicans)* strains. Cup plate method was used to detect the antimicrobial activity of the extracts using amoxicillin and ketoconazole as standards. Chloroform extract showed marked antibacterial potential against Gram negative strains with zone of inhibition (ZOI) of 23mm whereas the maximum antifungal activity was possessed against Aspergillus niger with ZOI of 15mm[26].

The effect of mycorrhizal, non-mycorrhizal with plant growth promoting rhizomicroorganisms (PGPR's) treated extracts (aqueous, ethanol, petroleum ether, benzene, chloroform and methanol) were tested against two Gram positive (*Bacillus subtilis*, *Staphylococcus aureus)*, two Gram negative *(Escherichia coli*, *Pseudomonas aeruginosa)* bacterial strains and two fungal strains *(Candida* sp., *Aspergillus* sp.*)*. Petroleum ether extract and ethanolic extract of mycorrhizal, non-mycorrhizal and mycorrhizal with PGPR's treated extracts were highly effective against the tested microbes. The antibacterial activity of petroleum ether and 80% ethanolic extracts exhibited moderate activity against all the four bacterial strains when compared with standard ciprofloxacin by zone of inhibition. Better antifungal activity was observed against *Aspergillus flavus*[18].

All the extracts generally have shown moderate activity against all bacterial and fungal strains used. Among the various parts of plant and extracts used, petroleum ether and chloroform extract

of the aerial parts of the plant showed comparatively better activity against different strains. These results may be due to the presence of non-polar bioactive compounds in the plant.

Antioxidant Potential

Antioxidant potential of the ethanolic leaf extract has been evaluated by in vitro radical scavenging assay. The results showed a dose dependent inhibition of DPPH activity (IC_{50} of 47.11 µg/ml) when compared to the standard ascorbic acid (IC_{50} of 50.11 µg/ml). The extract also showed superoxide radical scavenging activity at a dose of 100 µg/ml ($P<0.05$) in a dose dependent manner [20].

Hepatoprotectivity

Hepatoprotective activity of methanolic extract of the bark was evaluated against paracetamol induced toxicity in mice. Paracetamol was induced orally at a dose of 2.5g/kg to make liver damage. The experimental animals were divided in to five groups of six animals each. Group I normal control, Group II paracetamol control group, Group III positive control (Silymarin treated), Group IV and V were test groups. Animals were sacrificed on the day 8 and blood samples and organs were evaluated for biochemical and histochemical parameters respectively, and the results were compared with the positive and negative control. Biochemical studies showed significant reduction ($p<0.05$) in the levels of SGOT, SGPT and ALP in the test groups when compared to the paracetamol control group and the treatment group also showed remarkable improvement in the total serum protein level. Histopathological examination of the liver tissue confirmed the hepatoprotective activity by the extract. The extract almost maintained the normal architecture of the liver when compared to the paracetamol control group which had hemorrhage and necrosis in extensive areas of liver parenchyma and also the hepatocytes had vacuolated cytoplasm and a number of inflammatory cells and siderophages. The extract treated group also showed normal glomeruli and intestinal tissue rather than glomerular oedema and vacuolated epithelial lining of renal tubules in silymarin treated group and paracetamol control group. The protection against paracetamol induced toxicity has been preferred as a confirmatory test for hepatoprotective activity and at the same time various investigations suggest that the biochemical aspects of liver marker enzymes and pathological aspects of liver damage can be considered as study tools for hepatoprotective activity[26].

Anticancer Activity

Cytotoxicity of acetone and methanolic extracts were showed good activity against DLA and EAC cells, but the dose of the extracts used are not mentioned[12]. Cytotoxicity of hydro alcoholic extract of the plant was evaluated against MCF-7 by MTT assay. Different dilutions such as 5-100 µg/ml were used and the IC_{50} values were calculated using methotrexate as standard. The IC_{50} values of the extract and standard were found as 3.68µg/ml and 3.31µg/ml, respectively. The IC_{50} values confirmed the high cytotoxic potential of the extract towards MCF-7 but the author has concluded that the extract is only moderately cytotoxic[27].

Acute Oral Toxicity Studies

A study was performed to evaluate acute toxicity of the extracts on healthy Wilstar albino rats with reference to OECD guidelines423. Four groups of animal were selected randomly with 5 animals in each group. Group I-III were administered orally with extracts of 100, 500, 1000mg/kg body weight respectively and IV^{th} group (control group) was administered the vehicle alone. The extracts administered through oral gavage and the animals were observed for every 4h to monitor the changes in autonomic and behavioural responses such as spontaneous activity, irritability, corneal reflex, urination and salivation. In this study, no mortality was observed in rats with the maximum dose of extracts administered through oral route. The result revealed that the extracts are nontoxic and safe for in vivo use[27].

Antidiabetic Activity

Antidiabetic activity of aqueous and alcoholic extracts of whole plant has been studied on streptozotocin-nicotinamide induced experimental diabetic rats. Among the two extracts, the aqueous extract showed a significant reduction in blood sugar level when compared to normal rats. Various parameters such as acute toxicity, oral glucose tolerance, normoglycemic study were performed prior to antidiabetic screening. The experiment was designed in such a way that the rats were divided in to four groups (n=6). They were grouped as group-I (normal rats), group II (diabetic rats), group III (diabetic rats administered with aqueous extract), group IV (diabetic rats administered with alcoholic extract). Type-2 DM was induced in overnight fasted rats by intraperitoneal injection of streptozotocin in single dose of 60mg/kg. The study was designed for 21 days, the blood samples were collected retro-orbitally and the serum was separated. Tissue samples also collected from experimental animals and were studied for different biochemical parameters. Experimental animals treated with aqueous extract at oral doses of 200mg/kg and 400mg/kg for 21 days, showed 44.02% and 53.64% reduction in blood glucose level, respectively when compared with untreated rats. The estimated lipid levels in treated and untreated diabetic rats indicate significant reduction of elevated levels of triglycerides and total cholesterol after the treatment period. The study also revealed a significant increase in liver glycogen level to 6.25-13.22% after the treatment period. Lowering of serum lipid concentration in the treated animals was taken as an indication of decreased risk of vascular diseases[28]. The antidiabetic activity of the ethanolic extract of the whole plant was evaluated by α-glucosidase and α-amylase inhibition assay. α-Glucosidase inhibitory activity of the ethanolic extract was higher (IC_{50} of 21.90mg/ml) than their α-amylase inhibitory activity (IC_{50} of 462.49mg/ml). This could be an effective strategy for the treatment of type-2 DM, a mild inhibition of α -amylase and strong inhibition of α-glucosidase[19].

Antiinflammatory Activity

Antiinflammatory activity of the petroleum ether, ethanolic and aqueous extracts of root and aerial parts of the plant was carried out by Human RBC membrane stabilization method. The membrane stabilizing activity of the extracts were studied at different concentrations of as10, 50 and 100 µg/ ml on heat induced lysis of HRBC membrane. All the extracts tested showed high protection (59.18%) with a dose of 50µg/ml and the effect was equipotent with the standard diclofenac (56.35%) [29].

Analgesic Activity

In vivo analgesic activity of the plant extract was performed by tail clip method. Different doses such as 100 mg/kg and 200 mg/kg were evaluated against the standard pentazocin at a dose of 5mg/kg. The extract showed significant inhibition of tail clipping at various time intervals in a dose dependent manner and also showed significant ($P<0.01$) increase in the mean latency of biting of the tail clip after 30 minutes[27].

DNA Protective Effect

DNA protective effect of the ethanolic leaf extract against H2O2 induced DNA damage in cultured lymphocytes were studied by Comet assay. The experiment was designed in such a way that the cells were divided in to four groups, group I (control-0.05% DMSO), group II (500 µm H_2O_2), group III (60 µg/ml extract

pre-treated+ 500 µm H_2O_2) and group IV (60 µg/ml extract). The extent of DNA damage induced by administering H_2O_2 was significantly reduced in the cultured lymphocytes pre-treated with 60 µg/ml of the extract[20].

Antioxidant Activity

Antioxidant potential of ethanolic leaf extract was evaluated by in vitro radical scavenging assay. The result showed a dose dependent inhibition of DPPH activity with an IC_{50} 47.11 µg/ml when compared to the standard ascorbic acid with IC_{50} 50.11 µg/ml. The extract also showed superoxide radical scavenging activity at a dose of 100 µg/ml ($P<0.05$) in a dose dependent manner[20].

Antiviral Activity

Since there are reports on the use of roots and leaves of the plant by ayurvedic practitioners as treatment for various viral ailments, the author of this review was interested in carrying out investigation on plant extracts and bioactivity guided fractionation to confirm their activity. The preliminary study carried out using petroleum ether and chloroform extracts of the leaves showed a very good antiviral potential towards HSV-I and HSV-II against $2TCID_{50}$ and $10TCID_{50}$ challenging doses. Further studies are in progress. In this context, it may be pointed out that the plant *Strobilanthes cusia* from the same genus has been shown to exhibit very good antiviral potential against RNA viruses[29, 30]. Lupeol has shown weak antiviral activity in several studies, but it has served as a lead drug for the generation of more effective compounds. Lupeol isolated from *Strobilanthes cusia* root reveal an EC_{50} of 11.7µM against HSV-I and shown 100% inhibition of virus plaque formation at 58.7µM[31]. However, betulinic acid has exhibited better activity against HSV-I with an EC_{50} of 5.7 µM for reducing plaque formation, a CC_{50} value of 35.5 µM and a therapeutic index of 6.2[32]. The compound is well known for its anti HIV activity[33]. Studies on structure- activity relationship carried out reveal that the side chain at C_3 position, an ester group with terminal carboxylic acid and the isovaleryl domain together contributes the potent anti HIV activity[34,35,36].

CONCLUSION

The main objective of the present article was to thoroughly review the recent scientific studies that have been made on the plant to explore its pharmacological efficacy and the chemical moieties present in the plant. Although some active constituents have been isolated, the author feels that there is a need for carrying out further extensive studies to document all the phytoconstituents present in the plant. With the development of new molecular targets, there is an increasing demand for novel molecular diversity for bioactivity screening. Now there has been a worldwide interest in scientific validation of the old traditional medicines for their therapeutic efficacy. However research up to now has shown that that the plants are valuable sources for novel compounds. Emergence of combinatorial chemistry opens a wide platform to create natural product libraries from the base molecules isolated. This review brings out the promising pharmacological potential of the various parts of *S.ciliatus* and may provide useful lead for the development of drug able moieties from the plant.

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