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STEREOSPERMUM TETRAGONUM; AN EMERGING MEDICINALLY IMPORTANT TREE

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ABSTRACT

Stereospermum tetragonum is ayurvedic medicinal tree and used in ayurvedic formulations. It has shown that activities like antimicrobial, antioxidant, antivenom and antidiabetic and moreover in ethnomedicinal purpose it used as antiulcer, antipyretic and diuretic. It widely seen throughout the India. The plant has important phytochemical constituents like phenols, alkaloids, p-coumaric acid, glycosides, flavonoids and saponins. So, in this review article mainly discuss about the overall features of *Stereospermum tetragonum*.

KEYWORDS: Stereospermum tetragonum, Antidiabetic, antimicrobial, antioxidant and antivenom activity.

INTRODUCTION

Stereospermum tetragonum is a ayurvedic medicinal tree and belongs to the family Bignoniaceae and it is a large straight stemmed deciduous tree 18-30 min height and 2.8 min girth found throughout in moist regions of India up to an altitude of about 1200m, chiefly in deciduous forests.[1] The plants are having bitter, a stringent and acrid property. The medical significance of *Stereospermum tetragonum* was earlier reported.[2] It also called as yellow snake tree or trumpet flower tree. It is used in folk medical practices to treat DM in certain remote villages of Thirunelveli district of Tamilnadu. In ethnomedical practices, the plant is also used as diuretic, treat antiulcer, anti-pyritic etc.[3] It is used in the preparation of Chyavanprash (a popular Ayurvedic tonic), it is an ingredient of Dashamoola and used in other Ayurvedic formulations such as Sahachardithailam and Dhanwantharamthailam (An

excellent massage oils). It has antimicrobial, antiprotozoal and anti-inflammatory properties.[4] Phytochemical analysis showed the presence of tannins, phenol, glycosides, terpenoids, coumarins, in the active fraction and moreover gas chromatography of the plants revealed other significant constituents like Carbonochloridic acid, methyl (2-propynyl) hydrazone acetic acid, phytosterol and5-n-pentadecyl-2,4- dinitro-1-hydroxy-benzene.[5] Preliminary studies have showed promising antihyperglycemic activity of the roots of *Stereospermum tetragonam*. Other studies showed the active fraction showed presence of antidiabetes mellitus activity in type-1 and type-2 diabetic rats. Two active principles were isolated and characterized by spectral data. One of them was identified as an iridoid type glycoside and the other one was a lapachol like compound.[6,7] The plants also used as anodyne, appetizer, constipating, diuretic, litho tropic, expectorant, cardio tonic, aphrodisiac, anti-inflammatory, antibacterial, febrifuge tonic, antiemetic, antipyretic and antivenom activity.[2] Especially the root has shown the promising therapeutic activity rather than other parts.

Botanical description

Morphology.

Roots.

Roots are light brown in colour the fracture and texture found to be hard. Astringent and slightly bitter in taste.

Flowers

Large, pale yellow, trumpet shaped flowers occur in panicles and slightly curved about 2cm upper lip lobed, lower lip 3-lobed, tomentose at mouth, tube terete.[8]

Fig. 1: *Stereospermum tetragonum tree*, Flower, Pods.[4]

Table 1: Taxonomical classification of *Stereospermum tetragonum*.

Kingdom	Plantae
Division	Tracheophyta
Class	Magnoliopsida
Order	Lamiales
Family	Bignoniaceae
Genus	*Stereospermum*
Species	*S.tetragonum*

Binomial name: *Stereospermum tetragonum* DC

Synonyms: *Stereospermum colais*, *Stereospermum personatum*.[8]

Other species of *stereospermum*

Twenty four known *Stereospermum* species are widely distributed in Western Ghats - South, Central and south Maharashtra Sahyadris. The genus *Stereospermum* is further organized into groups including *S. acuminatissimum* K.Schum, *S. angustifolium* Haines, *S. annamense* Dop, *S. arcuatum* H.Perrier, *S. boivini* (Baill.) H.Perrier, *S. chelonoides* (L.f.) DC., *S. cylindricum* Pierre ex Dop, *S. euphorioides* DC., *S. fimbriatum* (Wall. ex G.Don) DC, *S. harmsianum* K.Schum., *S. kunthianum* Cham, *S. leonense* Sprague, *S. longiflorum* Capuron, *S. nematocarpum* (Bojer) DC, *S. neuranthum* Kurz, *S. rhoifolium* (Baill.) H.Perrier, *S. strigillosum* C.Y. Wu & W.C. Yin, *S. strigilosum* C.Y.Wu, *S. tetragonum* DC., *S. tomentosum* H.Perrier, *S. undatum* H. Perrier, *S. variabile* H.Perrier *S. zenkeri* K.Schum. ex De Wild.[9]

Ethnobotanical uses

Ethnobotanical uses of *Stereospermum tetragonum* are summarized in table 2.

Table 2: Ethnobotanical uses of *stereospermum tetragonum*.

Part used	Indicators
Root	Antidiabetic activity, Antipyretic, Diuretic
Fruits	Migraine
Bark	Piles
Whole plant	Anti-inflammatory, Antimicrobial and Antiprotozoal activity

Phytochemistry

1. Phytochemical investigation on leaves of *Stereospermum tetragonum* which showed the presence of bioactive compounds like Cardio glycosides, Flavonoid, Quinones, Terpenoids, Alkaloids and Steroids and inorganic elements like magnesium, iron,

sulphate, phosphate, chloride and fluoride were reported in leaves part. While pods of plants shows the presence of carbohydrate, protein, saponin, coumarin and flavonoid and inorganic elements like iron, sulphate and chloride.[10]

2. HPTLC study of *Stereospermum tetragonum* root showed p-coumaric acid presence.[11]
3. The gas chromatography of the plants revealed other significant constituents like Carbonochloridic acid, methyl (2-propynyl) hydrazone acetic acid, phytosterol and5-n-pentadecyl-2,4- dinitro-1-hydroxy-benzene.[5]

Pharmacological activities

Antidiabetic activity

Stereospermum tetragonum (Bignoniaceae) roots were extracted with distilled water by maceration process. The water extract of plant was precipitated with ethanol and separated into alcohol soluble fraction and precipitate fraction. The alcohol soluble fraction showed significant antidiabetic activity. Mainly two studies done in this activity like Streptozotocin induced method and alloxan induced diabetic activity.

In Streptozotocin-induced type-2 diabetic rats were produced by injecting 80 mg/kg streptozotocin (i.p.) in citrate buffer on the 5th day of their birth and this model is called neonatal-5 STZ induced rat model of type-2 DM.8 Eight weeks later, blood samples were drawn and glucose levels were determined to confirm induction of diabetes. The diabetic rats which showed blood glucose levels in the range of 11.5e12.0 mmol/L were selected for efficacy evaluation of the herbal drug.

To evaluate the efficacy, the streptozotocin diabetic rats were divided into three groups of six each. The diabetic control group was given 1.5 mL of water, p.o., daily. The test group was given daily dose of AF (25 mg/kg). This dose was found to be the optimum dose in our glucose tolerance test (GTT). The third group received glibenclamide (0.5 mg/kg). Weight and sex matched six normal rats were kept as control group (4th group). Treatment was continued for 21 days. Blood samples were collected on days 1, 7, 14 and 21. On day 21, animals were killed after blood collection and liver samples were removed for glycogen estimation. This study reports for the first time the anti-DM (type-2) activity of the active fraction (AF) isolated from *S. tetragonum* root. The anti-DM activity of the active fraction (25 mg/kg) was almost comparable to that of glibenglamide (500 mg/kg).[12]

In alloxan induced diabetic study ,development of induced diabetes mellitus was confirmed on fifth day after alloxan administration by examining the glucose level in the blood taken from tail vein. The blood sugar of rats was estimated by Glucometer Gx using commercially available glucostix reagent strips. An experiment was conducted in Wister rats (190-210g body weight) of uniform age. The rats were divided into four groups of six each. Group I was kept as control which received an equal volume of vehicle only. Group II are diabetic control rats. Group 111 diabetic rats treated With (25mg/kg) active fraction. Group IV diabetic rats treated with insulin (5 IU/Kg ip) the treatment was continued for 12 days.

Alloxan, in low doses, has been reported to produce non-insulin-dependent diabetes mellitus-like state which can progress to a gradual recovery or to an insulin-dependent diabetes mellitus stage. The present findings suggest that the active fraction of *Stereospermum tetragonum* shows antidiabetic activity in rats and is scientifically verified by histological studies. The active fraction of *Stereospermum tetragonum* is an attractive material for further studies for the development of conventional medicine for diabetes.[13]

Antivenom activity

Stereospermum tetragonum plant extracted by maceration process and used for neutralisation efficacy of this plant against Russell's viper (Daboia russelli) snake venom was determined by both in vitro and in vivo conditions.[5]

***In vitro* study**

Indirect hemolysis assay (PLA2 activity)

Phospholipase A2 activity was measured using an indirect hemolytic assay on agarose-erythrocyte-egg yolk gel plate by the method.[14] Increasing concentrations of Daboia russelli venoms (in µg) were added to wells in agarose gels containing 1.2% sheep erythrocytes, 1.2% egg yolk as a source of lecithin and 10mM CaCl2. Slides were incubated at 37˚C overnight and the diameters of the hemolytic halos were measured. Control wells contained 15µl of saline. The efficacy of *Stereospermum tetragonum* extracts in neutralizing the phospholipase activity was estimated by mixing a constant amount of venom (µg) with different amount of plant extracts (µl) and incubated for 30 min at 37°C. Then, aliquots of 10 µl off to the mixtures were added to the wells in agarose-egg yolk-sheep erythrocyte gels. Control samples contain venom without plant extract. Plates were incubated at 37°C for 20 hours. Neutralization expressed as the ratio mg plant extract/mg venom able to reduce by

50% the diameter of the hemolytic halo when compared to the effect induced by venom alone.[14]

Procoagulant activity

Various amounts of venom dissolved in 100µl PBS (pH 7.2) were added to human citrated plasma at 370C. Coagulation time was recorded and the Minimum Coagulant Dose (MCD) was determined as the venom concentration, which induced clotting of plasma within 60 seconds. Plasma incubated with PBS alone served as control. In neutralization assays constant amount of venom was mixed with various dilutions of plant extract. The mixtures were incubated for 30min at 37°C. Then 0.1ml of mixture was added to 0.3ml of citrated plasma and the clotting times were recorded. In control tubes, plasma was incubated with either venom alone or plant extract alone. Neutralization was expressed as effective dose (ED50), defined as the ratio µl anti venom (plant extract) /mg venom at which the clotting time increased three times when compared with clotting time of plasma incubated with two MCD of venom alone.[15]

Proteolytic activity

Proteolytic activity was determined according to the method using 2% casein as substrate in 0.02M Tris-HCl buffer (pH 8.5). Venom 200µg (1mg/ml) and different dilutions of plant extract 200µg, 250µg, 300µg were pre-incubated with 1ml of substrate for 2h at 37 °C. The undigested casein was precipitated by the addition of 1.5ml of 0.44M trichloroacetic acid (TCA) and centrifuged. The digested casein in the supernatant was determined using Folin ciocalteu's reagent. Venom without plant extract was considered as control or 100% activity.[16]

***In vivo* study**

Various doses of venom in 0.2ml of physiological saline were injected into the tail vein of mice, using groups of 3-5 mice for each venom dose. The LD50 was calculated with the confidence limit at 50% probability, by the analysis of deaths occurring within 24 h of venom injection. The anti-lethal potentials of plant extracts were determined against 2LD50 of Daboia russelli venom. Various amounts of plant extracts (µl) were mixed with 2LD50 of venom sample and incubated at 37°C for 30min and then injected intravenously into mice. 3-5 mice were used in each anti venom dose. Control mice received the same amount of venom without anti venom (plant extracts). The median Effective Dose (ED50) calculated from the

number of deaths within 24 hours of injection of the venom/anti venom mixture. ED50 was expressed as µl anti venom/mouse and calculated by probit analysis.

In in vitro study, the inhibitory effect of *Stereospermum tetragonum* extracts on the acetyl cholinesterase activity of venom was determined. During this experiment, direct hemolysis of Daboia russelli venom produced 93.50% hemolysis. *Stereospermum tetragonum* plant extract neutralized the hemolysis of RBC's produced by the venom up to 24%.

In phospholipase activity (PLA2) 10µg of Daboia russelli venom was able to produce 11mm diameter hemolytic halo, which is considered to be 1Unit. *Stereospermum tetragonum* extract can capable of inhibiting PLA2 dependent hemolysis of sheep RBC's induced by Daboia russelli venom in a dose dependent manner. In procoagulant activity 100µg of Daboia russelli venom was found to clot human citrated plasma in the 60s.

In the neutralization assay, the absence of clot formation shows the neutralizing ability of plant extract. High concentration of venom caused rapid clotting that required very high concentration of plant extracts to neutralize.

In in vivo study, assessment of venom lethality (LD50) of Daboia russelli venom was assessed and calculated. Assessment of venom toxicity (LD50) of the venom was assessed by LD50 range-finding test and the median lethal dose (LD50) assay using mice (18-20g). LD50 of Daboia russelli venom was calculated and found to be 0.602µg/g. Venom-neutralizing potency test (ED50) using *Stereospermum tetragonum* extract was carried out by pre-incubating constant amount of venom (2LD50) with various dilutions of the plant extracts prior to injection. Calculation of ED50 of *Stereospermum tetragonum* against 3LD50 of venom was done by Miller and Tainter method and found to be 10.47mg/3LD50 venom.

The in vitro enzymatic analysis reveals that the *Stereospermum tetragonum* plant extract could inhibit most of the toxic enzymes of the Daboia russelli. The result from in vivo and in vitro analysis showed that *Stereospermum tetragonum* plant extract possesses neutralizing potential against venoms, in present investigation Daboia russelli neutralize by the aqueous extract of *Stereospermum tetragonum* shows a good anti-venom activity.[5]

Antioxidant activity

Estimation of enzymatic antioxidants

Superoxide Dismutase assay

0.5 ml of leaf extract was taken in test tube and mixed with 1 ml of sodium carbonate (125 mm), 0.4 ml of nitro blue tetrazolium (NBT 25 µM) and 0.2 ml of EDTA (0.1 mm). The reaction was started after the addition of 0.4 ml of Hydroxylamine hydrochloride (1 mm) in the mixture. The absorbance was measured at 560 nm at every 5 minute interval. Units of SOD were expressed as amount of enzyme required for inhibiting the reduction of NBT by 50% and the activity was expressed in terms of units per mg of protein.[17]

Catalase assay

One ml of plant extract was added to 5 ml of phosphate buffer (300 µM, pH 6.8) containing hydrogen peroxide (100 µM) and incubated at 25°C for 1 minute. The reaction was terminated by the addition of 10 ml of sulphuric acid (2%), and the residual H2 O2 was titrated with potassium permanganate until pink colour appeared. Enzymatic activity was calculated by the decomposition of H2 O2 per minute per mg protein.[18]

Ascorbic acid oxidase assay

0.1 ml of leaf extract was added to 3 ml of substrate solution (8.8 mg of ascorbic acid in 300 ml phosphate buffer, pH 5.6) and the change in absorbance at 265 nm was measured at 30 sec intervals for 5 min. One enzyme unit was expressed as to 0.01 OD change per minute per mg protein.[19]

Reactive oxygen species (ROS) like Superoxide anion, hydroxyl radical and hydrogen peroxide produced during cellular metabolism are highly toxic to cellular macromolecules. Superoxide anion is considered as a weak oxidant, as it can produce hydroxyl radical and singlet oxygen, generated during oxidative stress. Catalyse is another antioxidant enzyme widely distributed in animal tissue. They protect the cell from hydroxyl radicals by hydrogen peroxide decomposition. Depletion of this enzyme may enhance oxidative stresses. In vitro antioxidant activity of *Stereospermum tetragonum* in water extract was found to be 27.80±0.14, in ethanol 31.60±0.12 and in methanol 41.53±0.16. From the present study, it can be concluded that Methanolic extract of *S. tetragonum* have significant reducing / in vitro antioxidant activity.[20]

Antimicrobial activity

Antibacterial activity of plant extract was determined by disc diffusion method[21] 100 µl from each standard bacterial stock suspension was spread on the surface of the solidified agar using sterile spreader. Sterile Whatman filter discs (6 mm dia.) was made and socked with

plant extract and placed on the inoculated plate. Each plate also contained one filter disc, as control, soaked in the respective solvent. Plates were then incubated at 37°C for 24h and the inhibition zones (in mm.) measured. The cup-plate agar diffusion method was adopted with some minor modifications to assess the antifungal activity of prepared extract. From each of the fungal stock suspension, 100 µl was thoroughly mixed with 20 ml of sterile molten potato dextrose agar (45°C - 50°C), poured into sterile Petri-dishes and allowed to solidify. cup-shape wells (10 mm dia.) were made and filled with plant extract using sterile p5 pettes. In Petri dish, one well was filled with the respective solvent as a control. Plate was incubated at 25 + 2°C for 72h and the inhibition zone (in mm dia.) was measured.

The findings indicate that the chosen plant serve as a very effective source of new antimicrobial agents for use in pharmaceuticals industries and medicine. Ethanolic and Methanolic extracts of tested plant show good antibacterial capability against tested bacteria. Antimicrobial activity *S. tetragonum* which shows no antibacterial effect with the aqueous extract but activity shows in methanolic and ethanolic extract. Plant extracts were more effective against Gram positive bacteria than Gram negative bacteria. This can be due to the difference in cell wall composition between them that effect permeability and susceptibility of these bacteria to different compounds. The antimicrobial effect is associated with some compounds which can penetrate the outer membrane and reach its site of action, partial by their size and shape. Several mechanisms can be involved in antimicrobial activity of the plant extract such as disruption in protein and cell wall, inactivation of enzyme and interfering in DNA replication. All the standard antibiotics used as positive control showed antibacterial activity against the tested microorganisms.[20]

CONCLUSION

So, this present review article concluded that *Stereospermum tetragonum* is an emerging ayurvedic medicinal important tree has several therapeutic properties. It is used as Antimicrobial, antidiabetic, antioxidant and antivenom agents. The herbal drugs has advantages rather than chemical drugs. In this present article concluded that *Stereospermum tetragonum* is an emerging medicinal important tree and possess several therapeutic properties.

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