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Phytochemical Screening, Antioxidant and Antibacterial Activity of *Strychnos colubrina L.* as an important Endangered Medicinal Species in Eastern Ghats

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Abstract:
To investigate phytochemical screening in qualitative analysis showed the active compounds presence in high concentration, such as flavonoids, steroids, tannins, alkaloids, glycosides, phenols, reducing sugars. Free radical scavenging activity was recorded highest in methanol extracts than ethyl acetate and aqueous root extracts to compare the ascorbic acid and Butylated hydroxyl toluene (BHT). In case of antibacterial activity of methanol extracts showed more inhibition zones than the ethyl acetate and aqueous root extracts to compare the streptomycin. First report of Phytochemical screening, antioxidant and anti-bacterial activity of all the crude extracts of the *Strychnos colubrina L.* root part. The phytochemicals such as alkaloids, phenols, saponins, Flavonoids and reducing sugars may have potentially significant application against antioxidant and microorganisms. This study offers a valuable source for the discovery of alternatives to the present antibacterial and antioxidant drugs.

Key words: *Strychnos colubrina L.*, Phytochemical Screening, antioxidant activity, DPPH method, anti-bacterial activity etc.

INTRODUCTION:

Medicinal plants besides therapeutic agents are also a large source of information for a wide variety of chemical constituents which could be developed as drugs with precise selectivity. These are the reservoirs of potentially useful chemical compounds which could serve as newer leads and clues for modern drug design [1]. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds [2]. Since 1980, the World Health Organization has been encouraging countries to identify and exploit traditional medicine and phytotherapy. The main Indian traditional system of medicine namely Ayurveda and Siddha are primarily plant based systems only. The evolution of new drugs especially phytochemically obtained materials has again opened new vistas for research and development. It is estimated that nearly 70-80% of the world population depends on the traditional medicines which are derived from plant based compounds. It is estimated that nearly 25% of prescribed drugs contain plant extracts or active ingredients obtained from or modelled on plant compounds. For instance, the most popular analgesic drug-aspirin, most valuable anticancer agents-paclitaxel and vinblastine are derived exclusively from plant sources [3]. There exists a well maintained balance between antioxidant mechanism and free radical generation in a normal healthy individual. These balances, however, shift towards the production of excessive free radicals or deficit in antioxidant defence in diseased states and leads to condition called oxidative stress. This oxidative stress is implicated in hundreds of diseases or disorders such as diabetes, cardiovascular diseases, neurological disorders such as Alzheimer's disease and Parkinson's disease, cancer and others. Antioxidants are substances which, when present at low concentrations, in relation to oxidative processes. Endogenous antioxidants such as ascorbic and tocopherols, glutathione, uric acid, thiols etc., and antioxidant enzymes such as superoxide dismutase and catalase defend the body against oxidative stress. However, in pathophysiological conditions, there is an extra requirement for antioxidants from external sources especially food and medicinal plants [4-8]. Microbial infections are the cause of a large burden of diseases and bacteria are listed in the first position among the common microorganisms responsible for opportunistic diseases occurring associated with AIDS. Therapy of bacterial infections is a frequent problem due to the emergence of bacterial strains resistant to numerous antibiotics [9-10].

1. Morphology of *Strychnos colubrina L.*

2. 2. Friuting of *Strychnos colubrina L.*

Figure:1 - Description,Distribution and Medicinal importance of the Plant:

Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years and in many plant parts around the world. Hence, researchers have recently paid attention to safer phytomedicines and biologically active compounds isolated from plant species use. In the present study, we have concentrated on the Phytochemical Screening, antioxidant and antimicrobial activity of *Strychnos colubrina L.*

MATERIAL AND METHODS:

Description:

Large, climbing shrubs ,tendrils simple or bi-fid, leaves 3.5-7x1.5-3.5 cm, elliptical-ovate,acute,base cuneate or obtuse rounded 3-nerved ,coriaceous.Flowers in cymes. Coralla tube and lobes almost equal in length,the throat with woolly tomentum.Ovary and Style hirsute. 2.5-3 mm across. Creamy white in 2-3 cm long.Cymes axillary and on old wood.Berries 1-1.8 cm across, globose, orange-yellow with crustaceous pericarp; Seeds 1-3,0.6-1.2 cm diameter, circular flat. Flowering and Fruiting in May to August. Fig-1

Distribution:

Rare in the moist shades along ravines. While this taxon is known to occur in Western Peninsular India covering parts of Karnataka and Kerala, it is so far recorded only at Veligonda hills of Nellore district in the entire Eastern Peninsular India. It was first collected by Ramaswamy in 1906 (FPM l.c) from Veligonda and again it could be recovered in "Flora of Nellore District, Andhra Pradesh" [11]. The existence of the plant also recorded in Chittoor Distict particularly at Kambakkam hills, Ambakkam, Sadhumalammakona, Brahmadevudigundam(Mamandur) [12]. First Red list of Medicinal plants of Andhra Pradesh, India – Conservation Assessment and Management planning is reported to a Endangered Species in Andhra Pradesh, India [13]. I have recorded at Kilasa kona near to Puttur in Chittoor District.

Medicinal importance:

Wood is used to cure fever. Root is anthelmintic, also useful to the treatment of Rheumatism, cutaneous disorders. Fruit is used to prevent Mania. The tribal people of Veligonda hills used the root part of this plant for the snake bite.

phytochemical screening:

Plant collection and identification:

The plant material roots of *Strychnos colubrina L*. belongs to the family Loganiaceae, were collected from the Veligonda hills area in Eastern Ghats, Nellore district, Andhra Pradesh in January 2011. The plant was identified with the help of' The flora of Nellore district' and 'Flora of Madras'. The roots of *Strychnos colubrina L.* plant material were shade dried, powdered and stored in desiccators till further use. These powders were subjected to solvent extraction process individually. In August 2011. the plant was identified and the Voucher specimen was deposited in the herbarium of department of Botany, S.V.University Tirupati, Chittoor District, Andhra Pradesh.

Preparation of Plant Extract:

The extraction step by Soxhlet apparatus was the least developed part of most analytical procedure and solvent extraction by Soxhlation method developed by Soxhlet in 1879, was adopted in this present work. 500g of fine powdered plant material was taken and soxhlated for 6-8 hrs using soxhlet apparatus with solvents based on the increasing polarity using hexane solvent initially followed by Hexane, ethyl acetate and methanol, Aqueous to get their soluble parts. The components were separated to the solvents based on their polarity. The extract was subjected to rotary evaporator at 40 ^{0}C to remove the excess solvent from the extract. Further all these crude fractions were tested for the detection of secondary metabolites.

Preliminary Screening Tests for Secondary Metabolites:

Preliminary phytochemical analysis of the hexane, ethyl acetate and methanolic crude extracts were carried out to detect the different classes of secondary metabolites in the selected plant materials by adopting standard qualitative methods as described by Gibbs (1974); Dey and Harborne (1989); Evans (1989); Gokhale*et al*., 1993 ; Harborne (1998) [14-20].

Preparation of Test Solutions:

The preliminary tests, for the detection of secondary metabolites, were carried out for the hexane, ethyl acetate and methanolic extracts. 5 grams of each extract was dissolved in 100 ml of the respective solvent and filtered through Whatman No.1 filter paper. Thus, the filtrates obtained were used as test solutions for the following preliminary phytochemical screening tests.

Antioxdant activity of *Strychnos colubrina.L*:

DPPH radical scavenging activity :

DPPH (1, 1- dipheny-2-picrylhydrazyl) solution (0.1mM) was prepared in methanol by dissolving 1.4 mg of DPPH in Methanol. The solution was kept in darkness for 30 minutes to complete the reaction. The free radical scavenging activity of the *Strychons colubrina L.* extracts was determined by DPPH. The antioxidant activity was measured by following method described [21-23].

Briefly, 1ml of 0.1mm methanolic DPPH solution was added to 3ml of different plant crude extracts, at different concentrations (50, 100, 150, 200, 250, 300, 350, µg/ml). The mixture was vigorously shaken and left to stand for 30 minutes under subdued light. The absorbance was measured at 517 nm in a UV spectrophotometer. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. Ascorbic acid Vitamin – C and Butylated hydroxyl toluene (BHT) a good antioxidants, was taken as a standard in this study. As a positive control, synthetic antioxidant Vitamin – C and BHT were used. All determinations were performed in triplicate.

The scavenging percentage (*S%*) was calculated as

$S\% = ((A\text{control} - A\text{sample})/A\text{control}) \times 100$,

where *A*control is the absorbance of the blank control (containing all reagents except the extract solution) and *A*sample is the absorbance of the test sample.

Antibacterial activity:

Different plant extracts was studied for antibacterial activity against pathogenic bacteria (clinical isolates) using agar well diffusion assay method. Antibacterial activity of solvent extracts was tested against three Gram-

positive(*C.perfringens, B.subtilis, S.aureus*)and one Gram-negative (*S.typhi*). The bacteria were maintained on sterile Nutrient agar (HiMedia, Mumbai) slants in a refrigerator. The test bacteria were inoculated into sterile nutrient broth (HiMedia, Mumbai) tubes and incubated overnight at 370 C. The test bacteria were aseptically swabbed on nutrient agar plates using sterile cotton swabs. With the help of a sterile cork borer, wells of 6mm diameter were punched in the inoculated plates all strains were clinical isolates obtained from the Microbiological Laboratory, Botany Department, S.V.University, Tirupati. Chittoor District Andhra Pradesh. The effects were compared with Streptomycin is a positive control. Each concentration of the *S.colubrina L.* plant extracts was tested against different bacterial pathogens. The plates were examined for the zone of inhibition, which appeared as the clear area around the wells. Inhibition zone diameter was measured. The readings were taken in three different fixed directions in all 3 replicates and the average values were tabulated [24-25].

RESULTS:

Phytochemical screening:

Preliminary screening for secondary metabolites was done using colour forming and precipitating chemical reagents on the dried roots of *S. colubrina L.* to generate preliminary data on the constituents of the plant extracts. Qualitative analysis of secondary metabolites from *S. colubrina L.* root extracts is shown in Table-1. The results of the qualitative screening of phytochemical analysis from *S. colubrina L.* Flavonoids, steroids, Phenols, tannins, glycosides, alkaloids and reducing sugars are present in Ethyl acetate, methanolic and Aqueous extracts. But all are absent in hexane. These phytochemical compounds are known to support bioactivity.

DPPH radical scavenging activity :

In the present study, all extracts were found to be effective scavengers against DPPH radical. They were superior to Vitamin – C, Butylated hydroxyl toluene (BHT) and their activities increased in a concentration dependent manner (Fig-2). The Methanol extracts showed the highest DPPH radical scavenging activity than the weakest scavengers of aqueous extracts and compared to the Vitamin – C and BHT at the concentration of 50µg/ml - 350 µg/mL (Table-2). This reports was first regarding antioxidant activity of *S.colubrina L.* The results of the DPPH free radical scavenging assay suggested that root part had potent antioxidant property of scavenging free radicals. These species could be used as a potent source for the cancer chemo protective therapy. Few research studies have been undertaken on the antioxidant activity in medicinal plants using DPPH scavenging assay.

Antibacterial test:

The antibacterial activity of the crude extracts i.e, Ethyl acetate, Methanol and Aqueous extracts of *S.colubrina* root were studied in different concentrations (20 mg/ml, 40 mg/ml, 60 mg/ml, 80 mg/ml) against 4 pathogenic bacterial strains, three Gram-positive(*C.perfringens, B.subtilis, S.aureus*)and one Gram-negative (*S.typhi*). The Methanol extract of *S.colubrina* root exhibited potent antimicrobial activity towards all the microbes. The zones of inhibition values compared with streptomycin(10 mg/ml) are presented in Table No *B.subtilis* was found to be more susceptible towards the methanol extract of root with a maximum inhibitory zone 19.2 mm, followed by Ethyl acetate 15.4 mm, and aqueous extract 4.6 mm. *C.perfringens* was found to be more sensitive to the Methanol extract with a maximum inhibitory zone 18.1 mm, Ethyl acetate and aqueous extract did not show any inhibition against *C.perfringens*. *S.typhi* was found to be more sensitive to the Ethyl acetate extract, with a maximum inhibitory zone 13.7mm, followed by Methanol 9.5 mm. Aqeous extract did not show any inhibition against *S.typhi*. *S.aureus* was found to be more sensitive to the Ethyl acetate extract of root with a maximum inhibitory zone 13.7 mm, followed by Methanol 6.3 mm and least sensitive exhibited to the aqueous extract 2.1mm. The results obtained shows that Methanol extract of roots showed very significant antimicrobial activity against the tested organisms, followed by Ethyl acetate and Aqueous extract.

Table -1:Qulitative analysis of secondary metabolites from *S.colubrina L.* root extracts.

Secondary metabolite	Name of the test	Root			
		I	II	III	IV
Alkaloids	Dragendorff reagent test	-	+	+	+
	Wagners test	-	+	+	+
Flavonoids	Shinoda's test	-	+	+	+
	Ferric chloride test	-	+	+	+
	Zinc- Hcl reduction test	-	+	+	+
	Lead – acetate test	-	+	+	-
glycosides	Kellar-kilini test	-	+	+	-
	Conc.H_2SO_4 test	-	+	+	+
	Molish's test	-	+	+	-
Lignin	Lignin test	+	+	+	-
	Labat test	+	+	-	-
Phenols	Phenol test	-	+	+	+
	Ellagic acid test	-	+	+	+
Saponins	Foam test	-	-	+	+
steroids	Salkowski test	-	+	+	+
Tannins	Gelatin test	-	+	+	-
	Ferric Chloride test	-	+	+	-
	Lead acetate test	-	+	+	-
Reducing sugars		-	+	+	+

' + ' Present, ' – ' Absent, I : Hexane, II : Ethyl acetate, III: Methanol, IV: Aqueous extracts.

Table-2: DPPH radical scavenging activity :

S.No.	Ug/ml	Ethylacetate % Inhibition	Methanol Extract % Inhibition	Aquoeus Extract %Inhibition	Vitamin-C	BHT
1.	50	24.16±0.37	37.23±0.37	18.56±0.35	53.56±0.41	49.63±0.25
2.	100	30.96±0.55	47.1±0.45	22.46±0.45	63.46±0.35	58.6±0.2
3.	150	37.03±0.55	52.13±0.61	31.63±0.25	70.53±0.40	66.6±0.26
4.	200	42.2±0.45	59.3±0.43	34.6±0.3	77.43±0.41	75.6±0.3
5.	250	49.03±0.55	64.33±0.40	39.1±0.45	85.6±0.4	83.5±0.2
6.	300	55.43±0.50	76.16±0.30	42.5±0.4	90.4±0.36	89.53±0.30
7.	350	61.26±0.47	84.3±0.4	47.5±0.45	97.6±0.3	96.4±0.32

Table-3: Antimicrobial activity *Strychnos colubrina* root extracts

Name of organisms	Ethyl acetate Extract				Methanol Extract				Aqueous Extract				Standard value (Streptomycin)
	Zone of Inhibition mm				Zone of Inhibition mm				Zone of Inhibition mm				
	20 mg/ml	40 mg/ml	60 mg/ml	80 mg/ml	20 mg/ml	40 mg/ml	60 mg/ml	80 mg/ml	20 mg/ml	40 mg/ml	60 mg/ml	80 mg/ml	10 mg/ml
C.perfringens	0.0	0.0	0.0	0.0	10.2±1.89	12.8± 0.2	14.2± 1.53	18.1±1.12	0.0	0.0	0.0	0.0	23.4± 1.04
S.typhi	10.5±1.12	12.6±1.23	13.2±1.56	13.7±0.99	6.2±0.98	6.4±0.45	7.3±1.02	9.5±0.86	0.0	0.0	0.0	0.0	13.2±0.02
B. subtilis	8.2± 0.25	10.5±1.12	13.6.±1.56	15.4±2.12	12.4±1.53	15.4±2.03	17.8±2.12	19.2±2.09	2.1±1.5	3.2±1.0	4.1±0.7	4.6±0.8	24.4±0.15
S.aureus	8.3±1.12	11.2±0.96	11.5±1.56	13.7±2.01	2.4±0.02	4.2±0.12	5.2±1.02	6.3±0.89	0.0	0.0	0.0	2.1±0.5	17.6±1.04

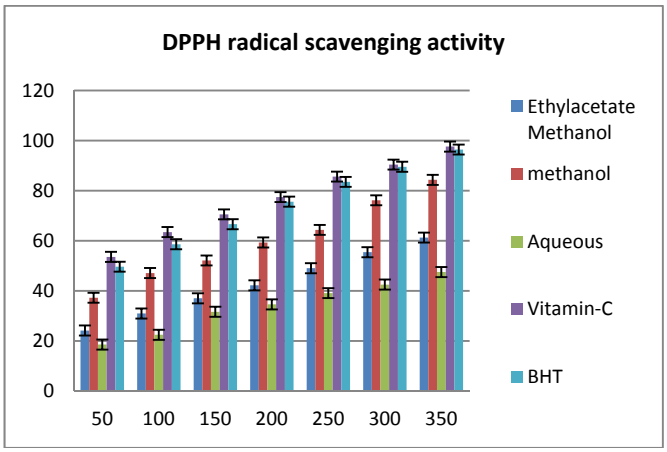
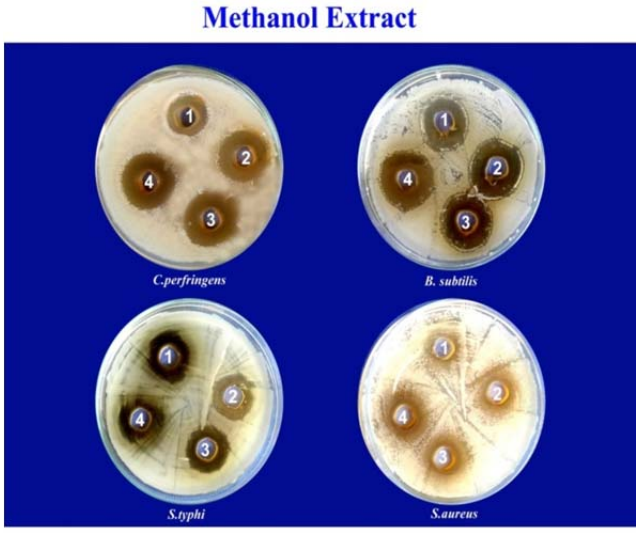
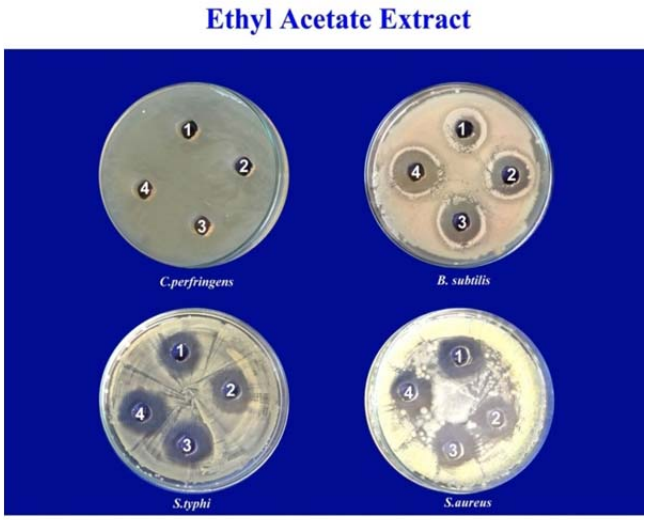


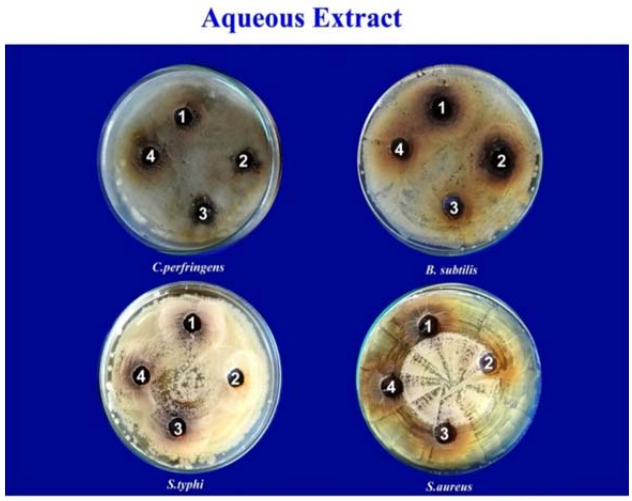
Figure: 2 –DPPH radical scavenging activity:



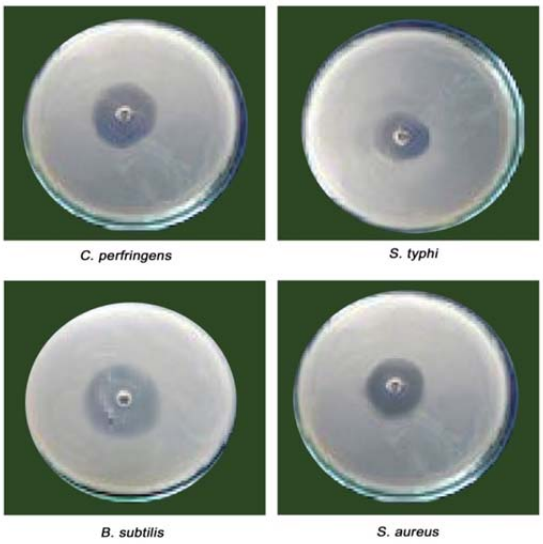
1-20 mg, 2-40 mg, 3-60 mg, 4- 80 mg



1-20 mg, 2-40 mg, 3-60 mg, 4- 80 mg



1-20 mg, 2-40 mg, 3-60 mg, 4- 80 mg



Streptomycin

Figure: 3 – Anti-bacterial activity of Ethyl acetate, Methanol and Aqueous extract

DISCUSSION:

Phytochemical screening, antioxidant and anti-bacterial activity of all the crude extracts of the *Strychnos colubrina L*. root part. The phytochemicals such as alkaloids, phenols, saponins, Flavonoids and reducing sugars may have potentially significant application against antioxidant and microorganisms. The preliminary phytochemical screening of *S.colubrina L.* has revealed the presence of secondary metabolites of therapeutic importance. It is evident from the literature that the genus *strychnos* which is consisting of about 200 species, is known for therapeutic role especially the primary, secondary, tertiary, and quaternary type indole alkaloids as antimicrobials.[26-27].

S.colubrina L. root extracts have a significant antimicrobial activity against the broad spectrum of microorganisms. The antibacterial studies of the extracts against *C.perfringens*, *S.typhi*, *B.subtilis* and *S.aureus*, showed the most promising antimicrobial properties indicating the potential discovery of novel drugs from plants. The order of the antimicrobial efficacy is Methanol extract > Ethylacetate extract > Aqeous extract. Several authors have reported the antimicrobial activity of crude extracts of various strychnos species, such as *S.wallichiana*, *S.nux-vomica* .[28,29].

The result clearly shows that alkaloids, phenols, glycoside's steroids, and flavonoids were responsible for the antioxidant and antimicrobial activity *of S.colubrina L.* root. This study offers a valuable source for the discovery of alternatives to the present antibacterial and antioxidant drugs. However, further study is needed to isolate, characterize and elucidate the structure of bioactive compounds present which were responsible for potent antioxidant and antimicrobial activity. The *S. colubrina L.* Plant parts contain medicinally important bioactive constituents.

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