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Pharmacological evaluation of *Strychnos colubrina* L., an endangered medicinal plant

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Abstract
Plants have been used since ancient times to cure diseases and to improve health and wellbeing of the populations. Plant diversity is a great source of medicines, while it provides direct economic benefits in the form of timber, food, fibre, industrial enzymes, flavours, cosmetics, emulsifiers, dyes, plant growth regulators, bio-pesticides and biofertilizers etc. The secondary metabolites, alkaloids, saponins, quinones, indoles, anthraquinones, terpenoids, flavonoids, phenolic compounds and amino acids are localised in cells. These have therapeutic properties and responsible for phytochemical markers to identify the genuine drug. *Strychnos colubrina* L. was screened for the presence of secondary metabolites using standard protocols as given by Harborne (1973),Gibbs(1974), Kokate (2001)and Khandelwal (2006), followed by pharmacological evaluation. Methanolic leaf extracts are tested for antioxidant and antimicrobial activities. The results were expressed as standard ± mean deviation (SD) of five replicates. Where ever applicable, the data were subjected to one-way and 2-way ANOVA and differences between samples were determined by with Scheffe's post hoc test, Dunnet's test using the program Statistical Analysis System (SASS V25). P Values < 0.05 were regarded as significant. In the preliminary phytochemical studies, the tannins, phenolic compounds, Alkaloids, flavonoids, Saponins, fats, oils, terpenoids have *shown positive test in Strychnos colubrina.* Glycosides, Lignins, Quinones, Anthraquinones, Anthocyanides, Coumarins, Carbohydrates, reducing sugars and Aminoacids are found to be absent. Sterols are absent in *Strychnos colubrina.* Administration of methanolic leaf extracts of *Strychnos colubrine* (200mg/kg) to have significant detrimental effect on one of 3 bacterial strains used i.e, *Staphylococcus aureus* and it has no effect on other 3 microbes namely, *Bacillus subtilis, Pseudomonas aeruginosa* and a fungal strain namely *Candida albicans.* Methanolic extract of plant leaves of *Strychnos colubrina* has not earlier been reported to have anti-microbial activity. The statistical analysis of the DPPH and NO_3 free radical tests with the *Strychnos colubrina* methanol extract has shown significant difference in results with the control groups in both the comparisons. The extracts solution exhibited significant anti-oxidant properties in the both tests Present study is significant and fills the gap in the ethno-medico-botanical repository of Andhra Pradesh.

Keywords: methanolic extract, snake wood tree, phytochemicals, antioxidant, antimicrobial, scheffe's post hoc test, dunnet's test

Introduction

Plants have been used since ancient times to heal and cure diseases and to improve the health and the wellbeing of the populations. Products made out plants for therapeutic or other human health benefits contain either raw or processed ingredients from one or more plants, is the focus of this research. The plant diversity is a great source of medicines, provides direct economic benefits in the form of timber, food, fibre, industrial enzymes, flavours, fragrance, cosmetics, emulsifiers, dyes, plant growth regulators, bio-pesticides and biofertilizers. The biochemicals present in vast majority of the plant species are the great reservoirs of new and potential drugs. They can be used for monitoring the environmental changes. They are the key resources for new genes and biotechnological valuable compounds (Marotrao Dalvi, 2010). Parts of any plant that are used for therapeutic purpose or used a precursor for making a useful drug. This makes it possible to differentiate between medicinal plants whose therapeutic properties and constituents have been established scientifically, and plants that are considered as medicinal but which have not yet been subjected to a thorough scientific study (Sofowora *et al.,* 2013). Medicinal plants are important constitute of flora and are widely distributed in India. The importance of medicinal plants and traditional health systems to solve healthcare problems of the world is gaining increasing attention. Because of this resurgent interest, the research on plants of medicinal importance is growing phenomenally across the world, often to the detriment of natural habitats and mother populations in the countries of origin. In most of the developing countries traditional medical practices have adopted as an integral part of their culture. Medicinal plants form an important pool for the identification of novel drug leads which have proven their value as a source of molecules with therapeutic potential since ancient time (Atanasov *et al.,* 2015). According to Harshberger (1896) the study on ethnobotany has now become a critical need of time.

India-An 'Emporium of Ethnomedicinal Plants'.

India has been one of the oldest countries having well-orchestrated medical practices based on plant resources. So

far on the globe about 4, 80, 000 plant species are discovered. India is known as an 'Emporium of Medicinal Plants' due to occurrence of more than 48,000 plant taxa, of which 18,000 are flowering plants, among these 10,000 plant species are estimated to be used by 4635 ethenic communities for human and veterinary health care.

The plant materials and recipes from herbs used traditionally by various human societies is a challenging field of research in ethnobotanical studies. The indigenous systems of medicine have their roots in folk medicine still practiced in remote tribal and rural areas and aboriginal societies where modern civilization has not yet made in roads. There are some miraculous medicines known to the tribals and rural folk and much acquired knowledge through experience of ages is usually passed on from one generation to other generation as a guarded secret of certain families. Recent studies have shown that groups having common or similar climate and environments but different ethnic and /or religion backgrounds have a very different knowledge and use of medicinal plant. It is not yet clear what factors influence this diversity in utilization.

Fig 1: Strychnos colubrina Plant

Materials and Methods

Leaves of *Strychnos colubrina* were collected from Penchalakona forest of Nellore district Andhrapradesh, India. The plants were identified by comparing with herbarium specimens. The plant material was purified with distilled water and was shade dried for one month. Leaf sample of the material was ground into fine powder and stored in air tight containers at room temperature. The powdered material was then extracted using solvent, methanol in the ratio 1:10 using Soxhlet apparatus. (Khan *et al*., 1988). The solvent was evaporated under reduced pressure in rotatory evaporator to get methanol and ethanol extracts. After extracting all colouring material, the solvent was removed by evaporating in a water bath, which gave rise to a solid isolate of extract used for antimicrobial and antioxidant activities along with phytochemical analysis.

I. Phytochemical analysis: The phytochemical analysis of the leaves was done to find out the presence of bioactive compounds with the help of standard qualitative protocols. (Harborne, 1973 and Gibbs, 1974, Kokate, 2002.)

Tests performed for the presence of phytochemicals

A. Tests for Flavonoids

- **Shinoda test (Magnesium hydrochloride reduction test):** To the test solution few fragments of magnesium ribbon and concentrated hydrochloric acid were added drop wise and reddish to pink colour was resulted.
- **Zinc Hydrochloride reduction test:** To test the sample solution for the flavonoids added a mixture of zinc dust and concentrated hydrochloric acid results in red colour.
- **Lead acetate test:** When aqueous basic lead acetate was added to test sample produces reddish brown precipitate.

Ferric chloride test: To few ml of test samples taken separately, few drops of ferric chloride were added which resulted in the formation of blackish red precipitate.

- **Alkaline reagent test**: To detect the presence of flavonoids, sodium hydroxide solution is added to turn the test sample solution green. When few drops of diluted acid is added the solution turns colourless to indicate the presence of flavonoids.

B. Tests for Saponins

- Foam test: 5ml of extract was vigorously shaken to obtain a stable froth, which was added with olive oil (3 drops). Presence of emulsion indicates the existence of saponins.

C. Tests for Glycosides

- **Kellar Kiliani test:** 1ml of concentrated sulphuric acid was taken in a test tube then 5ml of extract and 2ml of glacial acetic acid and ferric chloride (one drop) and observed for blue color formation.
- **Raymond's test:** Test solution when treated with dinitrobenzene in hot methanolic alkali giving a violet colour.
- **Molisch test:** Alpha naphthol with conc.H2SO4 when added to test sample gives reddish violet ring at the junction of 2 layers.
- **Conc.H2SO4 test:** Conc.H2SO4 was added to test sample which resulted in appearance of reddish colour.
- Legal's test: The test samples when treated with pyridine and sodium nitroprusside solution blood red colour will be developed.
- **Bromine water test:** When bromine solution is added the test solution gives yellow precipitate.

Tests for Alkaloids

- **Dragendorff's test:** 1 ml of the sample solution is taken in a test tube, and few drops of potassium bismuth iodide solution (Dragendorff's reagent) was added. The presence of alkaloids was determined by the appearance of reddish-brown precipitate.
- **Meyer's test:** 1ml of the sample solution is added with few drops of potassium mercuric chloride solution (Meyer's reagent). A creamy white precipitate was formed indicating the presence of alkaloids.
- **Hager's test:** To 1 ml of each of the sample few drops of Hager's reagent (Picric acid) was added. Yellow precipitate was formed reacting positively for alkaloids.
- **Tannic acid test:** When few ml of 10% Tannic acid was added to 1ml of each sample, a buff colour precipitate was formed giving positive result for alkaloids.

- **FeCl 3 test:** One drop of FeCl 3solution was added to each of the test sample, formation of yellow precipitate was resulted reacting positively for alkaloids.

D. Tests for Sterols

- **Libermann-Buchard test:** When few drops of acetic anhydride and few drops of concentrated sulphuric acid were added to the test samples if a brown ring shows up at the junction of two layers, it indicates presences of steroids. 2. Salkowski test: The presence of sterols can be detected by adding few drops of concentrated sulphuric acid to the test samples in chloroform, the lower layers of solution turns green on sterols presence.

E. Tests for Tannins and Phenolic Compounds

- **Gelatin test:** When gelatine and water were added to test samples formation of white precipitate was resulted.
- **Lead acetate:** Few ml of test samples were taken in different test tubes followed by the addition of aqueous basic lead acetate. It results in the formation of reddish brown bulky preceipitate.
- **Alkaline reagent:** The test solution will give a yellowish red precipitate when sodium hydroxide solution was added.
- **Ellagic acid test:** Presence of phenols in the test solution can be detected by adding 5% each of glacial acetic acid and Sodium Nitrite. If the solution turn Niger brown colour, it indicates phenols in solution.

F. Tests for Fats and Oils

- **Stain test:** when we a small quantity of extract between two filter papers, the stain on filter papers gives the presence of the oils.
- **Saponification test:** Added a few drops of 0.5N alcoholic potassium hydroxide to various extracts with a drop of phenolphthalein separately and heat on water bath for 1-2hours. If the solution produces soap or partial neutralization of alkali, it's a sign of presence of oils and fats.

G. Tests for Lignins

- **Labat test:** The test sample turns olive green colour on addition of gallic acid when lignins are present.
- **Furfuraldehyde test:** The test sample turns red colour on addition of fur furaldehyde when lignins are present.

H. Tests for Quinones

- **Alcoholic KOH test:** When alcoholic KOH was added to the test samples red to blue colour appears reacting positively for quinones.

I. Terpenoids and steroids test

- 50% H_2SO_4 is added along the sides of the test tube containing a mixture of methanolic HCl and acetic anhydride. If there is any change in color, from green to blue-green (sometimes via red or blue) indicates the presence of terpenoids and steroids Anthraquinones test
- 5 g plant powder was shaken with 20 ml of benzene and filtered. To the filtrate 5 ml of 10% ammonium hydroxide solution was added and shaken well. Presence of pink red or violet color, in the ammonical phase indicates the presence of free anthroquinones (Fransworth, 1966).

J. Anthocyanidin test

- To the plant extract was added equal volume of methanolic HCl. Appearance of red or purple color indicates the presence of anthocyanidins.

K. Coumarin test

- When few drops of sodium hydroxide are added to the methanolic extracted test solution, if the solutions turning yellow indicates presence of coumarins.

L. Proteins test (Millions test)

- 2 ml of methanolic extract was boiled with a few drops of Million's reagent (Millions reagent is a solution of mercuric nitrate in nitric acid) results in the formation of red color indicates the presence of proteins.

M. Carbohydrate test (Molish test)

- To the methanolic extract, c-naphthol solution (1gm dissolved in 100 ml of ethanol w/v) was added. Then conc. H_2SO_4 is added gently along the walls of the inclined test tube. Appearance of a red to violet color at the interface is taken as a positive reaction.

N. Indole test

- If a violet color was developed on adding Ehrlich reagent to the alcoholic extract, it is considered as a positive reaction for indoles.

O. Test for reducing sugars

- To the 5 ml of methanolic extract, 5 ml of Benedict's reagent was added in a boiling test tube. The test tubes were incubated in boiling on water bath for 15-30 minutes. The formation of an orange red precipitate indicated the presence of reducing sugars.

P. Test for amino acids

- To the methanolic extract, was added few drops of Ninhydrin solution and boiled. The formation of violet colour indicates the presence of amino acids.

Determination of In vitro Antioxidant Activity of *Strychnos colubrina* methanolic leaf extracts Using DPPH Scavenging Method, Nitric oxide radical scavenging method.

A. DPPH (1, 1-Diphenyl-2picrylhydrazyl) free radical scavenging activity:(**Blois, 1958)**

1. The ability of the sample to scavenge DPPH radicals were determined
2. 0.5 ml of aliquot of test at different concentrations (100 µg/ml and 200 µg/ml) in methanol was taken and to this, 100mM methanolic solution (0.5 ml) of 1, 1-Diphenyl-2picryl hydrazyl (DPPH) was added.
3. The mixture was incubated for 30 min in darkness and at ambient temperature.
4. Absorbance was recorded at 517 nm.

B. Nitric oxide radical scavenging activity: (Ganesh *et al*., 2004).

- The standard used here was Ascorbic acid. 100 µg/ml and 200 µg/ml solution of standard in methanol and ethanol were used for assay.
- Sample solution was prepared by dissolving the sample in methanol and ethanol

- 10 mg of each extract were dissolved in methanol and ethanol and solutions of 100 µg/ml and 200 µg/ml concentrations were prepared.
- In this assay 0.5 ml of Sodium nitro prusside (Ganesh *et al*., 2004). (5 mmol) in phosphate buffered saline pH 7.4, was mixed with different concentration of the sample and incubated at 25°C for 3 hrs.
- A control without the test compound, but an equivalent amount of methanol and ethanol were taken. Buffer solution containing tube is acted as control.
- Then 1.5 ml of Griess reagent (1% sulphonyl amide, 2% phosphoric acid and 0.1% N-1-naphthyl ethylene diamine dihydrochloride) was added and incubated for 30 minutes.
- The absorbance of the chromophore formed during diazotization of nitrite ions with suphanilic acid and its subsequent coupling with Napthyl ethyl enediamine was read at 546 nm. The experiment was repeated in triplicate.
- Absorbance was measured at 546 nm and the percentage scavenging activity was measured.

$$\text{Percentage inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of Test X } 100}{\text{Absorbance of Control}}$$

- The percentage of inhibition for each reaction was then calculated, and EC50 values (µg/mL) were calculated from the linear equation from the curve between the percentage of inhibition and the solution's concentration. Each experiment was conducted in triplicate. The EC50 value of each extract was expressed as the mean˘SD. The assays were performed as previously described.

Determination of Antibacterial Activity activity of *Strychnos colubrina* methanolic leaf extracts using Disc Diffusion Assay

Kirby-Bauer method (Bauer *et al* 1966) was followed to perform sensitivity test using disc diffusion with standard antibiotics. The assessment of antimicrobial activity was done based on the measure of diameter of inhibition zones, Sensitivity tests were performed by disc diffusion (*NCCLS, 1998*). The assays were performed against three clinically pathogenic bacteria *Bacillus subtilis MTCC 441*, *Staphylococcus aureus MTCC 737*, *Pseudomonas aeruginosa MTCC 1035* and a fungal pathogen *Candida albicans MTCC 3018.* Each extract was dissolved in 95% Methanol or sterile water at different concentrations (250, 500 and 1000 mg/mL). Then each solution was impregnated on to a small disc of filter paper and placed on the top of agar containing 100 µL of bacterial solution at a concentration of 1.5ˆ108 CFU/mL per plate (adjusted by comparing to 0.5 McFarland). In the same manner each plate has carried a blank disk by keeping solvent control only in the centre, and antibiotic discs (6mm diam) of 20 µg/ml, Streptomycin sulphate (for bacteria) and Nystatin 20 µg/ml, (for fungal) were used as positive control. All of the plates were incubated at 37°C for 18 hours for bacteria and at 28°C for 48 hours for fungi. The zones of growth inhibition around the discs were measured after 18 hours of in incubation at 37°C for bacteria and 48 hours for fungi at 28°C, respectively. The size of inhibitory zone is taken as a measure of sensitivity of micro-organism to the plant extract.

Statistical Analysis

Each test is conducted in three sets. The average value is calculated duly following statistical methods. Experimental results were expressed as standard ± mean deviation (SD) of five replicates.

Where ever applicable, the data were subjected to one-way analysis of variance (ANOVA), 2-way analysis of variance (ANOVA) methods and differences between samples were determined by with Scheffe's post hoc test, Dunnett's post hoc test using the Statistical Analysis System (SAS, 1999) programme. P Values < 0.05 were regarded as significant.

Results

Phytochemical studies

Table 1: Analysis of Phytochemicals from methanolic leaf extracts of *Strychnos colubrina.*

S. NO.	Name of the phytochemical	Name of the test	Methanolic leaf extract of *Strychnos columbrina*
1.	Flavonoids	Shinoda test	+
		Zn-HCl reduction test	+
		Lead acetate test	-
		$Fecl_3$ test	+
		Alkaline reagent test	+
2	Saponins	Frothing test/Foam test	+
3	Glycosides	Keller-Kilianii test	-
		Raymond's test	-
		Molisch test	-
		Conc.H_2SO_4 test	-
		Legal's test	-
		Bromine water test	-
4	Alkaloids	Dragendorff test	+
		Mayer's test	-
		Wagner's test	+
		Hager's test	-
		Tanicacid test	+
		$Fecl_3$ test	+
5	Sterols	Lieberman Burchardt test	+
		Salkowski test	+

6	Tannins and Phenolic Compounds	$FeCl_3$ test	+
		Gelatin test	-
		Lead acetate test	+
		Alkaline reagent test	-
		Mitchell's test	+
		Elagic acid test	+
7	Fats and Oils	Stain test	+
		Saponification test	+
8	Lignins	Labat test	-
		Lignin(furfuraldehyde) test	-
9	Quinones	Alcoholic KOH test	-
10	Terpenoids	50% H_2SO_4 Test	+
11	Anthraquinones	Benzene+ammonium hydroxide test	-
12	Anthocyanides	Methanolic HCl test	-
13	Coumarins	Alcoholic Sodium hydroxide	-
14	Proteins	Million's test	-
15	Carbohydrate test	Molish test	-
16	Indole test	Ehrlich reagent +alcoholic extract	-
17	Reducing sugars	Benedict's reagent test	-
18	Aminoacids	Ninhydrin test	-

'+' Present, '--' Absent

Anti-oxidant properties of *Strychnos colubrine*

DPPH Method

Table 2: DPPH Mean values for Anti-oxidant properties of *Strychnos colubrine*

Treatments	Concentration (µg/ml)	Absorbance at 517 nm	% Absorbance at 517 nm
Control	-	1.68 ± 0.03	0.00%
Standard(Ascorbic acid)	100	0.78 ± 0.03	53.57%
	200	0.47 ± 0.06	72.02%
Methanolic Leaf extract of *Strychnos columbrina*	100	0.82 ± 0.03	51.19%
	200	0.57 ± 0.03	66.07%

Table 3: One-way Anova of DPPH for Anti-oxidant property of *Strychnos colubrine*

ANOVA					
Absorbance Value					
	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	2.734	4	.684	490.579	.000
Within Groups	.014	10	.001		
Total	2.748	14			

Table 4: Dunnett's Post Hoc Test Analysis for DPPH of *Strychnos colubrine*

Multiple Comparisons					
Dependent Variable: Absorbance Value					
Dunnett t (<control)[a]					
(I) Treatment	**(J) Treatment**	**Mean Difference (I-J)**	**Std. Error**	**Sig.**	**95% Confidence Interval Upper Bound**
Standard (Absrobic Acid) 100	Control - DPPH solution	-.89667*	.03048	.000	-.8215
Standard (Absrobic Acid) 200	Control - DPPH solution	-1.20667*	.03048	.000	-1.1315
strychnos columbrina 100 mg	Control - DPPH solution	-.86000*	.03048	.000	-.7849
strychnos columbrina 200 mg	Control - DPPH solution	-1.10667*	.03048	.000	-1.0315
*. The mean difference is significant at the 0.05 level.					
a. Dunnett t-tests treat one group as a control, and compare all other groups against it.					

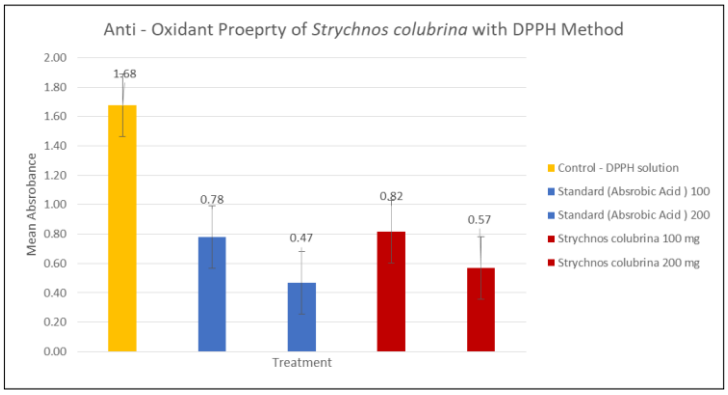

Fig 2: For DPPH method for Anti-oxidant property of *Strychnos colubrina*

Nitric Oxide (NO_3) free radicals absorption method

Table 5: Mean values for NO_3 Free radical scavenging of *Strychnos colubrine*

Treatments	Concentration (µg/ml)	Absorbance at 546 nm	% Absorbance at 546 nm
Control	-	1.5 ± 0.03	0.00%
Standard (Ascorbic acid)	100	0.63 ± 0.06	58.00%
	200	0.34 ± 0.04	77.33%
Methanolic Leaf extract of *Strychnos columbrina*	100	0.66 ± 0.06	56.00%
	200	0.42 ± 0.04	72.00%

Table 6: Anova of NO3 free radical scavenging test of *Strychnos colubrine*

ANOVA					
Absorbance Value					
	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	2.550	4	.638	289.829	.000
Within Groups	.022	10	.002		
Total	2.572	14			

Table 7: Dunnett's Post Hoc Analysis of NO3 test of *Strychnos colubrine*

Multiple Comparisons					
Dependent Variable: Absorbance Value					
Dunnett t (<control)[a]					
(I) Treatment	**(J) Treatment**	**Mean Difference (I-J)**	**Std. Error**	**Sig.**	**95% Confidence Interval**
					Upper Bound
Standard (Absrobic Acid) 100	Control -nitric oxide	-.87333*	.03830	.000	-.7789
Standard (Absrobic Acid) 200	Control -nitric oxide	-1.15667*	.03830	.000	-1.0622
strychnos columbrina 100 mg	Control -nitric oxide	-.84000*	.03830	.000	-.7456
strychnos columbrina 200 mg	Control -nitric oxide	-1.07667*	.03830	.000	-.9822
*. The mean difference is significant at the 0.05 level.					
a. Dunnett t-tests treat one group as a control, and compare all other groups against it.					

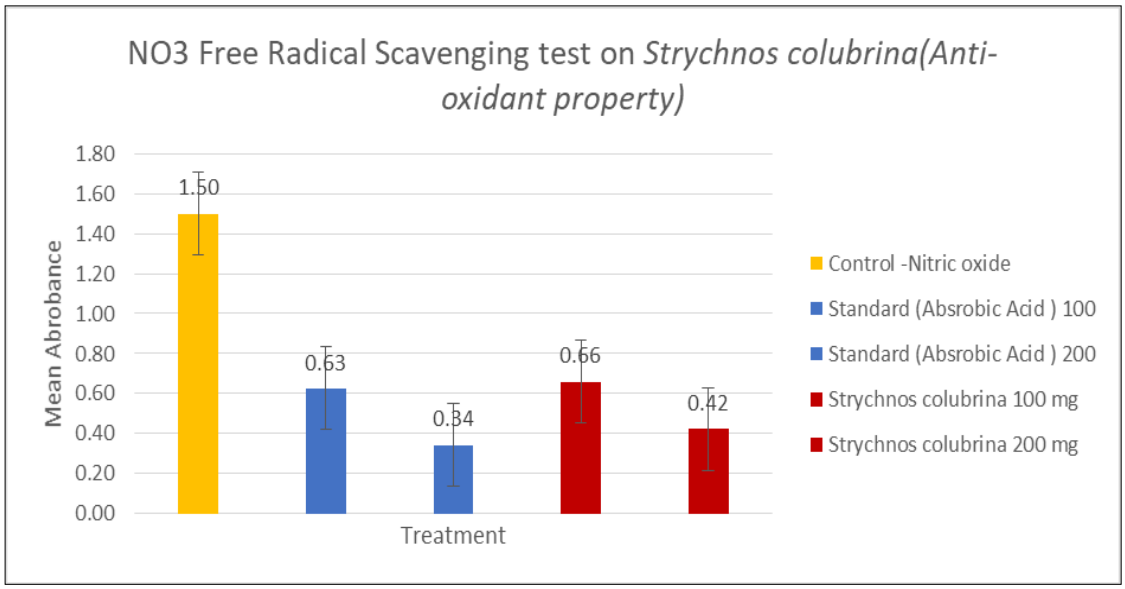

Fig 3: For NO3 Free Radical scavenging test of *Strychnos colubrina*

Anti-microbial activity of and *Strychnos Columbrina*

Table 8: Anti-microbial property of *Strychnos Colubrina*

Solvent extracts	Zone of inhibition in mm											
	Names of microbes											
	***Bacillus subtilis* (MTCC 441)**	***Staphylococcus Aureus* (MTCC 737)**	***Pseudomonas aeruginosa* (MTCC 1035)**							***Candida albicans***		
Quantity (Micrograms)	1000	500	250	1000	500	250	1000	500	250	1000	500	250
Methanolic extract of *Strychnos Columbrina*	-N-	-N-	-N-	17.7 ± 1.2	16.5 ± 0.5	15.5 ± 0.4	-N-	-N-	-N-	-N-	-N-	-N-

Table 9: 2-way Anova of Anti-microbial property of *Strychnos Colubrina*

Tests of Between-Subjects Effects					
Dependent Variable: Zone of Inhibition in mm					
Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Corrected Model	1867.310[a]	11	169.755	1055.474	.000
Intercept	620.010	1	620.010	3854.984	.000
Dosage	1.820	2	.910	5.658	.010
Microbe	1860.030	3	620.010	3854.984	.000
dosage * microbe	5.460	6	.910	5.658	.001
Error	3.860	24	.161		
Total	2491.180	36			
Corrected Total	1871.170	35			
a. R Squared =. 998 (Adjusted R Squared =. 997)					

Table 10: 2-Scheffe's Post Hoc analysis of Anti-microbial property of *Strychnos Colubrina*

Multiple Comparisons						
Dependent Variable: Zone of Inhibition in mm						
Scheffe						
(I) microbe	**(J) microbe**	**Mean Difference (I-J)**	**Std. Error**	**Sig.**	**95% Confidence Interval**	
					Lower Bound	**Upper Bound**
Bacillus subtilis	Staphylococcus aureus	-16.6000*	.18905	.000	-17.1680	-16.0320
	Pseudomonas aeruginosa	.0000	.18905	1.000	-.5680	.5680
	Candida albicans	.0000	.18905	1.000	-.5680	.5680
Staphylococcus aureus	Bacillus subtilis	16.6000*	.18905	.000	16.0320	17.1680
	Pseudomonas aeruginosa	16.6000*	.18905	.000	16.0320	17.1680
	Candida albicans	16.6000*	.18905	.000	16.0320	17.1680
Pseudomonas aeruginosa	Bacillus subtilis	.0000	.18905	1.000	-.5680	.5680
	Staphylococcus aureus	-16.6000*	.18905	.000	-17.1680	-16.0320
	Candida albicans	.0000	.18905	1.000	-.5680	.5680
Candida albicans	Bacillus subtilis	.0000	.18905	1.000	-.5680	.5680
	Staphylococcus aureus	-16.6000*	.18905	.000	-17.1680	-16.0320
	Pseudomonas aeruginosa	.0000	.18905	1.000	-.5680	.5680
Based on observed means. The error term is Mean Square (Error) =. 161.						
*. The mean difference is significant at the. 05 level.						

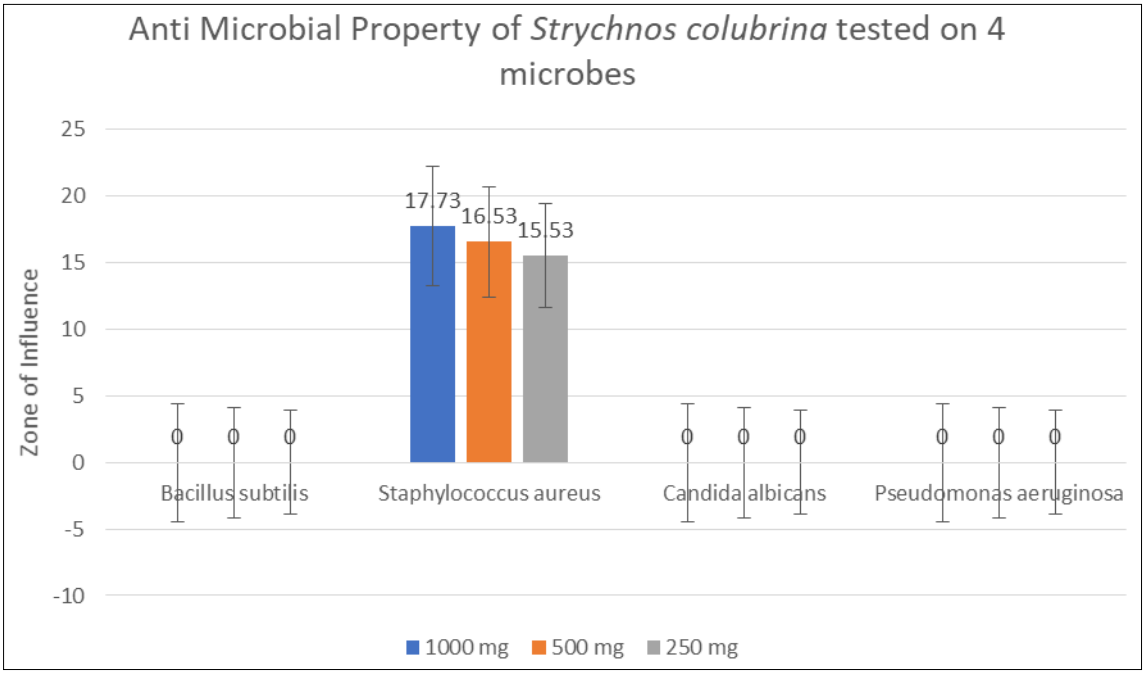

Fig 4: Anti-microbial property of *Strychnos Colubrina*

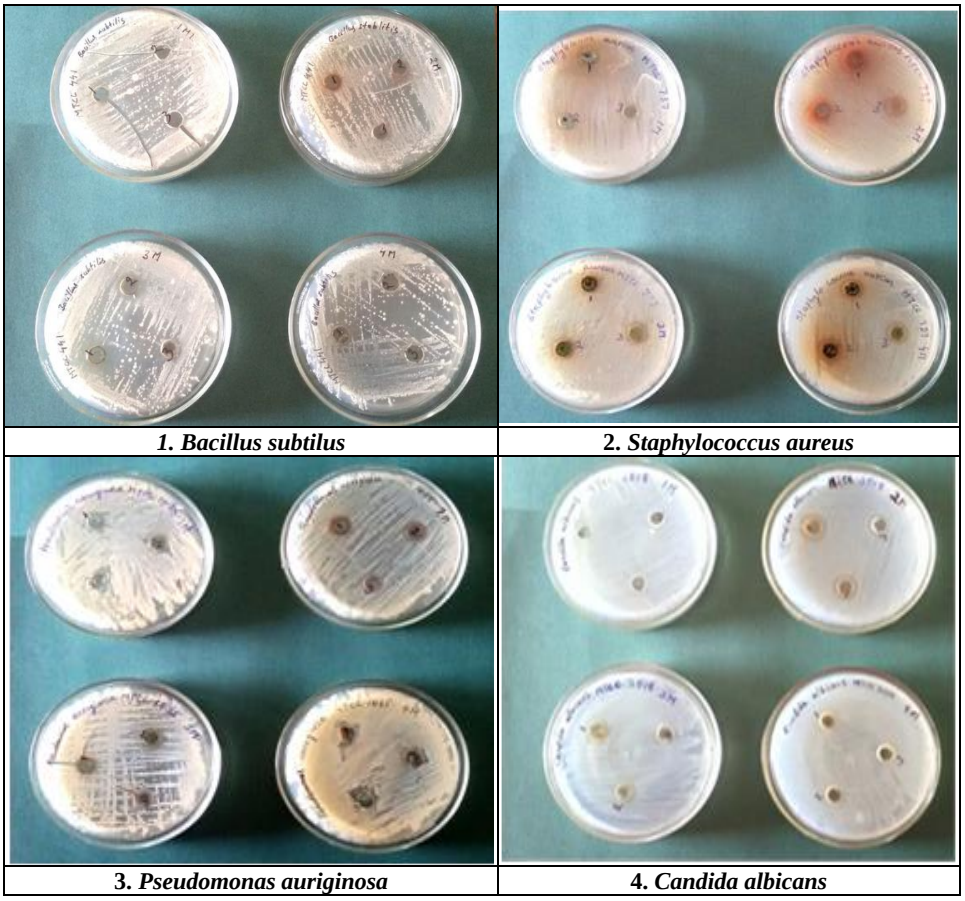

Fig 5: Plates with Anti-microbial activity

Conclusion

The phytochemical studies: These studies have been carried out during the present study for preliminary screening of secondary metabolites and qualitative analysis of whether these compounds are present or not in various taxa. These studies act as an identification marker of genuine plant-based drugs. In the preliminary phytochemical studies, the tannins, phenolic compounds, alkaloids, flavonoids, saponins, fats, oils, terpenoids are known to present in *Strychnos colubrina.* Whereas glycosides, lignins, quinones, anthraquinones, anthocyanides, coumarins, carbohydrates, reducing sugars and aminoacids and Sterols are found to be absent in this plant

The anti-oxidant activity of *Strychnos Colubrina:* Phytochemical analysis of the extracts has shown the presence of some flavonoids and phenolics. These compounds give antioxidant effect, and this can be used for drug extractions from *Strychnos colubrina* in the future. Quantitative analysis of these bio-active constituents in *Strychnos colubrina* leaves have to be further investigated in the future. They can become potent antioxidants.

Anti-microbial activity of *Strychnos Colubrina:* The present study reveals that *Strychnos Colubrina* leaf extracts have significant detrimental effect on one of three bacterial strains used i.e, *Staphylococcus aureus* and it shows no effect on other two bacteria namely, *Bacillus subtilis*, *Pseudomonas aeruginosa* and a fungal strain namely *Candida albicans*. Methanolic plant extract generally contain various potent phytochemicals in mixed form. This may be the reason for the lesser inhibitory effect shown by leaf extract when compared to that exerted by proven antibiotics. For the study of plant extracts, the number of microbial strains was reduced in accordance with their known function as food destroying agents and human pathogens. The methanolic extract of plant leaves of *Strychnos colubrina* has not earlier been reported to have anti-microbial activity.

As discussed above, medicinal plants are at the mainstay in the treatment of diseases for the vast majority of tribal and rural people. As the present study reveals the potential of these medicinal plants, further research can be directed to probe into the structure and mechanism of action of the bioactive therapeutic principles for development of drugs to treat various diseases that affect the mankind. Phytochemical screening of ethno-medico-botanical species indicated the presence of numerous potential chemical constituents which have high therapeutic value. Thus, the phytochemical studies of this species may lead for further investigations to find out drugs of importance. The present study concludes that the plant species with numerous highly effective phytochemical compounds like saponins, alkaloids, terpenoids, steroids, lignins, tannins, indoles, anthroquinones and several types of anthocyanidins, phenolic compounds, flavonoids and lipid compounds having many medicinal applications, used in wide range of formulations, commercial, higher therapeutic and economic values. Biological activity conducted in screening the medicinal properties of selected plant material of methanolic extracts of leaves of *Strychnos colubrina* has proven as antibacterial and anti-oxidant, agent. It is, therefore, implied that the isolation and proper characterization of the active

constituents from the extracts of the select plant species as possible antioxidant, antimicrobial agent is possible. Quantitative analysis of the active compounds in *Strychnos colubrina L.* leaf extracts should be further studied. Hence this report provides insights into the therapeutic potential of the phytomedicine and scope for further research on these drugs. Further investigations must be carried out from this research for the benefit of human welfare. The present screening may also be useful for continuing, future research mainly in the field of pharmaco toxicology.

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