Research Article

Phytochemical Screening, Antioxidant and Antibacterial Activity in Various Leaf Extracts of *Psychotria bisulcata* Wight & ARN. (Rubiaceae) from the Southern Western Ghats

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ABSTRACT

Aim: Rubiaceae is the largest family which comprises of various medicinal plants which has numerous phytopharmacological properties. The aim of the research is to analyse the secondary metabolites, antioxidant, and antibacterial effectiveness of several leaf extracts of *Psychotria bisulcata*.

Materials and Methods: The leaf powder has been extracted with five solvents and analysed for phyto components and radical scavenging activities with standard protocols. Three strains of gram-negative and gram-positive bacteria; *Staphylococcus aureus*, *Bacillus subtili, Streptococcus pneumonia, Klebsiella pneumonia, Salmonella typhi* and *Pseudomonas aeruginosa* were inhibited by well diffusion method.

Results: The preliminary phytochemical evaluation indicated the presence of abundant secondary compounds likely glycosides, flavono glycosides carbohydrates, proteins, amino acids, phytosterols, alkaloids, tannins, phenols and flavonoids in addition the absence of gums & mucilages, fixed oils & fats. The major phytochemicals were quantitatively analysed, with higher results in the ethanolic extract. Antioxidant activity was evaluated using two methods: DPPH and the Reducing Power assay, with the ethanolic extract demonstrating the maximum radical scavenging activity. The antibacterial activity of the ethanolic extract exhibits a better inhibition zone when compared to other extracts.

Conclusion: The study confirmed that the plant has various secondary metabolites, better radical scavenging activity and anti-bacterial activity which can be utilized by the pharmaceutical industries.

Keywords: *Psychotria bisulcata*, Secondary metabolites, Antioxidant activity, Antibacterial activity.

INTRODUCTION

One of the two main kingdoms of life forms is the plant kingdom. They are the only life on the earth that can use solar energy to produce their own food[1]. Plants are essential to the habitats they live in and help to improve the environment. A medicinal plant is defined as any plant that contains chemicals that can be used for therapeutic reasons or that act as precursors to the manufacture of valuable pharmaceuticals[2]. Several plants have been utilised in traditional medicine for many years. Secondary metabolites produced by plants constitute novel sources of medications, additives to food, flavouring and other commercial commodities, and the use of plant cell cultures has faced several hurdles in their production[3]. *Psychotria bisulcata* belongs to the family Rubiaceae. Natural therapies often use plants from the genus Psychotria (leaves, barks, roots and rhizomes) to cure bronchial, digestive disorders such as cough, bronchitis, ulcer and pain in abdomen[4-6].

MATERIALS AND METHODS

Collection of the plant samples

Psychotria bisulcata plant was collected in The Nilgiris District, Tamil Nadu. The plant was validated by the botanical survey of India, Southern Circle, Coimbatore using the deposited specimen. The voucher number for the specimen is BSI/SRC/5/23/2021/Tech-204. The leaf was shade dried and roughly pulverised. The powder was stored in an airtight container and used for further extractions.

Solvent extraction

To extract nonpolar and polar compounds, the pulverised plant sample was extracted with petroleum ether, chloroform, acetone, and ethanol using Soxhlet equipment at 55-85^{o}C for 8-10 hours. The aqueous extract was subsequently extracted using cold maceration method. Extract solvents were brought down to ambient temperature and kept at 4^{o}C for future usage.

Extract preparation

Plant samples extracted with petroleum ether, chloroform, acetone, ethanol, and aqueous were concentrated under decreased pressure using a rotating vacuum evaporator to remove water molecules. The lyophilized powder was kept at -20^{o}C for future investigations.

Phytochemical Screening

Secondary metabolites are chemicals produced by plants that help them to compete in their own environment. Phytochemical screening indicates the plant's physiological and therapeutic activity. Assays were performed to identify the preliminary phytochemical screening according to the standardized protocol [7-11].

Estimation of Secondary Metabolites

Establishing bioactive entities from plant sources will help identify viable drugs and objectively confirm the existing conventional method. The overall flavonoid, tannin and phenol composition of the *P. bisulcata* leaf extracts were validated employing aforementioned standard methodologies[12-14].

Antioxidant analysis

Natural antioxidants are derived from plants, and their effectiveness varies in response to plant species, diversity, extraction procedures, along with growth environments. Two methods was used to determine antioxidant activity: 2,2 diphenyl-1- Picryl-Hydroxyl assessment and the reducing power assay[15,16].

Antibacterial activity

Antibacterial activities of *P. bisulcata* leaf were studied using six bacterial pathogenic strains obtained from the Department of Biochemistry, Bharathiar University, Coimbatore. The bacterial strains used were *Bacillus subtilis, Staphylococcus aureus, streptococcus pneumonia, Salmonella typhi, Klebsiella pneumonia,* and *Pseudomonas aeruginosa.* The impact of different extracts of leaf on the six bacterial pathogenic strains has been evaluated using the Agar well diffusion method[17].

Statistical Analysis

The results were expressed as Mean ± SD/SEM. The data were statistically analyzed using SPSS version 21 by means of one-way ANOVA.

RESULTS AND DISCUSSION

Plants produce secondary metabolites known as phytochemicals. They are biologically functional substances which exist naturally in plant life. Phytochemical physiological functions comprise of antioxidant and antibacterial qualities, detoxification of enzymatic modulation, systematic immune activation and metabolism of hormone regulation[18]. The phytochemical properties of the plant *P.bisulcata* were summarised in the table. 1. The results indicated the presence of a therapeutically active chemical. All the five leaf extracts contained glycosides, flavono glycosides, proteins, amino acids, carbohydrates, tannins, flavonoids, alkaloids, phenols and phytosterols, to that absence of gums & mucilages and fixed oils & fats in all the extracts.

Phenolic and flavonoid chemicals are naturally occurring substances with high antioxidant activity that can be found in many parts of plants. Phenolic and flavonoid molecules can receive electrons from reactive oxygen species, producing phenoxyl radicals that are far more stable[19]. The total flavonoid content in the leaf extracts of *P.bisulcata* are expressed in rutin equivalents and presented in table 2. The maximum flavonoid content was obtained in the ethanolic leaf extract 169.71 ± 0.35 mgRE/g, while the aqueous extract exhibited a relatively lower level of flavonoids 8.48 ± 0.2 mgRE/g. The ethanolic extract had the highest phenolic and tannin content 700.28 ± 2.7 mgGAE/g and 635.77 ± 2.42 mgGAE/g, respectively, while the petroleum ether extract had lower levels 36.41 ± 2.70 mgGAE/g for phenols and 18.37 ± 2.23 mgGAE/g for tannins. The existence of phenolic compounds in medicinal plant extracts enhance the ability of free radical scavenging capacity of live cells. Flavonoids are plant secondary metabolites carrying a polyphenolic structure that are frequently encountered in fruits, vegetables, along with certain beverages[20]. Catechins and other flavanols can serve as a plant's defence mechanism against pests due to their astringency[21]. These flavonoids have been identified to have anti-cancer, anti-microbial, anti-oxidant, anti-inflammatory, ulcer-preventing, and anti-edematogenic properties. 30% of the research seen concentrated on anti-oxidant functions of flavonoids, which are crucial in protecting the body from free radicals and oxidative stress[22]. Polyphenols are secondary metabolities from plants that are crucial for sustaining plants from UV radiation and pathogenic attacks[23]. Multiple research investigations examined the anti inflammatory, anti- aging, antiproliferative, and antioxidant effects of phenolic compounds[24]. Tannin is a natural polyphenolic compound[25].

The efficacy of *P. bisulcata* samples to scavenge free radicals was examined using the DPPH radical scavenging assay, and the results were compared to standards comprised of rutin and BHT. The lowest inhibitory concentration was obtained in ethanol 24.85 µg/mL and the highest in petroleum ether extract 40.44 µg/mL. (Figure 1). The Figure 2 shows the reducing power ability of the plant extracts. The ethanolic extract had the maximum reducing power (0.378 ± 0.002 GAE/L), whereas the petroleum ether extract had the lowest (0.190 ± 0.21 GAE/L).

P.bisulcata plant was tested for antibacterial properties. *Bacillus subtilis*, a rod-shaped spore-forming bacteria, was inhibited in various solvents for zone formation; the species had no zone of inhibition in petroleum ether, but the highest zone of inhibition was found in ethanol (19.23mm). *Staphylococcus aureus* showed no zone development in chloroform extract and a significant zone formation in ethanol (17.65 mm). *Streptococcus pneumonia* was least in petroleum ether (10.14 mm) and substantially in ethanol (17.64 mm). *Salmonella typhi* inhibits minimum with petroleum ether (14.32 mm) and most significant with ethanol (24.78 mm). The chloroform (9.75mm) extract of *Klebsiella pneumonia* was the least effective, while the ethanol (15.78mm) extract performed better. In *Pseudomonas aeruginosa*, petroleum ether extract shows no zone of inhibition, whereas ethanol (15.26mm) has better results. (Table 3). *Bacillus subtilis* is an aerobic, Gram-positive soil bacterium[26]. *Bacillus* genus, which is frequently spotted in soil, water and several non-dairy fermented foods, also occur in human and animal guts[27]. In the present investigation, several leaf extracts of *P. bisulcata* demonstrated a broad spectrum of activity against all

microorganisms tested. According to the analysis, a significant proportion of plant extracts show antibacterial effect. The petroleum ether extract showed no appreciable action against the microorganisms tested. According to Jayasinghe *et al*., *Psychotria* sp. extracts exhibited substantial antibacterial potential against *E. coli, B. subtilis, and A. niger*[28]. The current investigation clearly reveal that *Psychotria* extracts have antibacterial effects against *Bacillus* and Salmonella typhi. Khan *et al*., discovered that methanol extracts of *Psychotria microlabastra* roots, leaves, stems, and barks have extensive antibacterial qualities against *S. aureus, E.coli, K. pnemoniae, B. subtilis, S. pneumoniae, and P. aeruginosa*[29]. *P. bisulcata* leaf extracts demonstrated strong antibacterial effect when tested[29]. In accordance with Giang *et al*., the polar fractions of *Psychotria reevesii* Wall. Exhibit a greater quantity of tannin content, which is reportedly essential for its antibacterial capabilities, in addition to its strong inhibiting potency against *Pseudomonas aeruginosa* and *Staphylococcus aureus*[30]. The ethanolic leaf extract of *P. bisulcata* was highly effective against *Salmonella typhi* and *Bacillus subtilis*.

Table 1: Phytochemical screening of *P. bisulcata* leaf extracts.

Sample	Leaf				
Phytochemical	**P.E**	**C.F**	**A**	**E**	**AQ**
Carbohydrates	L	L	M	AB	M
Proteins	L	L	L	AB	M
Amino acids	L	L	L	AB	M
Alkaloids	L	L	M	M	L
Saponins	A	A	L	M	M
Phenol	M	L	M	M	M
Tannins	M	L	L	M	L
Flavonoids	L	L	M	AB	M
Glycosides	L	L	L	M	L
Flavonos glycosides	L	L	M	M	M
Cardiac glycoside	L	L	L	M	M
Phytosterols	L	M	M	M	L
Fixed oils & fats	A	A	A	A	A
Gums & mucilages	A	A	A	A	A

AB- Abundantly present; M- Moderately present; L- Low; A- Absent

Table 2: Quantitative phytochemical screening of *P. bisulcata* leaf extracts.

Solvent	Flavonoids (mg RE/g extract)	Phenols (mg GAE/g extract)	Tannins (mg GAE/g extract)
Petroleum ether	21.34 ± 0.11	36.41 ± 2.70	18.37 ± 2.23
Chloroform	60.8 ± 0.23	141.17 ± 2.22	91.92 ± 2.56
Acetone	138.35 ± 0.31	473.95 ± 3.66	423.88 ± 4.47
Ethanol	169.71 ± 0.35	700.28 ± 2.7	635.77 ± 2.42
Aqueous	8.48 ± 0.2	171.14 ± 0.48	170.48 ± 0.26

RE- Rutin equivalent; GAE- Gallic acid equivalent

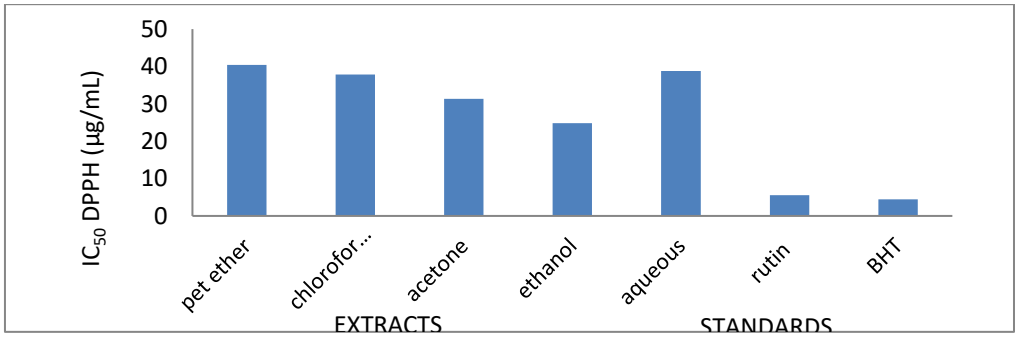


Figure 1: DPPH scavenging activity of *P. bisulcata* leaf extracts.

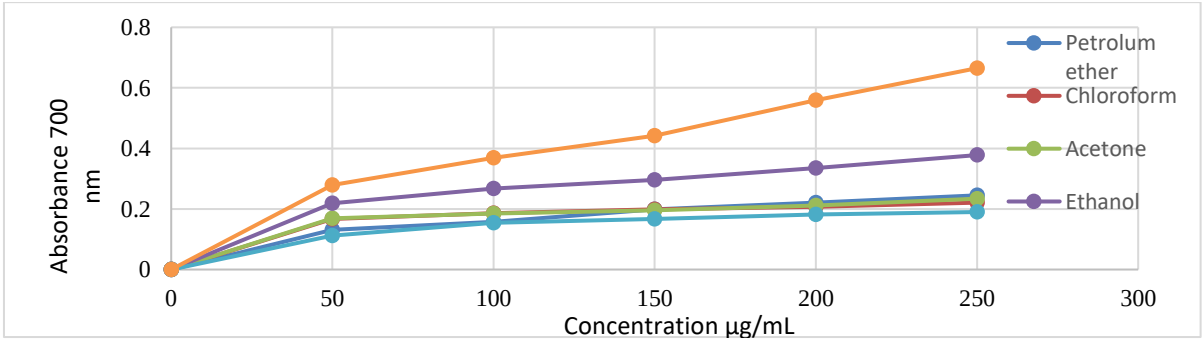
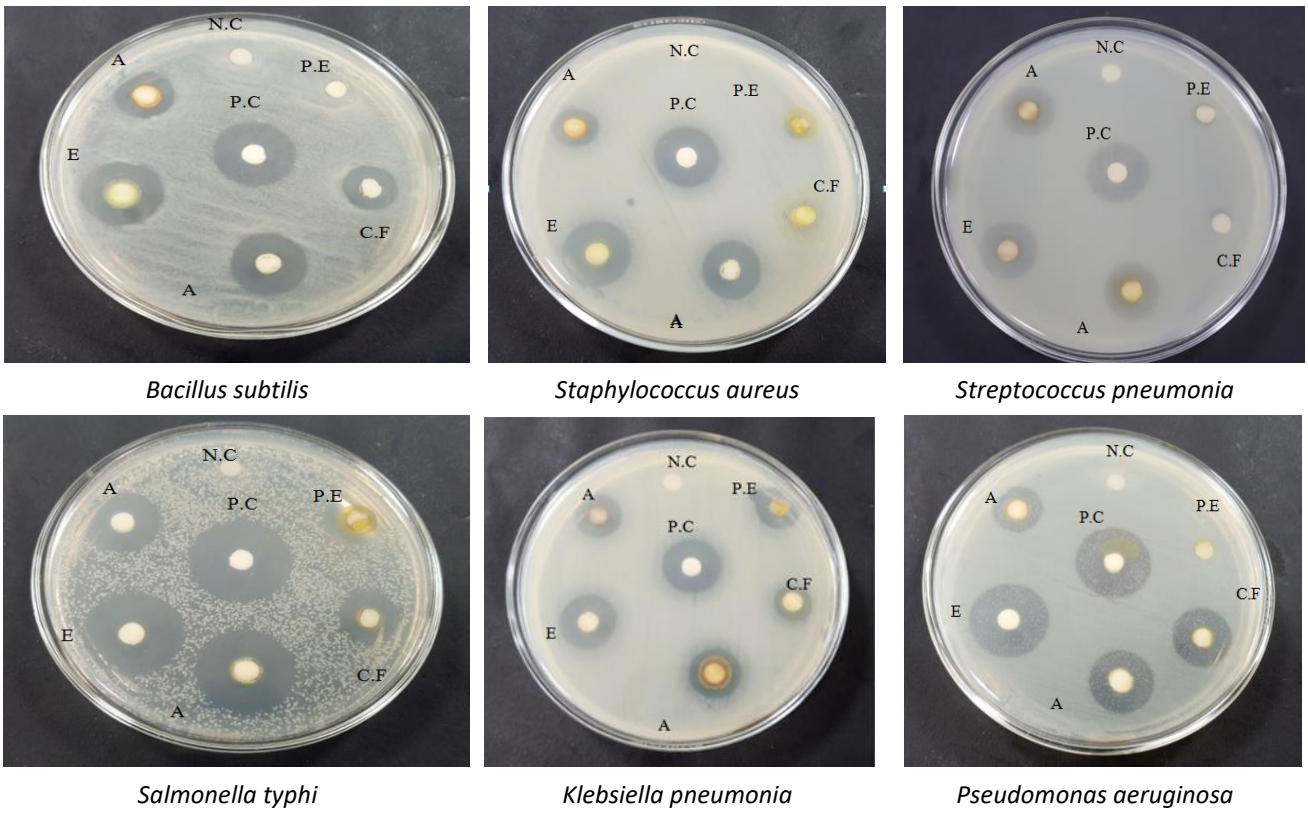


Figure 2: Reducing power assay of *P. bisulcata* leaf extracts.

Table 3: Anti-bacterial activity of *P. bisulcata* Leaf Extracts.

Solvent	Concentration (mg/mL)	Diameter of Zone of inhibition (mm)					
		Gram Positive			Gram Negative		
		Bacillus subtilis	***Staphylococcus aureus***	***Streptococcus pneumonia***	***Salmonella typhi***	***Klebsiella pneumonia***	***Pseudomonas aeruginosa***
Negative Control	-	-	-	-	-	-	-
Petroleum ether	10	-	7.12 ± 0.12	10.14 ± 0.85	14.32 ± 0.12	10.42 ± 0.19	-
Chloroform	10	12.3 ± 0.12	-	11.48 ± 0.54	14 42. ± 0.15	9.75 ± 0.27	8.15 ± 0.08
Acetone	10	17.41 ± 0.34	14.36 ± 0.08	12.45 ± 0.36	20.15 ± 0.36	13.41 ± 0.36	13.45 ± 0.5
Ethanol	10	19.23 ± 0.26	17.65 ± 0.45	17.64 ± 0.24	24.78 ± 0.46	15.78 ± 0.56	15.26 ± 0.54
Aqueous	10	14.26 ± 0.19	11.39 ± 0.28	14.35 ± 0.42	16.42 ± 0.16	12.28 ± 0.5	11.45 ± 0.55
Positive Control (Streptomycin)	1	21.47 ± 0.43	19.74 ± 0.48	18.34 ± 0.42	26.43 ± 0.16	16.46 ± 0.75	17.56 ± 0.46



NC- Negative Control; PC- Positive Control; PE- Petroleum ether; CF- Chloroform; AC- Acetone; E- Ethanol; A- Aqueous.

Figure 3: Anti-bacterial activities of the various solvent Leaf Extracts of *P. bisulcata.*

CONCLUSION

Medicinal plants have been traditionally used in healthcare since ancient times. The pharmaceutical industry uses many of the secondary metabolites produced by plants. Flavonoids exhibit a wide range of pharmacological actions, comprising of antioxidant, anti-biotic, anti-inflammatory, anti-mutagenic, anti-cancer, anti-diabetic, immunomodulatory, and anti-oestrogenic properties. Phenolic antioxidants seem to be the most significant secondary metabolities, as they have shown promising radical scavenging activity in *in-vitro* assessments. As a result, presence of phytochemicals, antioxidants and strong antibacterial properties of the plant *Psychotria bisulcata* can be employed in the pharmaceutical industry to produce drugs.

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ABBREVATION

DPPH: 2,2-diphenyl-1-picrylhydrazyl, RE - Rutin Equivalents, GAE - Gallic Acid Equivalents, BHT- butylated hydroxytoluene.

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