

Pharmacognostic Assessment of the Endemic and Vulnerable Medicinal Climber-*Cayratia pedata* (Lam.) Gagnep. var. *glabra* Gamble and Its Antibacterial Activity

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ABSTRACT

Objective: The objective of this study is to evaluate a meticulous pharmacognostic crum is to supplement constructive information with regard to its identification, characterization, and standardization of endemic and endangered medicinal climber *Cayratia pedata* var. *glabra* and also screening the antibacterial activity of this climber. **Materials and Methods:** The morphological characters of study plant, microscopic examination of leaf powder, anatomy of young stem, physicochemical analysis of leaf powder, extractive values, phytochemical analysis, powder with different chemical reagents, fluorescence analysis of plant powder, and other World Health Organization (WHO) recommended for standardization were analyzed. The antibacterial activity of this study plant is also analyzed. **Results:** *C. pedata* var. *glabra* belongs to the family Vitaceae, commonly known as "Kattuppirandai" is one such endemic and endangered species in Thaisholai, Nilgiris South Division, Western Ghats. With the patronage of veteran ethnic group traditional knowledge of this region, the species *C. pedata* var. *glabra* was selected for the pharmacognostical examination and antibacterial screening. There were no pharmacognostical reports of this plant, specifically to determine the anatomical and other physicochemical standards required for its quality control. The current study deals with pharmacognostical parameters for the aerial parts of study plant, which mainly consists of macromorphological and microanatomical characters, physicochemical constants (ash values and extractive values), fluorescence analysis, and phytochemical screening, one of the WHO accepted parameter for the identification of medicinal plants. The pharmacognostical exploration was undertaken for this species with the purpose of sketch the pharmacopeial standards. The antibacterial activity of this plant confirms the therapeutic power. **Conclusion:** The information obtained from pharmacognostical studies will be of used for supplementary pharmacological and therapeutical evaluation

of the species and will assist in standardization for quality, purity, and authentication with the help, of which adulteration and substitution can be prevented. The antibacterial activity of this plant confirm the traditional knowledge of local healers on the wound healing property of this species and also suggest this plant species can be used as a promising source for the development of new pharmaceuticals that address the therapeutic needs to cure infectious diseases.

Key words: Antibacterial, *Cayratia pedata* var. *glabra*, macromorphology, pharmacognostic, pharmacological

SUMMARY

- The species *C. pedata* var. *glabra* was selected for present research work, since this species is listed in Red data book and has a wider use for different ailments among the tribal population of Thiashola due to its high medicinal value. Pharmacognostical profile was generated from macroscopical analysis, microscopical studies, powder analysis, physico-chemical constituent values, fluorescence analysis and preliminary phytochemical evaluation. The antibacterial activity of this plant confirms the therapeutic power.

Macromorphology → Microanatomy → Pharmacognostical studies → Antibacterial activity

Abbreviations Used: WHO: World Health Organization; IUCN: International Union for the Conservation of Nature.

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INTRODUCTION

Medicinal plants have a long-standing history in many indigenous communities and persist to provide useful tools for treat various diseases. The practices of traditional medicine are based on hundreds of years of belief and observations, which predate the development and spread of modern medicine.^[1] It is no wonder that the world's one-fourth population, i.e., 1.42 billion people, are dependent on traditional medicines for the treatment of various ailments.^[2] However, a key obstacle, which has hindered the acceptance of the alternative medicines in the developed countries, is the lack of documentation and stringent quality control. There is a need for documentation of research work carried out on traditional medicines. With this backdrop, it becomes extremely important to make an effort toward standardization of the medicinal plant material to be used as a medicine.

Plant assortment

Cayratia pedata var. *glabra* belongs to the family Vitaceae, commonly known as "Kattuppirandai" is one such endemic and endangered

species in Thaisholai, Nilgiris South Division, Western Ghats. Sholas are the places of high biodiversity, which includes many endemic, endangered, and rare species of both flora and fauna. Because of the well-organized structure, the sholas provide ecobalance as well. The Todas, a tribal community lives in the pockets of Western Ghats of Tamil Nadu, are inhabitants of Thiashola. Toda culture is remarkably resilient. With the patronage of veteran ethnic group traditional knowledge, the species *C. pedata* var. *glabra* was selected for the pharmacognostical examination and antibacterial screening since

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this species is listed in Red data book as per the International Union for the Conservation of Nature red list categories, which has a wider use for diverse ailments among the Toda tribes due to its augment medicinal value.

Traditional use

The pharmacognostical studies give helping hand to the researcher in resolving the identity enigmas of the crude drug. In Indian traditional system of medicine, the study genus is used as astringent, diarrhea, refrigerant, hysteria, wound healing, and ulcers. *C. pedata* var. *glabra* is a weak climber of the family Vitaceae. This species can be found in Thiashola and Korakundah range. It is used as medicine in Siddha, Ayurveda, and Folk medicine for treating ulcers, microbial disease, and hepatoprotective. A decoction is administered in uterine and other flukes.

As there is no strong pharmacognostic work recorded on this medicinally potent climber, the present work was undertaken to lay down the standards which could be useful for establishing its authenticity. The findings of the current study can be useful to progress and surge further scientific investigation on the aerial parts of this species and also developing a standardized profile of aerial parts of *C. pedata* var. *glabra* which would be of immense use to identify and establish the authenticity of the study plant and also sustain the antibacterial activity of this plant species.

Plant description

The study plant *C. pedata* var. *glabra* is commonly known as kattuppirandai, ainthilai kodi (5-pedata) in tamil, goalilata in hindi, godhapadi in sanskrit, and velutta sori valli in malayalam. It is a perennial climber grows up to a height of 8–12 m with nodes and internodes having 5-foliolate leaves with (2–3 cm) long petioles and elliptic to oblong leaflets. Tendrils are leaf-opposed, branched, and wiry coiled on the stem. Inflorescence arises just opposite the leaves on the stem. It is axillary, corymbose cymes, and divaricate. Flowers are bisexual, greenish and fruiting peduncles (2–5 cm) long. At young stage the flower is yellow in color later it is white.^[3] Fruits are slightly green color, fleshy, and bilobed shape at apex. It is the important differentiable characterization among the other genus.^[4]

Antibacterial study

Infectious diseases are the world's leading cause of premature deaths, killing almost 50,000 people every day. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world.^[5] The abusive and indiscriminate use of antimicrobial compounds over many years is the main factor responsible for the appearance of the phenomenon of bacterial resistance.^[6]

MATERIALS AND METHODS

Chemicals and instruments

All the chemicals used were of laboratory grade. Compound microscope, watch glass, glass slides, cover slips, Petri dishes, and other common glasswares were used in this experiment. Photographs were taken with using Nikon Labphot 2 Microscopic Unit and trinocular microscope. Ethanol solvent and reagents used for staining different sections such as toluidine blue, safranin, and IKI-Lugol's iodine were procured from The Precision Scientific Co., Coimbatore, India.

Collection and authentication of plant material

Aerial plant of *C. pedata* var. *glabra* [Figure 1] was collected from Thiashola, Manjoor, Nilgiris South Division, Western Ghats

before that we got proper permission from the Principal Chief Conservator of Forests, Chennai, and the District Forest Officer, Ooty, under Section 28 (i) of Wildlife Protection Act, 1972, in the month of October, and the voucher herbarium specimen was processed followed by standard methods.^[7] The collected plants were identified with the help of the existing floras^[8-10] and compared with type specimens available in the herbarium of Botanical Survey of India, Southern Circle, TNAU Campus, Coimbatore (No. BSI/SRC/5/23/2010-11/Tech. 1300), Tamil Nadu and the type specimens were deposited for further reference.

Macroscopic study and microscopic analysis

The macroscopic and microscopic studies of this plant were carried out according to the method of Johansen and Wallis.^[11,12] Fresh and healthy leaves, stem, and tendrils were separated from the plant and thoroughly washed with running water to remove the adherent impurities for anatomical studies. Some quantities of leaves and total aerial parts were air-dried, powdered, and stored in air-tight containers for further studies. Fresh stem was used for free-hand section cutting and was fixed in Formalin Acetic Acid and dehydrated with thiobarbituric acid as per the schedule given by Sass, 1940.^[13] The paraffin-embedded specimens were sectioned with the help of Rotary Microtome. Dewaxing of the sections was done by customary procedure.^[11] The sections were stained with Toluidine blue, Safranin, and IKI-Lugol's iodine as per the method of O'Brien *et al.*, 1964.^[14] After clearing the T.S, various microscopical studies were carried out in the study plant. For studying the leaf constants such as stomatal morphology and trichome distribution Jeffrey's maceration fluid Sass, 1940^[13] were prepared. Photographs of different magnifications were taken with NIKON ALPHA PHOTO-2 microscopic unit. Bright field was used for normal observations whereas polarized light was employed for the detailed study of crystals and starch grains. Descriptive terms of the anatomical features are taken from the standard anatomy book Esau, 1964.^[15]

Shade drying and powdering of the collected plant material

Freshly collected aerial plant parts were cleaned to remove adhering dust and then shade dried at 31°C for 15 days. The shade dried plant materials were mechanically ground to coarse powder and passed through a Wiley Mill to get 60-Mesh size and used for physicochemical, phytochemical, and fluorescence analysis. Samples were stored in the good grade plastic containers which are maintained at room temperature until analysis.

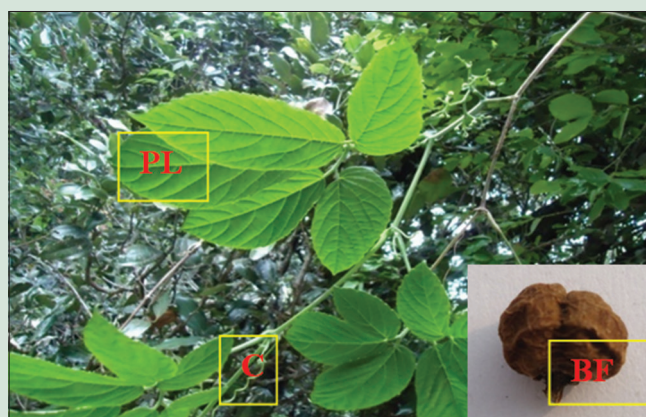


Figure 1: Macroscopic characteristics of *Cayratia pedata* var. *glabra*. PL: Pedate Leaf; C: Climber; BF: Bilobed Fruit

Physicochemical analysis

The parameters such as actions of plant powder with different chemical reagents, total ash, acid-insoluble ash, water-soluble ash, sulfated ash, extractive values of petroleum ether and ethanol, and fluorescence analysis were studied according to the official method^[16,17] and the World Health Organization guidelines on quality control methods for medical plants material.^[18]

Soxhlet extraction

Dried and powdered aerial plant powder (50 g) of *C. pedata* var. *glabra* was filled in the thimble and extracted successively with petroleum ether and ethanol (50 g/250 ml) using a Soxhlet extractor for 5–6 h. Extracts thus obtained will be concentrated in rotavapor and separated in glass vials and stored at 4°C in refrigerator for further use. Different dilutions of the extracts will be prepared in dimethyl sulfoxide (DMSO). The working concentrations of the extracts were 100, 200, and 300 µg/ml DMSO, respectively. The extracts were subjected to phytochemical analysis and antibacterial activity assay.

Preliminary phytochemical screening

Phytochemical screening of different successive solvent extracts was carried out using the standard procedure described by Kokate *et al.*, 1995.^[19]

Antibacterial screening

Preparation of inoculums

Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loop full of cells from the stock cultures to test tubes of liquid nutrient medium and were incubated without agitation for 24 h at 37°C. The cultures were diluted with fresh liquid nutrient broth to achieve optical densities corresponding to 2–10⁶ colony forming units/ml for bacteria.

Bacterial strains used

In vitro antibacterial activity was examined for the aerial plant part extracts of the species, *C. pedata* var. *glabra* against three bacterial strains which include the Gram-positive strains, namely, *Staphylococcus aureus* (ATCC 25212), *Bacillus subtilis* (ATCC 25413), and Gram-negative organism *Escherichia coli* (ATCC 25932) using Nutrient Agar Medium. All these bacteria were obtained from the Department of Microbiology, Nandha College of Pharmacy, Erode. Petroleum ether and ethanolic extracts were used to test their antibacterial activity by disc diffusion method.^[20]

Preparation of sterile disc

Whatman's No. 1 filter paper (1.5 mm thick) was punched into 5 mm diameter disc form and they are sterilized in the hot air oven. Presterilized each sterile disc was incorporated individually with 20–30 µl of extracts (100, 200, and 300 µl of plant extracts, one unit solution of streptomycin, 1000 µl of DMSO) using micropipette. The condensed extracts were applied in small quantities on discs and they were allowed to dry in air. After sometimes another doses of extracts were applied on discs. Then, they were stored at 4°C. The 20 ml of sterilized Nutrient Agar Medium was poured into sterile petriplates, after solidification, 100 µl of fresh culture were swabbed on the respective plates. The discs were kept over the agar plates using sterile forceps at various concentrations (100, 200, and 300 µl). DMSO as negative control and streptomycin (one unit solution) were used

as positive control for *S. aureus*, *B. subtilis*, and *E. coli*. Triplicate of each plate were prepared and incubated at 37°C for 24 h. The zone of inhibition was calculated by measuring 10 minimum dimension of the zone of no bacterial growth around the well.

Determination of minimum inhibitory concentration

The minimum inhibitory concentration (MIC) was determined through the broth dilution method.^[21] Bacteria were grown in nutrient broth for 6 h. After this, 200 µl of 10⁶ cells/ml were inoculated in tubes with (1800 µl) nutrient broth supplemented with seven different concentrations of (100–700 µg/ml) plant extracts. The serially diluted (single fold) plant extracts were used according to the National Committee for Clinical Laboratory Standards, 2000. For the determination of MIC, streptomycin 100 µg/ml was used as positive control, and the pure solvent (ethanol and petroleum ether) 100 µl was used as negative control. All the tubes were incubated at 37°C for 24 h and they were examined for visible turbidity. The MIC values were taken as the lowest concentration that inhibited the visible growth of the tested organisms.^[22,23]

RESULTS AND DISCUSSION

Microscopic results of leaf powder

The present anatomical study provides a set of characters specific for *C. pedata* var. *glabra* with which one can establish the identity of the plant in fragmentary form. Microscopic examination of leaf powder is one of the methods adapted to find the adulteration of the powder form. Raphides and epidermal trichomes are seen in leaf powder. The raphides are short, cylindrical blunt bundles comprising several thin pointed needles. The raphide bundles are 40–80 µm long and 20–30 µm thick and appear bright under polarized microscope [Figure 2a and b]. Nonglandular covering types of trichomes are abundant on the adaxial as well as abaxial surfaces. They are 2–5 celled, uniseriate, unbranched, and thin-walled. They taper gradually into pointed tip. The cells are vertically oblong and wide. The trichomes are 300–750 µm long and 60 µm thick at the base [Figure 3a and b]. This is in accordance with the result of Pragasam, 2010.^[24]

Anatomical studies of young stem

The anatomy of young stem shows initial stage of secondary growth. It is 1.7 mm thick. The epidermal layer is thin continuous all around the stem, and it is cylindrical in shape. The epidermal cells are thin walled. Dense epidermal trichomes are seen on the epidermal layer [Figure 4a and b]. The cortex is wider and is differentiated into outer zone of about 8 layers of collenchyma cells and inner zone of 3 or 4 layers of parenchyma cells. In the middle, the vascular cylinders of several, wide, and wedge-shaped xylem segments are present which interlinked by thin layer of sclerenchyma tissue [Figure 4b]. The xylem segments have 1–3 radial short rows of wide, circular, thin-walled vessels, and

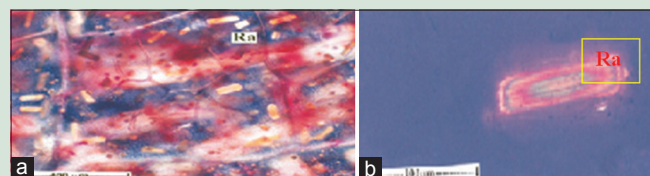


Figure 2: (a) Raphides as seen in cleared lamina. (b) Single raphide bundle as in leaf powder Ra: Raphide

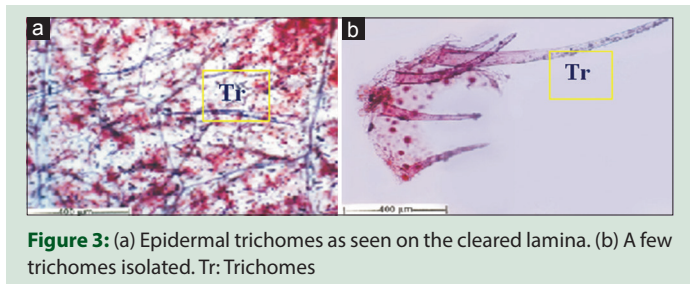


Figure 3: (a) Epidermal trichomes as seen on the cleared lamina. (b) A few trichomes isolated. Tr: Trichomes

thick blocks of phloem with sclerenchyma caps on the outer part. The pith is wide, parenchymatous, thin-walled, and compact. Calcium oxalate druses are sparsely seen in the cortical cells [Figure 4c]. Wheeler and LaPasha^[25] and Pragasam^[24] observed that anatomy of *Cissus* sp. supports the close.

Powder treated with different chemical reagents

The behavior of aerial plant powder with various reagents was observed and presented in Table 1. Slight yellowish difference (pale green to yellowish green, greenish yellow to pale yellow) was noted in the powder as such and treated with ammonium solution, ferric chloride, lead solution, antimony trichloride when compared to other reagents used. Powder treated with iodine solution, sodium nitroprusside, and potassium hydroxide showed brownish shades.^[26] The information collected from these tests was useful for standardization and obtaining the quality standards.^[16,17]

Fluorescence analysis

Fluorescence analysis of plant powder as such showed pale green to dark green in visible and ultraviolet (UV) light. Powder treated with 1 N HCl and 50% HNO₃ showed slight color differences such as greenish yellow to yellowish green to light green. Powder treated with 50% H₂SO₄ showed strong color difference (green to dark green). There are no distinct color differences seen in visible and UV light [Table 2]. Crude drugs are often assessed qualitatively in this way, and it is an important parameter for pharmacognostic evaluation.^[27]

Ash values and extractive values

Ash values are used to determine quality and purity of crude drug. It indicates the presence of various impurities such as carbonate, oxalate, and silicate.^[28] The results of ash values determination indicate that the sample contained 5.40% of total ash content. The water soluble ash value (31.49%) was lower than acid insoluble ash (52.17%). The sample contained (21.55%) sulfated ash. The percentage of extractive value was maximum in ethanol extract (17.20%) followed by petroleum ether (10%), respectively [Table 3]. Total ash value, fluorescence analysis, and extractive values will be helpful in identification, authentication, and useful for the determination of the exhausted or adulterated drug.^[29,30]

Preliminary phytochemical screening

To investigate the chemical constituents of plant powder of *C. pedata* var. *glabra*, the successive solvent extracts were subjected to qualitative phytochemical screening. The preliminary phytochemical screening revealed the presence of proteins, amino acids, alkaloids, anthraquinones, flavonoids, glycosides, phenols and tannins, steroids and sterols, triterpenoids, and volatile oil. The ethanol extraction was more efficient than petroleum ether extract because the ethanol extract contains more phytoconstituents [Table 4]. All extracts showed negative response for saponins.

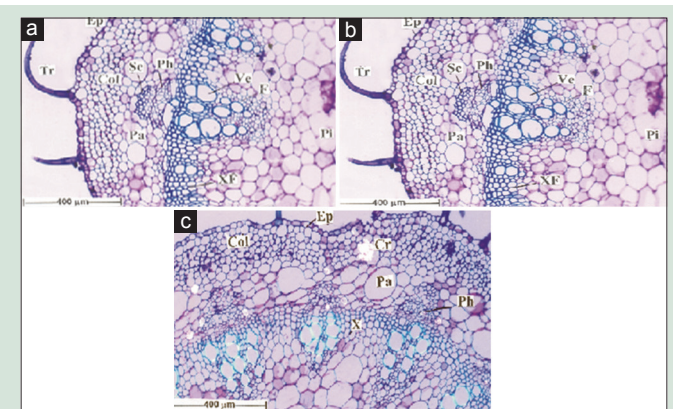


Figure 4: (a) T.S. of young stem – Ground plan. (b) T.S. of young stem – A sector enlarged. (c) T.S. of stem showing crystals (Under polarized light). Co: Cortex; Col: Collenchyma; Cr: Crystals; Ep: Epidermis; F: Fibres; Pa: Parenchyma; Pi: Pith; Ph: Phloem; Sc: Sclerenchyma cap; X: Xylem

Table 1: Behaviour of plant powder with different chemical reagents

Powder + reagents used	Colour of the powder
Powder as such	Pale green
Powder + ammonium solution	Yellowish green
Powder + ferric chloride	Greenish yellow
Powder + iodine solution	Pale brown
Powder + sodium nitroprusside	Yellowish brown
Powder + lead solution	Pale green
Powder + potassium hydroxide	Yellowish brown
Powder + anti-monytrichloride	Pale yellow

Table 2: Fluorescence behavior of plant powder

Reagents	Behavior of powder	
	Visible light	UV light
Powder as such	Pale green	Dark green
Powder + 1 NHCl	Greenish yellow	Yellowish green
Powder + 50% H ₂ SO ₄	Green	Dark green
Powder + 50% HNO ₃	Yellow	Light green

UV: Ultraviolet

Table 3: Physicochemical and extractive values of *Cyclanthera pedata* var. *glabra*

Physicochemical properties	Values
Total ash	5.40±1.20
Acid insoluble ash	52.17±0.06
Water soluble ash	31.49±1.06
Sulfated ash	21.55±0.97
Extractive values	Yield (%)
Petroleum ether	10.00
Ethanol	17.20

Values are means of triplicate determinations±SD. SD: Standard deviation; Kokoshi et al., 1958

Antibacterial assay

The antibacterial activity of petroleum ether and ethanolic extracts of *C. pedata* var. *glabra* were investigated using the disc diffusion method, and results were tabulated in table form [Figures 5, 6 and Tables 5, 6]. The synergistic activity of rich constituents may be the possible factor for the inhibition of the growth of bacterial colonies.^[31] Results of present investigation clearly indicated that all the three dilutions (100, 200, and

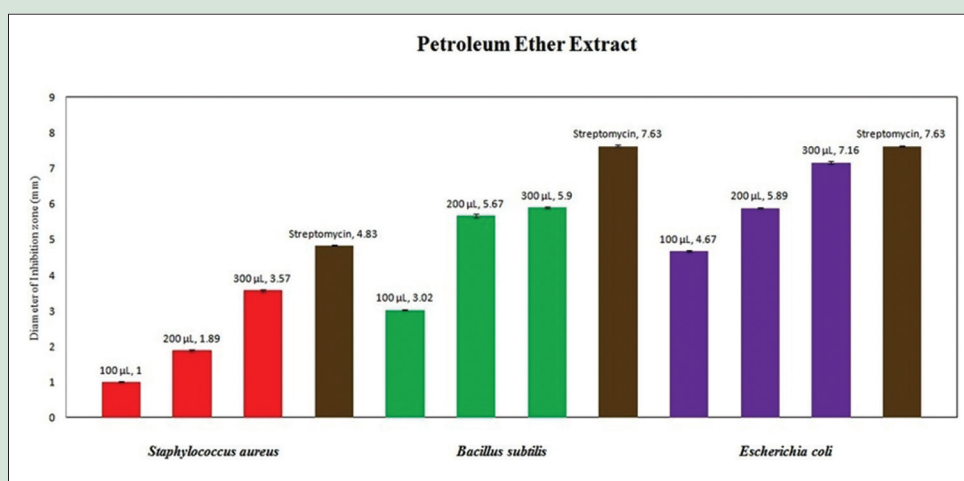


Figure 5: Antibacterial activity of petroleum ether extract of *Cayratia pedata* var. *glabra* on certain pathogenic bacteria

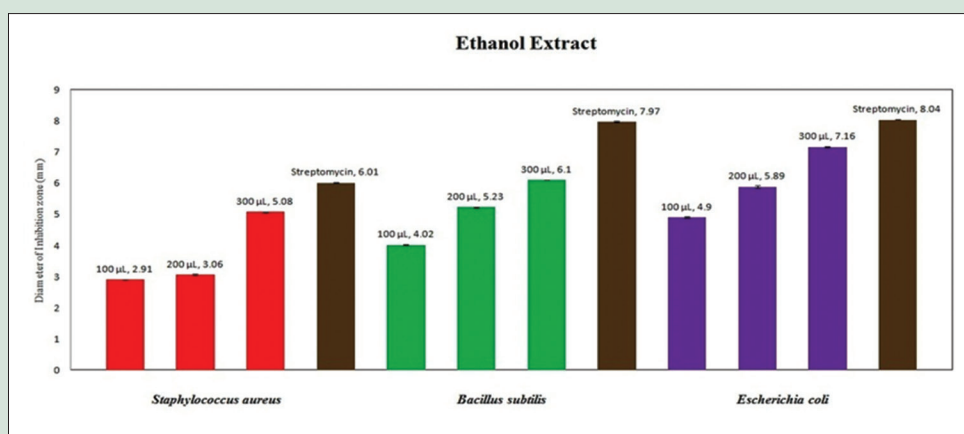


Figure 6: Antibacterial activity of ethanolic extract of *Cayratia pedata* var. *glabra* on certain pathogenic bacteria

300 µl) of both the extracts produced inhibitory effects against the tested microorganisms. This is in accordance with the result of Peter and Raj, 2012.^[32] DMSO solvent showed zero activity against bacterial pathogens, *S. aureus*, *B. subtilis*, and *E. coli*. The maximum zone of inhibition was observed by ethanolic extract against the bacterium, *E. coli* (7.16 ± 0.28) while the petroleum ether extract showed moderate zone of inhibition for all bacterial strains tested which was ranging between 1.00 ± 0.24 and 6.13 ± 0.15 [Figure 7]. The study plant extracts had antibacterial activity, which is more or less equal to the action of standard streptomycin. This is in agreement with the reports of Kumanan et al., 2014.^[33]

Zone of inhibition of streptomycin for *S. aureus* and *E. coli* was widely varied (4.83 ± 0.21 and 6.01 ± 0.17 ; 7.63 ± 0.35 , and 8.04 ± 0.09) and slight difference was noted in the inhibition zone of both the extracts on *B. subtilis* (6.63 ± 0.45 and 7.97 ± 0.56). From the overall concert, it is known that the ethanolic extract showed broad spectrum of antibacterial activity in comparison to that of the other solvent extract. It may be explained that the activity of antibiotics in plant extracts against bacterial growth may be due to their mechanism of action, chemical structure, or spectrum of activity.

Minimum inhibitory concentration

As the ethanolic extract showed more promising and effective determination of MIC compared to that of petroleum ether extract against 3 pathogenic bacteria of both Gram-positive and

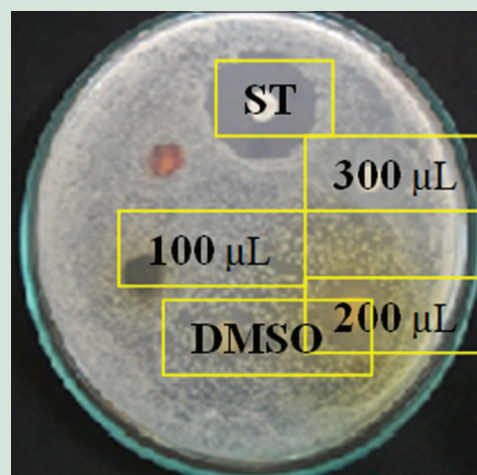


Figure 7: Antibacterial activity of ethanolic extract of *Cayratia pedata* var. *glabra* against *Escherichia coli*

Gram-negative strains [Tables 7 and 8]. The MIC of the petroleum ether extract was ranged between 200 and 500 µl, and ethanolic extract was ranged between 200 and 400 µl, respectively. *S. aureus* and *E. coli* are the most susceptible bacterial strain in two extracts.

Table 4: Qualitative phytochemical screening of plant powder extracts

Chemical constituents	Chemical tests	Petroleum ether	Ethanol
Carbohydrates	Molisch's test	-	-
	Barfoed's test	-	-
Proteins	Warming test	-	+
	Test with trichloroacetic acid	-	+
	Biuret test	-	+
Aminoacids	Millon's test	-	+
	Ninhydrin test	-	+
	Dragendorff's reagent	-	+
Alkaloids	Mayer's reagent	+	-
	Wagner's reagent	+	-
	Borntrager's test	-	+
Flavonoids	Alkaline reagent test	+	+
	Zinc hydrochloride test	+	+
Glycosides	Borntrager's test	+	+
	Ferric chloride test	+	+
Phenols and tannins			
Saponins	Foam test	-	-
Steroids and sterols	Salkowski test	-	+
	Sulfur test	+	-
Triterpenoids	Liebermann-Burchard test	-	+
Volatile oil	Sudan test	-	+

+ and -: The presence/absence of compounds

The most resistant bacteria were *B. subtilis*. This is in accordance with the results of Andreumont 2001;^[6] Selvarani and Viji Stella Ba, 2014.^[34]

CONCLUSION

The information obtained from pharmacognostical studies will be of used for supplementary pharmacological and therapeutical evaluation of the species and will assist in standardization for quality, purity, and authentication with the help, of which adulteration and substitution can be prevented. The present findings confirm the traditional knowledge of local healers (Toda's) on the antibacterial property of this species and also suggest the aerial part of this plant species can be used as a promising source for the development of new pharmaceuticals that address the therapeutic needs to cure infectious diseases. Results of present investigation clearly indicated that all the three dilutions (100, 200, and 300 µl) of both extracts produced inhibitory effects against the tested microorganisms. The study plant extracts had antibacterial activity, which is more or less equal to the action of standard Streptomycin. In conclusion, *C. pedata* var. *glabra* contains biologically active compounds that may serve as applicant for the discovery of new drugs in the treatment of antibacterial activities. Further evaluation of the antibacterial properties of these extracts and isolation of the compounds responsible for the antibacterial activity is required.

Table 5: Antibacterial activity of different extracts of *Cyclanthera pedata* var. *glabra* against two Gram-positive strains

Microorganisms	Extracts	Disc concentration (µg/mL)	Diameter of the inhibition zone (mm)	
<i>Staphylococcus aureus</i>	Petroleum ether extract	100 µL	1.00±0.24	
		200 µL	1.89±0.32	
		300 µL	3.57±0.70	
	Streptomycin	One unit solution	1000	4.83±0.21
			1000	-
			1000	-
	Ethanol extract	One unit solution	100 µL	2.91±0.19
			200 µL	3.06±0.45
			300 µL	5.08±0.23
	Streptomycin	One unit solution	1000	6.01±0.17
			1000	-
			1000	-
<i>Bacillus subtilis</i>	Petroleum ether extract	100 µL	3.02±0.21	
		200 µL	5.67±0.89	
		300 µL	5.90±0.36	
	Streptomycin	One unit solution	1000	6.63±0.45
			1000	-
			1000	-
	Ethanol extract	One unit solution	100 µL	4.02±0.43
			200 µL	5.23±0.35
			300 µL	6.10±0.10
	Streptomycin	One unit solution	1000	7.97±0.56
			1000	-
			1000	-

Values were performed in triplicates and represented as mean±SD. Negative control (DMSO); Positive control (streptomycin). SD: Standard deviation; DMSO: Di methyl sulfoxide

Table 6: Antibacterial activity of different extracts of *Cyclanthera pedata* var. *glabra* against Gram-negative strain

Microorganisms	Extracts	Disc concentration (µg/mL)	Diameter of the inhibition zone (mm)	
<i>Escherichia coli</i>	Petroleum ether extract	100 µL	4.67±0.32	
		200 µL	6.13±0.15	
		300 µL	5.23±0.7	
	Streptomycin	One unit solution	1000	7.63±0.35
			1000	-
			1000	-
	Ethanol extract	One unit solution	100 µL	4.90±0.36
			200 µL	5.89±0.67
			300 µL	7.16±0.28
	Streptomycin	One unit solution	1000	8.04±0.09
			1000	-
			1000	-

Values were performed in triplicates and represented as mean±SD. Negative control (DMSO); Positive control (streptomycin). SD: Standard deviation; DMSO: Di methyl sulfoxide

Table 7: Minimum inhibitory concentration of petroleum ether extract on certain pathogenic bacterial strain

Bacteria	MIC (mg/mL)								
	Solvent ^a	Control ^b	100	200	300	400	500	600	700
Gram-positive									
<i>Staphylococcus aureus</i>	+	-	+	+	-	-	+	-	-
<i>Bacillus subtilis</i>	+	-	+	+	+	+	-	-	-
Gram-negative									
<i>Escherichia coli</i>	+	-	+	+	-	-	-	-	-

^aNegative control (petroleum ether); ^bPositive control (streptomycin). (+) and (-): The presence and absence of bacterial growth, respectively. MIC: Minimum inhibitory concentration

Table 8: Minimum inhibitory concentration of ethanolic extract on certain pathogenic bacterial strain

Bacteria	MIC (mg/mL)								
	Solvent ^a	Control ^b	100	200	300	400	500	600	700
Gram-positive									
<i>Staphylococcus aureus</i>	-	-	+	+	-	-	-	-	-
<i>Bacillus subtilis</i>	+	-	+	+	+	+	-	-	-
Gram-negative									
<i>Escherichia coli</i>	+	-	+	+	-	-	-	-	-

^aNegative control (ethanol); ^bPositive control (streptomycin). (+) and (-): The presence and absence of bacterial growth respectively. MIC: Minimum inhibitory concentration

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