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Phytochemical screening, anti-oxidant, and antimicrobial profile of leaves of *Barleria stocksii* T. Anderson (Acanthaceae): An endemic species from the Deccan Peninsula.

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Abstract

Barleria stocksii T. Anderson (Acanthaceae) is endemic to Andhra Pradesh and Karnataka states of India. The present work is aimed to determine the phytochemical screening, antioxidant, and antibacterial activity of methanolic leaf extract of *Barleria stocksii*. Qualitative phytochemical analysis results reveal the presence of alkaloids, flavonoids, phenols, glycosides, tannins, steroids, saponins and anthocyanins. Methanolic leaf extract showed the highest number of secondary metabolites and is followed by ethanol and aqueous solvents. *B. stocksii* methanolic leaf extract showed intense DPPH and H_2O_2 scavenging activity with IC_{50} values of 90.36 and 109.57µg/ml respectively. Ferric reducing antioxidant power of *B. stocksii* methanolic leaf extract was found almost similar to the standard at 100 µg/ml concentration. Antibacterial activity of methanolic leaf extract of *B. stocksii* was investigated by using the Disc Diffusion method and the maximum growth inhibition was shown at 100 µg/ml concentration against *Escherichia coli*. The potential of the methanolic leaf extract to impede the growth of Bacteria is a sign of its broad spectrum antibacterial ability which may be utilized in the management of microbial infection.

Keywords: *Barleria stocksii*, Endemic plant, Antioxidant activity, Phytochemical analysis & Antibacterial activity.

Introduction

Since ancient times, many plants have been utilized for novel therapeutic applications. The utilization of plants in traditional medicine is increasing day to day. So, there is a frequent exploration of medicinal plants being done to meet the community needs. The medicinal plants have promising potential and are widely used without any intoxicating activity. Various chemical substances that have valuable properties in traditional medicine are phenols, flavonoids, alkaloids, tannins, steroids, triterpenoids, etc. To examine the medicinal properties and capabilities of the plant, the chemical analysis of phytochemical content and antioxidant activity assay from *B. stocksii* is important. Species of the genus *Barleria* are used as ornamentals[1-4] and also for medicinal purpose[5-7]. There is only a handful of evidence on phytochemical and antioxidant activity of *Barleria* species. Various classes of compounds like barlerionoside, barlerin, acetyl barlerin, shanzhiside methyl ester and 7-methoxysiserrosiden and pulinoside were isolated from *Barleria prionitis*[8-9]. The species of the genus *Barleria* are used for cough, bronchitis, inflammation[10], toothache, nervine disorders, boils, anemia, snake bite, diabetes, and lung disorders etc.[11]. In the human body, Antioxidants work as the very good scavengers of free radicals[12]. The phenolic compounds having many hydroxyl groups are effective primary antioxidants as they have the capability to donate H- atoms to the free radicals, resulting in the formation of unreactive phenoxyl radicals due to the occurrence of resonance stabilization[13]. Most of the antioxidants so far are extracted from plants and they belong to phenolic and polyphenolic compounds and also to the carotenoids and vitamins, of the other types. The principle of antioxidant action is explained by the structural properties of the molecules involved, the system in which they are present, the processing and storage conditions of the other[14]. *Barleria stocksii* T. Anderson belongs to the Acanthaceae family and is endemic to Andhra Pradesh (Anantapur, Chittoor, Kadapa and East Godavari districts) and Karnataka (Chikkaballapur, Gadag and Bagalkot districts) states, India[15-16].

Less than 15% of the global biodiversity is estimated to be in bioactive potentiality, intensifying the need to research a high amount of plant extracts. Thus, a sustainable quantity of natural products will be adequately available to the upcoming research works[17].

Microbes are being resistant to various drugs so far. It is a challenge for researchers to innovate new drugs to destroy the microbes. The activities of medicinal plants depend on the polarity of extracting solvent[18]**.** There are no reports on the phytochemical and antioxidant activity of *B. stocksii* regarding its medicinal properties. This study aims to provide information on the phytochemical screening, antibacterial, and antioxidant properties of *B. stocksii* to the best of our knowledge.

Material and methods

Plant material and preparation of sample

Barleria stocksii (Figure 1) was collected from Puttaparthi Hills (14° 05'35.86" N, 77°44'37.60" E), Anantapur, Andhra Pradesh during July 2018 and the voucher specimen (SVUTY/GS-57) were deposited in KN Rao Herbarium, Department of Botany, Sri Venkateswara University, Tirupati, A.P., India. Fresh leaves were separated and washed thoroughly with tap water, followed by distilled water and sliced. They were air dried at room temperature for 5-7 days. Dried plant material was refined using a blender. 100 g of plant powder was macerated with 100 ml of representative organic solvents (Benzene, Chloroform, Ethyl Acetate, Ethanol, Hexane, Methanol and Water) and placed on an orbital shaker with constant stirring overnight at 120 rpm. Finally, the extract was centrifuged at 10,000 rpm for 10 min. The supernatant was again filtered through Whatman No.1 filter paper and then the solvent was evaporated by a rotary evaporator until crude extract forms into a paste. The final residue obtained from each extract was dissolved in 100 mg/ml of concentrated Dimethyl sulfoxide (DMSO) and was processed to find out the secondary metabolites, antioxidant assay and antibacterial activity.

Figure 1. *Barleria stocksii* habit.

Phytochemical tests

Phytochemical tests for *B. stocksii* were performed according to the standard method Kokate et al. 2008[19].

Antioxidant activity

Determination of Scavenging activities

The quenching property of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical was estimated in accordance with Yamaguchi et al. 1998[20]. Briefly, 4 mg of DPPH was dissolved in 100 ml of methanol and was stored at 20^{o}C. 2 ml stock solution was added to 1 ml of different concentrations of *Barleria stocksii* methanolic leaf extract (BS-MLE) (20, 40, 60, 80 and 100 µg/ml). The reaction mixture was incubated at room temperature in a dark room for 45 min. The absorbance was recorded at 517 nm where ascorbic acid was used as a control. Hydrogen peroxide (H_2O_2) scavenging activity method was performed according to The Ruch et al. 1989[21]. Different concentrations of BS-MLE (20, 40, 60, 80 and 100 µg/ml) was added to 2 ml of H_2O_2 (40

mM) solution prepared in phosphate buffer (pH 7.4) and the reaction mixture was kept aside at room temperature (30 ± 2^{o}C) for 20 min and then absorbance reading was taken at 230 nm. The percentage of free radical quenching property was measured by using the following formula-

% Free radical quenching = [(Control absorbance- Sample absorbance)/Control absorbance] X 100

Reducing power assay

Ferric reducing antioxidant power (FRAP) assay was performed by the previously described, Oyaizu 1986[22] method. Different concentrations of (20, 40, 60, 80 and 100 µg/ml) BS-MLE were mixed with 2.5 ml of Phosphate buffer (0.2 m, pH 6.6) and 2.5 ml of 1% potassium ferricyanide and it was incubated at 50^{o}C for 20 min. After the incubation period, 2.5 ml of 10% trichloroacetic acid was added and centrifuged at 3000 rpm for 10 min. Finally, 2.5 ml of supernatant, 2.5 ml of distilled water and 0.5 ml of 0.01% $FeCl_3$ were added and absorbance was measured at 700 nm. Ascorbic acid was used as a positive control and phosphate buffer for blank. The experiments were carried out in triplicates.

Antibacterial activity

Antibacterial activity of BS-MLE was carried out by Kirby-Bauer disc diffusion method[23] against both gram –ve (*Klebsiella pneumoniae* (MTCC-741), *Escherichia coli* (MTTC-443)) and gram +ve (*Bacillus subtilis* (MTTC-441), *Staphylococcus aureus* (MTTC-731)) bacteria. 100 µl of actively growing overnight inoculum was spread on Nutrient agar plates. Each plate consists of different concentrations (10, 25, 50 and 100 µg/ml) of BS-MLE loaded on 6 mm Whatman No.1 paper discs and Ciprofloxacin (5 mcg). Nutrient agar plates were incubated for 12-24 h at 37± 2^{O}C and the inhibition zone was noted.

Results and discussions

The following tests (Table 1) were conducted to observe phytochemicals from the different solvents of leaf extract. Screening of different crude extracts of leaves of *B. stocksii* revealed the presence of secondary metabolites mostly glycosides, flavonoids, phenols, tannins and steroids which are represented in Table 2.

Table 1: Preliminary photochemical screening of *Barleria stocksii*

Test	Experiment	Observation	Inference
Test for Alkaloids	*Mayer's reagent test*: Few ml of plant extract was mixed with chloroform and the residue digested with 1 % HCl. Finally acid solution was mixed with Mayer's reagent.	Formation of precipitation and turbidity	Presence of Alkaloids
	Wagner's reagent test: Mayer's reagent test: Few ml of plant extract was mixed with chloroform and the residue digested with 1 % HCl. Finally acid solution was mixed with Wagner's reagent.	Yellowish white precipitation	Presence of Alkaloids
Test for Flavonoids	*Shinodon's test*: For few ml of plant extract few drops of Conc. HCl and small pieces of Magnesium ribbons were added one after the other.	Pinkish red color	Presence of Flavonoids
	Ferric chloride test: Few drops of ferric chloride solution was added to few ml of plant extract.	Formation of blackish red color	Presence of Flavonoids
Test for Phenol compounds	*Phenol test*: 2 drops of 1% ferric chloride solution was added to few ml of plant extract.	Formation of intense blue color	Presence of Phenol compounds
	Ellagic acid test: Few ml of plant extract was treated with few drops of 5% acetic acid and few drops of 5% sodium nitrate solution.	Formation of muddy or brown precipitation	Presence of Phenol compounds
Test for Glycosides	*Keller Kilani test*: 5 ml of extract and few ml of glacial acetic acid followed by 2 drops of ferric chloride were added. Total reaction mixture transferred into test tube containing Conc. H_2SO_4.	Formation of reddish-brown ring at the junction of two layers	Presence of Glycosides
Test for Tannins	*Gelatin test*: Residue from methanolic extract was dissolved in water and tested with 1% gelatin solution.	Formation of white precipitate	Presence of Tannins
	Ferric chloride test: To 5 ml extract, few drops of ferric chloride was added.	Formation blackish precipitate	Presence of Tannins
Test for Steroids	*Salkowski test*: Few ml of extract mixed with $CHCl_3$ followed by adding conc.H_2SO_4.	Formation of red color	Presence of Steroids
	Lieberman's Burchard test: Few ml of extract mixed with $CHCl_3$ followed by adding conc.H_2SO_4 along with test tube sides.	Formation of green color	Presence of Steroids
Test for Lignin	*Lignin test:* Few ml of extract was mixed with Conc. HCl and 2% furfuraldehyde.	No Observation	Absence of Lignins
	Labat test: Few ml of extract was added to Gallic acid.	No Observation	Absence of Lignins
Test for Saponins	Dried extract powder was added to distilled water and shaken vigorously for 15 min.	Formation of Honeycomb forth	Presence of
Test for Terpenoids	*Liebermann-Burchard's test*: Few ml of extract mixed with 0.5 ml of acetic anhydrate, 0.5 ml of $CHCl_3$ and 0.5 ml of Conc. H_2SO_4.	Reddish violet color	Presence of Terpenoids
Test for Anthocyanidins	To 5ml of extract equal volume of methanolic HCL was added.	Red or purple color	Presence of Anthocyanidins

Methanolic leaf extract showed the maximum number of secondary metabolites followed by Ethanol and Water. Therefore we chose methanolic leaf extract for antioxidant, antimicrobial and cytotoxicity studies. The presence of secondary metabolites in the plants is mainly influenced by several factors like, light intensity, temperature, salinity, drought stress, seasons, etc[24]. Phenolic compounds are one of the important secondary metabolites in medicinal plants[25]. Plant phenols constitute a certain group of compounds that work as primary antioxidants. Presence of hydroxyl groups makes them react with active oxygen radicals, like hydroxyl radicals, superoxide radicals and lipid peroxyl radicals and thus inhibit the lipid peroxidation at an early stage.

Table 2: Result of phytochemical analysis of *B. stocksii* leaf extract

	Solvents						
Compositions	Water	Methanol	Ethanol	Benzene	Chloroform	Ethyl acetate	Hexane
Alkaloids	-	+	+	-	-	-	-
Flavonoids	+	+	+	+	-	+	-
Phenols	+	+	+	+	-	-	-
Glycosides	+	-	+	-	+	+	+
Tannins	+	+	+	+	-	-	-
Steroids	-	+	+	+	+	+	+
Lignins	-	-	-	-	-	-	-
Saponins	+	+	-	-	-	-	-
Terpenoids	-	+	-	-	-	-	+
Anthocyanins	+	-	-	-	-	-	-
Note: '+' Presence of phytochemical substance; '-' Absence of phytochemical substance.							

In Vitro antioxidant activity of BS-MLE was evaluated by DPPH, H_2O_2 and FRAP assay. BS-MLE showed concentration-dependent scavenging activity i.e., an increase in the concentration of BS-MLE from 25 to 100 µg/ml showed increased scavenging activity which is statically significant ($P \leq 0.05$). Similarly, an increase in the concentration of BS-MLE from 25 to 100 µg/ml showed increased inhibition of H_2O_2 from 10 to 45%. BS-MLE showed maximum inhibition of 57.76 and 45.61% at 100 µg/ml against DPPH and H_2O_2 scavenging activity. IC_{50} values were determined as 90.36 mg/ml and 1109.57 mg/ml for DPPH and H_2O_2 scavenging assay respectively. *In Vitro* antioxidant activity of BS-MLE was estimated using the ferric reducing power method. The reducing power was estimated based on the extract efficiency to reduce the Fe^{+3} to Fe^{+2}.

Reducing power of BS-MLE was found almost similar to the standard at 100 µg/ml concentration. Antioxidant results were represented in Figure 2. DPPH undergoes reduction in the presence of hydrogen donating antioxidants. The scavenging ability of methanolic leaf extracts of *B. stocksii* for free radicals of DPPH exhibited pronounced scavenging activities. Phenolic compounds are a type of antioxidants that donate hydrogen atoms. Thus higher radical scavenging activity of hydro-alcoholic extract can be assigned to a large amount of hydrogen donating phenolic antioxidants in methanolic extract. The result obtained in this study justify the *B. stocksii* leaf methanolic extract show better antioxidant results compared to the previous studies on other *Barleria* species[26]. Tamboli and More[27] reported that *Barleria gibsoni* ethanolic leaf extract exhibited food antioxidant activity assessed by DPPH method recorded as 180 µg/ml. Kumari et al. 2017 [28] mentioned that the antioxidant activity of *Barleria lupina* leaf and stem methanolic extract determined by DPPH method was 48.86 and 60.82 µg/mL.

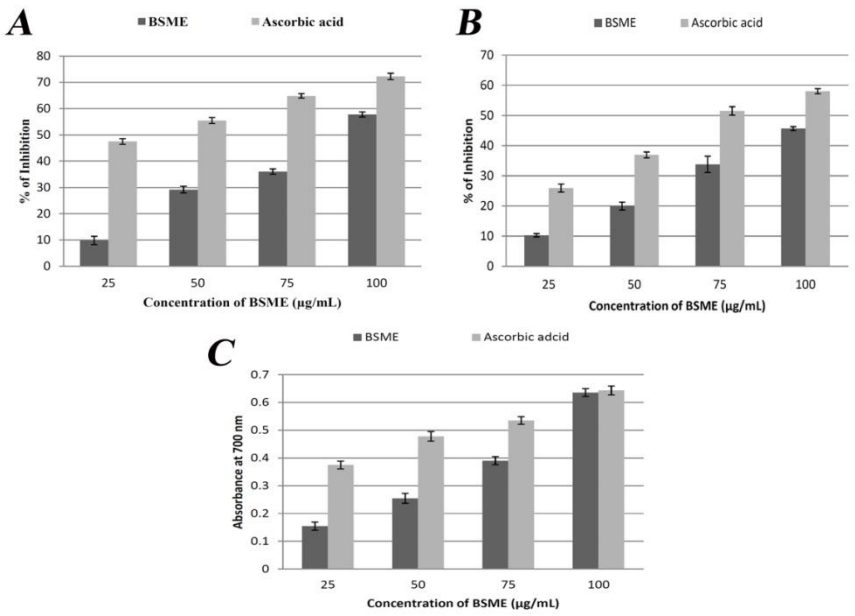


Figure 2. Antioxidant activity of BSMLE. **(A)** DPPH assay, **(B)** H_2O_2, **(C)** FRAP assay.

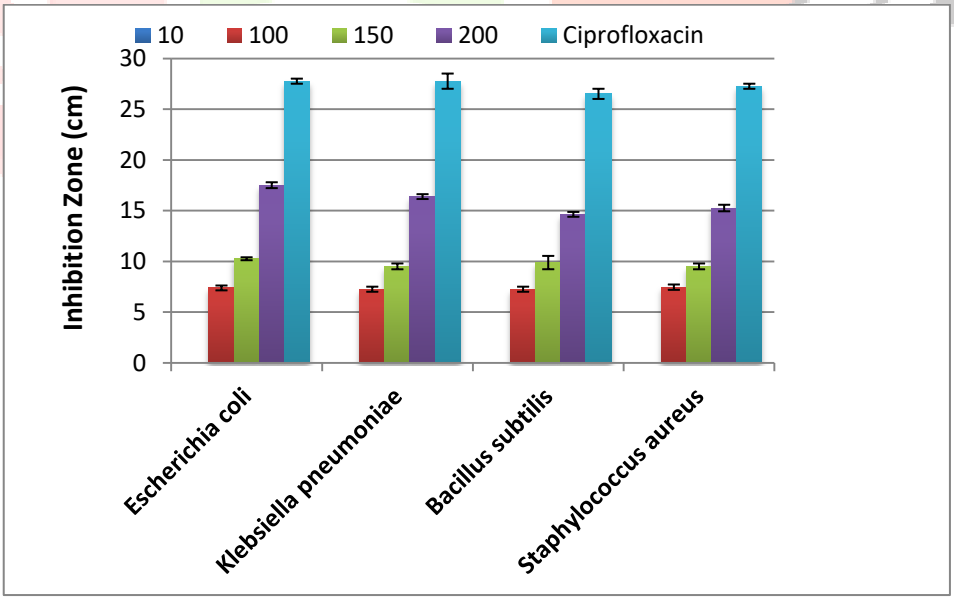
Antioxidant activity of the flavonoids is due to their potentiality to lessen the free radical formation and to scavenge free radicals. Various free radicals such as super oxides, hydroxyls, epoxyls, peroxynitrite (PAN) and singlet oxygen radicals are produced in the human diseases leading to severe oxidative stress. The oxidative stress can be rectified by natural antioxidant compounds and drugs[29]. In the human health system phytochemicals, especially polyphenols, flavonoids play a major role in dietary antioxidants[30].

Methanolic leaf extract showed significant inhibition zone against both gram positive and negative bacteria. The highest antibacterial activity showed at 100 µg/ml against gram negative and gram positive bacteria. The strong zone of inhibition is shown for *Escherichia coli* (17.50±0.28mm), followed by *Klebsiella pneumoniae* (16.37±0.23mm), *Staphylococcus aureus* (15.25±0.32mm) and *Bacillus subtilis*(14.62±0.23mm) after the 24 h incubation period when compared with the standard drug (Ciprofloxacin) (Table 3 & Graph 1) and (Figure 3).

Table 3: Antibacterial activity of methanolic leaf extract of *B. stocksii*.

	Zone of Inhibition (mm)				
Name of the organisms	10 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	Ciprofloxacin
Escherichia coli-(MTCC-443)	0	7.37 ±0.23	10.25±0.14	17.50±0.28	27.75±0.25
Klebsiella pneumoniae-(MTCC-741)	0	7.25±0.25	9.5±0.28	16.37±0.23	27.75±0.75
Bacillus subtilis-(MTCC-441)	0	7.25±0.25	9.87±0.65	14.62±0.23	26.5±0.5
Staphylococcus aureus-(MTCC-731)	0	7.45±.26	9.5±0.28	15.25±0.32	27.25±0.25

Graph1: Graphical representation of antibacterial activity of BS-MLE.



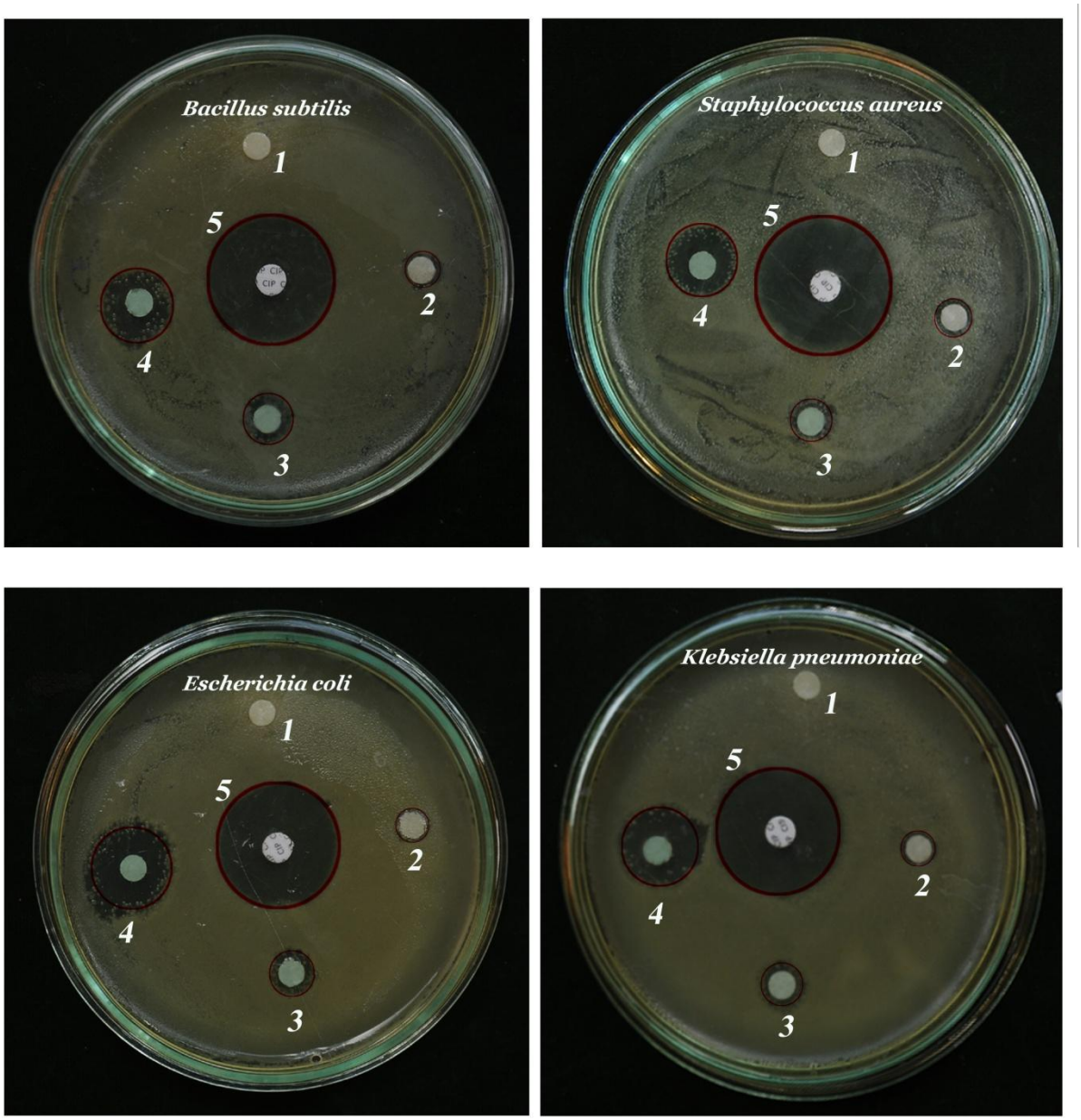


Figure 3. Antibacterial activity of BS-MLE. **1.** 10µg/mL, **2.** 25 µg/mL**, 3.** 50 µg/mL, **4.** 100 µg/mL, **5.** Ciprofloxacin (5 µg/mL)

The results revealed that inhibition against gram negative bacteria was supported by many earlier reports[31-33]. Flavonoids, terpenoids, saponins, triterpenoids and phenols[34-37] have good significant inhibitory action against bacteria. Phytochemicals involved in inhibiting the peptidoglycan synthesis disturb the structure of microbial membrane[38], inhibition of DNA replication, toxin action causing lysis of bacteria cells. Polyphenols have the ability to suppress the cell proliferation mechanism by blocking essential enzymes of microbial metabolism[39]. Saponins are involved in altering permeability of cell wall[40].

Conclusion

The present study evaluated the phytochemical screening, antioxidant and antibacterial activity of *B. stocksii* leaf extract, an endemic species from Andhra Pradesh and Karnataka, India. Leaf extract showed secondary metabolites such as alkaloids, flavonoids, phenols, glycosides, tannins, terpenoids, steroids, saponins and anthocyanins. Maximum number of secondary metabolites were present in methanolic leaf extract and followed by ethanol and aqueous extracts. The antioxidant capacity of the extract was tested using DPPH, H_2O_2 and FRAP assay. Finally, *B. stocksii* leaf methanolic extract exhibited great antioxidant and antibacterial activities. These results clearly indicate their unreported pharmaceutical and biomedical importance.

Conflict of interest

The authors declare no interest of conflict.

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