Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2016; 5(3): 256-262
Received: 25-03-2016
Accepted: 26-04-2016

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Preliminary phytochemical and antimicrobial properties of *Pavetta crassicaulis* Bremek. flower extracts collected from Western Ghats, Karnataka, India

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Abstract
Pavetta crassicaulis Bremek. (F.: Rubiaceae), an important ethno-medicinal shrub/small tree, belonging to the family Rubiaceae, grows up to 4 meter tall the plant is endemic to peninsular India. Plant parts like bark pulverized or in decoction is used for visceral obstructions arthritis. Leaves and roots used in poultices for boils and itches, decoction of leaves used externally for hemorrhoidal pains. *Pavetta crassicaulis* plant parts like roots used in the treatment of visceral problems and dropsy, bark is used on the victims of epilepsy, boiled leaves used to prepare decoction to cure hemorrhoids, urinary complaints, and anticephalagic. Fruits used as anthelmintic and flowers are eaten fried. For preliminary phytochemical analysis and antimicrobial assay, flower samples were collected, shade-dried for 21 days and subjected for soxhlet extraction. Polarity wise the solvents used to extract the crude compound are, hexane, petroleum ether, chloroform and ethanol. In the preliminary phytochemical analysis, hexane, petroleum ether showed negative results and chloroform crude extract shows positive result for flavonoids. The ethanolic crude extract showed positive reaction for Saponins, Flavonoids, Steroids, Glycosides, Phenols and Sterols. The flower crude extract of *Pavetta crassicaulis* Bremek. was screened against pathogenic four fungal strains and nine bacterial strains by zone inhibition test. For fungal pathogens hexane, petroleum ether, chloroform extract shows nil effect and ethanolic extract showed noticeable antifugal activity at 50 and 100% concentration, so that, the flower crude extract was good effect on tested fungal pathogens mainly Crysoporium and Trichophyton species. For bacterial strains the crude extract shows dose dependent inhibition. The maximum inhibition zone observed in ethanolic crude extract for *Escherichia coli, Staphylococcus aureus, Salmonella typhi* and *Klebsiella pneumonia*, the antibacterial properties may due the presence of flavonoids, glycosides, phenols, alkaloids, saponins and sterols in the ethanolic extract.

Keywords: *Pavetta crassicaulis* Bremek., Western Ghats, Karnataka, Preliminary Phytochemical analysis, antimicrobial activity.

Introduction
Medicinal plants have been used for centuries as remedy for human diseases. These plants are sources of biologically active chemical compounds and some of them are anti-microbial agents [1]. Several medicinal plants have been evaluated for possible antimicrobial activity and to get remedy for a variety of ailments of microbial origin [2-5]. Secondary metabolites such as flavonoids [6], terpenoids [7], steroids [8], saponins [9], glycosides [10] extracted from higher plants exhibited antimicrobial properties.
Bacterial pathogens were rapidly mutating against old chemosynthetic drugs [11]. Pathogens were getting resistance against the frequently used drugs due to this invention of new moderated drugs must necessary [12].
Pavetta is a genus of flowering plants in the Rubiaceae family. It comprises about 350 species of trees, evergreen shrubs and sub-shrubs. It is found in woodlands, grasslands and thickets in sub-tropical and tropical Africa and Asia [13]. *Pavetta crassicaulis* Bremek. is an important ethno-medicinal shrub or small tree belonging to the family Rubiaceae, grows up to 4 meter tall the plant is endemic to peninsular India. The leaves are often membranous with dark bacterial nodules, has small, white, tubular flowers, sometimes salviform or funnel-shaped with 4 spreading petal lobes. The flowers are carried on terminal corymbs or cymes, the leaves are elliptical-oblong to elliptic-lanceolate, 6-15 cm long, and pointed at both ends. The flowers are white, rather fragrant, and borne in considerable number in hairy terminal panicle which is 6-10 cm long. The sepals are very small and toothed. The flowers tube is slender and about 1.5 cm long, with obtuse petals above half the length of the tube.

The fruits are black when they dry, somewhat rounded and about 6mm in diameter [14].

In local Mizos of Mizoram, India in their folkloric medical methods *Pavetta crassicaulis* Bremek plant parts like bark pulverized or in decoction is used for visceral obstructions arthritis. Leaves and roots used in poultices for boils and itches, decoction of leaves used externally for hemorrhoidal pains. The root of this plant is bitter and is given in visceral problems and dropsy. The bark is used on the victims of epilepsy. Decoction and boiled leaves are used to cure hemorrhoids, urinary complaints, and anticephalagic. Fruits used as anthelmintic and flowers are eaten fried [15]. Rural and tribal of Odisha used *Pavetta crassicaulis* Bremek. leaf in the treatment of skin diseases. [16].

In Kachchh, Gujarat, India, *Pavetta crassicaulis* Bremek roots used by the locals and folklore medicinal practitioners in the treatment of dropsy [17].

In Pankaj Oudhia's medicinal plant data base, the local tribal medicinal practitioners in the region of Jagdalpur, Chhattisgarh, India used the treatment of hypertrophy of prostate gland, sexual disorders and in the treatment of obesity as a fat burner [18].

Local and tribal peoples of Uttara Pradesh, India, *Pavetta crassicaulis* Bremek roots were used in the treatment of visceral problems and dropsy. The bark is used on the victims of epilepsy. Decoction and boiled leaves are used to cure hemorrhoids [19].

The study conducted in Sahyadri-Konkan Corridor of Maharashtra Western Ghats revealed the abundant distribution of *Pavetta crassicaulis* Bremek. in Western Ghats region of Maharashtra India [20]. The wide distribution of *Pavetta crassicaulis* Bremek. in Kalbhairavanatha Sacred Grove,Terungan, Ambegaon Taluka, Pune, India, a part of Western Ghats [21].

A Case Study conducted on Satkosia Gorge Wildlife sanctuary and Its Surroundings, Orissa, India, on the topic of "Human induced fragment formation and Vascular Plants Species Diversity", revealed that, the shrub/ small tree *Pavetta crassicaulis* Bremek. was moderately distributed among the shrub species in that region with a count of 31 Total number of total occurrences (TNoO), and 1 Occurrence in number of plots (NoP) [22].

The Preliminary qualitative phytochemical analysis of *Pavetta crassicaulis* Bremek. methanolic crude extract revealed the presence of Alkaloids, steroids and terpenoid [23].

Despite of many work on this genus pavetta the species *Pavetta crassicaulis* Bremek. a very important medicinal plant was unexplored for many pharmacological activities used by the folklore and tribes. Therefore, the aim of the study was to provide basic data on the antimicrobial and qualitative preliminary phytochemical properties of the plant flower extract.

Materials and Methods

Plant collection and authentication

The flower material of *Pavetta crassicaulis* Bremek. were collected from Shringeri taluk, Karnataka in February 2015. The plant was identified by Prof. K G Bhat, Udupi and a voucher specimen was conserved under the reference number KU/AB/RN/AS/002.

Plant preparation and extraction

The flower samples were dried in shade for 20 to 25 days, mechanically powdered and subjected to Soxhlet extraction using hexane, petroleum ether, chloroform, and ethanol [24]. the crude extracts were collected in air-tight plastic containers and stored in cool condition.

Preliminary phytochemical screening

Air-dried and powdered flower materials and also all crude extracts were screened for the presence of alkaloids, saponin, glycosides, flavonoids, steroids/sterols and phenols using standard methods [25-27]

Microorganisms used

Pathogenic fungal strains like *Candida albicans, Chrysosporium merdarium, Trichophyton rubrum* and *Chrysosporium keratinophilum* and pathogenic bacterial strains like *Xanthomonas campestris* (MTCC-2286), *Pseudomonas syringae* (MTCC-1604), *Agrobacteirum tumefaciens* (MTCC-431), *Klebsiella pneumonia* (MTCC-7028), *Escherichia coli* (MTCC 1559), *Salmonella typhi* (MTCC-734), *Pseudomonas aeruginosa* (MTCC-1934), *Staphylococcus aureus* (MTCC-902). *Streptomyces pneumonieae* (MTCC-4734) obtained from the Institution of Microbial Technology (IMTECH), Chandigarh, India were used.

Medium used

Nutrient agar (NA) media for bacterial pathogens and Sabouraud dextrose agar (SDA) used for the culturing of experimental fungal pathogens.

Standard drug

Antifungal drug Bavistin (1mg/ml of sterile distilled water) and Antibacterial drug Ciproflaxin (1mg/ml of sterile distilled water) were used as standard antibiotic to compare with the plant crude extracts.

Preparation of microbial cultures

For fungi

The test fungi were aseptically inoculated in sterile test tubes using Sabouraud dextrose broth and incubated at 28 ^{0}C for 36-48 h. The plant crude extracts were dissolved in 10% DMSO to get desired concentrations of 12.5, 25, 50 and 100 mg/ml respectively. Sabouraud dextrose agar (SDA) plates were prepared and the broth cultures of fungal strains were uniformly swabbed with the help of tween-20. 6 mm diam wells were punched in the inoculated plates using a sterile cork borer. One hundred µl of different concentrations of extract and standard (Bavistin, 1mg/ml of sterile distilled water) and DMSO (10%) were filled into the respectively labeled wells and incubated for 36-48 h at 28 ^{o}C.

For bacteria

The test bacteria were aseptically inoculated in sterile test tubes using nutrient broth and incubated at 370C for 24 hours. The plant crude extracts were dissolved in 10% DMSO to get desired concentrations of 12.5, 25, 50 and 100 mg/ml respectively. The drug Ciproflaxin was used as standard antibiotic (1mg/ml of sterile distilled water) to compare with

the plant crude extracts. Nutrient agar plates were prepared and the broth cultures of bacterial strains were uniformly swabbed. 0.6 cm diameter wells were punched in the inoculated plates using a sterile cork borer. 100 µl of different concentrations of crude extracts and standard (Ciproflaxin, 1mg/ml of sterile distilled water) and DMSO (10%) were filled into the respectively labeled wells and incubate for 24 hours at 37 °C.

Results

Preliminary phytochemical screening

The soxhlet extraction of *Pavetta crassicaulis Bremek* flower (750 g) with hexane gives 07.05 grams, with petroleum ether gives 20.34 g, with chloroform gives 18.64 g and with ethanol gives 35.32 g yield (Chart 1). The results of phytochemical screening of *Pavetta crassicaulis* Bremek extracts indicate the presence of Alkaloids, Saponins, Flavanoids, Steroids/Sterols, Glycosides and Phenols in ethanol crude extract, the chloroform crude extracts shows positive results for Flavonoids. But, the petroleum ether crude extract gave negative results for all these compounds (Table 1).

Anti-microbial activity of different extract

Hexane crude extract against test pathogens

Hexane crude extract show nil effect on all tested microorganisms (Table 2) which indicates that Hexane crude extract is not effective against tested pathogens.

Petroleum ether crude extract against test pathogens

Petroleum ether crude extract show nil effect on all tested microorganisms (Table 3) petroleum ether crude extract also shows nil effective against tested pathogens.

Chloroform crude extract against test pathogens

Chloroform crude extract show negligible effect on all tested microorganisms (Table 4). In 100% concentration it shows negligible effect on all bacterial strains but chloroform crude extract shows nil effect on the all the tested fungal strains in all concentrations.

Ethanolic crude extract against test pathogens

Against fungal pathogens

The ethanolic crude extract showed nil effect on tested fungal pathogens in 12.5, 25% concentrations. In 50 and 100% it shows negligible antifungal activity in that *Crysoporium* and *Trichophyton* species more susceptible to the extract. The results revealed that the ethanolic crude extract has shown good activity on tested fungal pathogens (Table 5 and Chart 3).

Against bacterial pathogens

The ethanolic crude extract shows appreciable antibacterial activity against all tested bacterial strains in12.5, 25, 50 and 100% concentrations. The ethanolic extract shows highest zone of inhibition against bacterial strains against all tested pathogens. (Table 5).

In all the concentrations of ethanolic extract (12.5, 25, 50 and 100%) all the tested bacterial organisms were susceptible (Table 5 and Chart 2).

From the study, it revealed that bacterial strains like *Escherichia coli, Staphylococcus aureus, Salmonella typhi* and *Klebsiella pneumonia* were more susceptible to the ethanolic crude extract and *Xanthomonas campestris, Pseudomonas aeruginosa, Streptomyces pneumonia, Pseudomonas syringae* and *Agrobacterium tumefaciens,* were moderately susceptible to ethanolic extract. In the 100% concentration the ethanolic crude extract shows appreciable antibacterial activity which is comparable with the standard drug Ciproflaxin (1mg/ml of sterile distilled water),

The flower ethanolic extract is more effective against animal pathogens viz., *Staphylococcus aureus, Salmonella typhi, Klebsiella pneumonia, Escherichia coli,* and *Streptomyces pneumonia* than the plant pathogenic bacteria viz., *Agrobacterium tumefaciens, Pseudomonas syringae, Pseudomonas aeruginosa,*and *Xanthomonas campestris*.

On the other hand the flower ethanolic extract is more effective against Gram positive bacterial strains (*Staphylococcus aureus*, *Streptomyces pneumonia)* than the gram negative pathogens (*Salmonella typhi, Klebsiella pneumonia, Escherichia coli*, *Agrobacterium tumefaciens, Pseudomonas syringae, Pseudomonas aeruginosa,*and *Xanthomonas campestris*) in a small difference of 1 mm.

Standard drugs bavistin and Ciproflaxin were showed zone for all tested pathogens. Control DMSO did not show any zone of inhibition and it in turn confirms the positive activity of ethanolic crude extract. In present days pathogens are getting resistance against frequently used drugs so, discovery of new drugs were necessary. From our results it is concluded that the ethanolic flower crude extract shows appreciable antibacterial activity against tested pathogens it might be useful in the treatment of those infections. These antibacterial properties may due the presence of alkaloids, flavonoids, glycosides, phenols, saponins and sterols (Table 1).

Discussion

The flower crude extracts of *Pavetta crassicaulis* Bremek. Displayed concentration dependent antimicrobial activity. (Table 5 and Chart 4). The experiment revealed that only the ethanolic crude extract of *Pavetta crassicaulis* Bremek. Has effective antibacterial activity against all tested pathogenic bacterial strains with less antifungal activity. (Table 5 and Chart 2 and 3). Other three extract were showed nil effect on all the tested pathogens in all concentrations. (Table 2, 3 and 4). The flower ethanolic extract is more effective against animal pathogens viz., *Staphylococcus aureus, Salmonella typhi, Klebsiella pneumonia, Escherichia coli,* and *Streptomyces pneumonia* than the plant pathogenic bacteria viz., *Agrobacterium tumefaciens, Pseudomonas syringae, Pseudomonas aeruginosa,* and *Xanthomonas campestris*.(Table 5) and the ethanolic extract is more effective against *Crysoporium* and *Trichophyton* species (Chart 3).This result shows that the plant ethanolic crude extract might be useful in the treatment of these infections. This antimicrobial properties may due the presence of flavonoids, glycosides, phenols, alkaloids, saponins and sterols in ethanolic crude extract. (Table 1)

Table 1: Preliminary phytochemical Analysis

Secondary Metabolites	Type of tests	Hexane crude extract	Petroleum ether crude extract	Chloroform crude extract	Ethanol crude extract
Alkaloids	Mayer's test	-	-	-	-
	Wagner's test	-	-	-	+
Saponins	Foam test	-	-	-	+
Tannins	Ferric chloride test	-	-	-	-
Flavonoids	Shinda test	-	-	-	+
	Zinc -HCl reduction test	-	-	-	+
	Alkaline reagent test	-	-	+	+
	Lead acetate test	-	-	+	+
Steroids	Salkowaski test	-	-	-	+
Glycosides	Keller-Killianis test	-	-	-	+
	Brown water test	-	-	-	+
	Legal test	-	-	-	+
Phenols	Ferric chloride test	-	-	-	+
	Acetic acid test	-	-	-	+
Sterols	Liebermann burchad test	-	-	-	+

- : Negative result, +: positive result.

Table 2: Hexane crude extract of *Pavetta crassicaulis* Bremek. against test microbial strains\

Test organisms	Zone of inhibition in mm (Mean±SD)				
	12.5 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml	
Fungal strains					**Standard (Bavistin)**
Ca	0±0	0±0	0±0	0±0	35±0.05
Cm	0±0	0±0	0±0	0±0	34±0
Tr	0±0	0±0	0±0	0±0	39±0.05
Ck	0±0	0±0	0±0	0±0	38±0
Bacterial strains					**Standard (Ciproflaxin)**
Sa	0±0	0±0	0±0	0±0	34±0
St	0±0	0±0	0±0	0±0	35±0.11
Kp	0±0	0±0	0±0	0±0	37±0.05
At	0±0	0±0	0±0	0±0	35±0.05
Ps	0±0	0±0	0±0	0±0	32±0.05
Pa	0±0	0±0	0±0	0±0	34±0.05
Xc	0±0	0±0	0±0	0±0	31±0.05
Ec	0±0	0±0	0±0	0±0	34±0.05
Sp	0±0	0±0	0±0	0±0	36±0.05

Ca: *Candida albicans*, Cm: *Chrysosporium merdarium*, Tr: *Trichophyton rubrum*, Ck: *Chrysosporium keratinophilum*, Sa: *Staphylococcus aureus*, St: *Salmonella typhi*, Kp: *Klebsiella pneumonia*, At: *Agrobacterium tumefaciens*, Ps: *Pseudomonas syringae*, Pa: *Pseudomonas aeruginosa*, Xa: *Xanthomonas campestris*, Ec: *Escherichia coli*, Sp: *Streptomyces pneumonia*.

Table 3: Petroleum ether crude extract of *Pavetta crassicaulis* Bremek. against test microbial strains

Test organisms	Zone of inhibition in mm (Mean±SD)				
	12.5 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml	
Fungal strains					**Standard (Bavistin)**
Ca	0±0	0±0	0±0	0±0	35±0.05
Cm	0±0	0±0	0±0	0±0	34±0
Tr	0±0	0±0	0±0	0±0	39±0.05
Ck	0±0	0±0	0±0	0±0	38±0
Bacterial strains					**Standard (Ciproflaxin)**
Sa	0±0	0±0	0±0	0±0	34±0
St	0±0	0±0	0±0	0±0	35±0.11
Kp	0±0	0±0	0±0	0±0	37±0.05
At	0±0	0±0	0±0	0±0	35±0.05
Ps	0±0	0±0	0±0	0±0	32±0.05
Pa	0±0	0±0	0±0	0±0	34±0.05
Xc	0±0	0±0	0±0	0±0	31±0.05
Ec	0±0	0±0	0±0	0±0	34±0.05
Sp	0±0	0±0	0±0	0±0	36±0.05

Ca: *Candida albicans*, Cm: *Chrysosporium merdarium*, Tr: *Trichophyton rubrum*, Ck: *Chrysosporium keratinophilum*, Sa: *Staphylococcus aureus*, St: *Salmonella typhi*, Kp: *Klebsiella pneumonia*, At: *Agrobacterium tumefaciens*, Ps: *Pseudomonas syringae*, Pa: *Pseudomonas aeruginosa*, Xa: *Xanthomonas campestris*, Ec: *Escherichia coli*, Sp: *Streptomyces pneumonia.*

Table 4: Chloroform crude extract of *Pavetta crassicaulis* Bremek. against test microbial strains

Test organisms	Zone of inhibition in mm (Mean±SD)				
	12.5 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml	
Fungal strains					**Standard (Bavistin)**
Ca	0±0	0±0	0±0	0±0	35±0.05
Cm	0±0	0±0	0±0	0±0	34±0
Tr	0±0	0±0	0±0	0±0	39±0.05
Ck	0±0	0±0	0±0	0±0	38±0
Bacterial strains					**Standard (Ciproflaxin)**
Sa	0±0	0±0	0±0	8±0.05	34±0
St	0±0	0±0	0±0	6±0.05	35±0.11
Kp	0±0	0±0	0±0	7±0.05	37±0.05
At	0±0	0±0	0±0	6±0	35±0.05
Ps	0±0	0±0	0±0	8±0.05	32±0.05
Pa	0±0	0±0	0±0	7±0.11	34±0.05
Xc	0±0	0±0	0±0	8±0.05	31±0.05
Ec	0±0	0±0	0±0	8±0.05	34±0.05
Sp	0±0	0±0	0±0	7±0	36±0.05

Ca: *Candida albicans*, Cm: *Chrysosporium merdarium*, Tr: *Trichophyton rubrum*, Ck: *Chrysosporium keratinophilum*, Sa: *Staphylococcus aureus*, St: *Salmonella typhi*, Kp: *Klebsiella pneumonia*, At: *Agrobacterium tumefaciens*, Ps: *Pseudomonas syringae*, Pa: *Pseudomonas aeruginosa*, Xa: *Xanthomonas campestris*, Ec: *Escherichia coli*, Sp: *Streptomyces pneumonia.*

Table 5: Ethanolic crude extract of *Pavetta crassicaulis* Bremek. against test microbial strains

Test organisms	Zone of inhibition in mm (Mean±SD)				
	12.5 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml	
Fungal strains					**Standard (Bavistin)**
Ca	0±0	0±0	6±0.05	11±0	35±0.05
Cm	0±0	0±0	6±0.05	11±0.05	34±0
Tr	0±0	0±0	7±0.05	14±0.05	39±0.05
Ck	0±0	0±0	6±0.05	13±0.05	38±0
Bacterial strains					**Standard (Ciproflaxin)**
Sa	6±0.05	10±0	12±0.05	15±0.05	34±0
St	7±0.05	11±0.05	10±0.1	14±0.1	35±0.11
Kp	6±0.05	9±0.05	11±0.11	15±0.05	37±0.05
At	6±0.05	9±0.05	12±0.05	16±0.05	35±0.05
Ps	6±0.05	8±0.05	10±0.1	14±0.05	32±0.05
Pa	7±0.05	9±0.05	12±0.05	16±0.05	34±0.05
Xc	6±0.05	8±1.3	12±0.1	14±0.05	31±0.05
Ec	6±0.05	8±0.05	13±0.05	16±0.05	34±0.05
Sp	7±0.05	9±0.05	11±0.1	17±0.05	36±0.05

Ca: *Candida albicans*, Cm: *Chrysosporium merdarium*, Tr: *Trichophyton rubrum*, Ck: *Chrysosporium keratinophilum*, Sa: *Staphylococcus aureus*, St: *Salmonella typhi*, Kp: *Klebsiella pneumonia*, At: *Agrobacterium tumefaciens*, Ps: *Pseudomonas syringae*, Pa: *Pseudomonas aeruginosa*, Xa: *Xanthomonas campestris*, Ec: *Escherichia coli*, Sp: *Streptomyces pneumonia*.

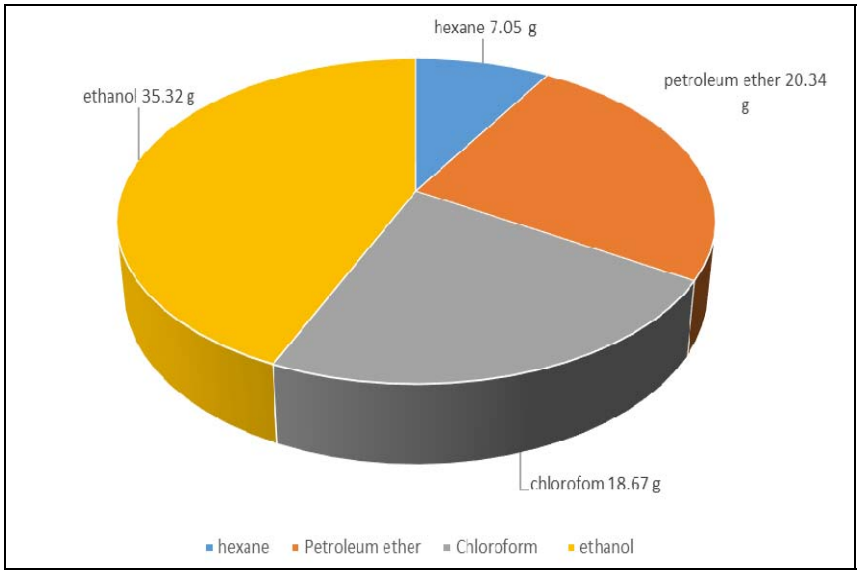

Chart 1: Crude extract yield in different solvent in grams

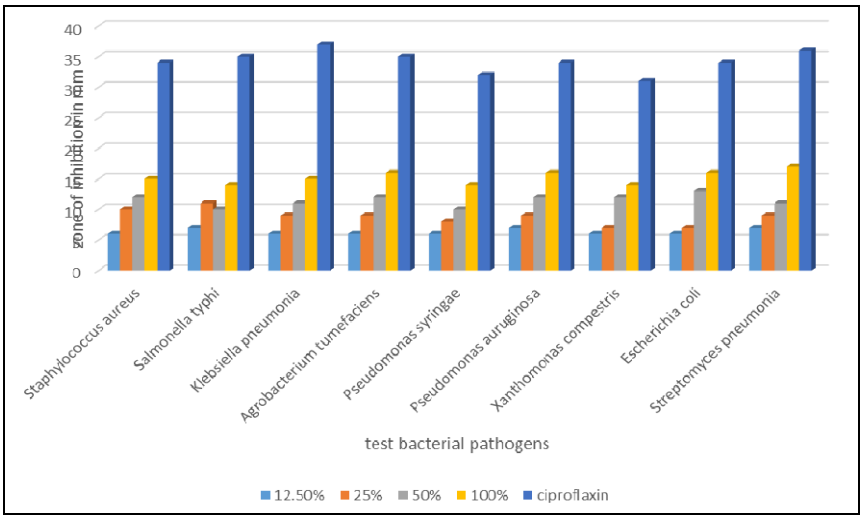

Chart 2: Activity of ethanolic crude extract against bacterial pathogens

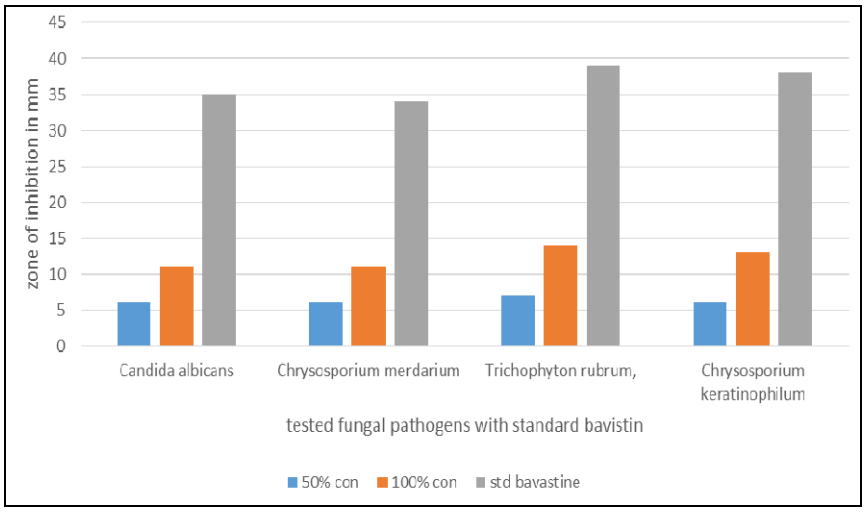

Chart 3: Activity of ethanolic crude extract against fungal pathogens in 50 and 100% con.

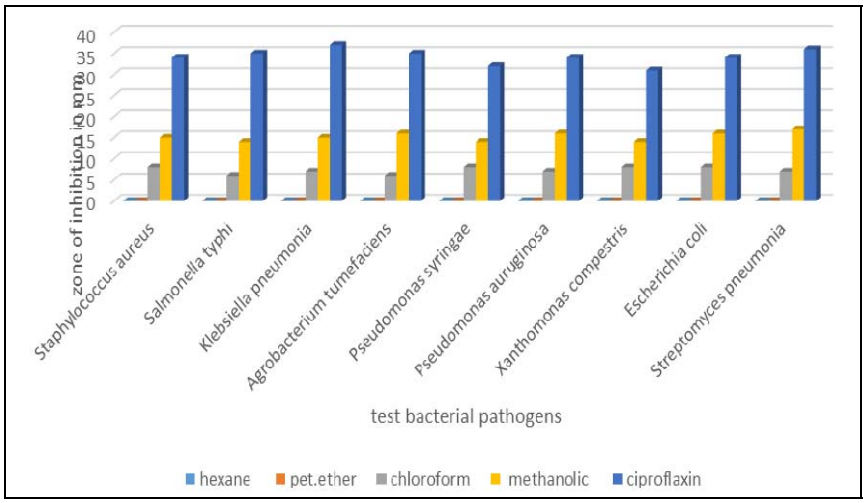

Chart 4: Different crude extracts performance in 100% concentration against pathogenic bacterial strains

Acknowledgement
The authors thankful to department of PG studies and research in Applied Botany, Jnanasahyadri, Shankaraghatta, Kuvempu University for providing facilities to conduct our experimental work. Authors also thankful to Adithya rao G S, Research Student, Department of PG Studies and Research in Applied Botany, Jnanasahyadri, Kuvempu University, Karnataka, India, for the crucial help in the collection of plant materials.

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