

Original Article

**PHYTOCHEMICAL EVALUATION AND *IN VITRO* ANTIMICROBIAL AND ANTIOXIDANT STUDIES OF LEAF AND STEM BARK EXTRACTS OF *POLYALTHIA FRAGRANS* (DALZ.) BEDD.- AN ENDEMIC SPECIES OF WESTERN GHATS**

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ABSTRACT

**Objective:** Phytochemical constituents of various solvent extracts of leaf and stem bark of *Polyalthia fragrans* (Dalz.) Bedd were screened. Antimicrobial and antioxidant activities of the extracts were evaluated.

**Methods:** Crude extracts of methanol, ethyl acetate, chloroform and aqueous of leaf and stem bark were evaluated for antimicrobial activity by disc diffusion method and antioxidant activity by DPPH (diphenyl-2-picrylhydrazyl) radical scavenging and reducing power assay. Quantitative analysis of total phenolics was done by the Folin-Ciocalteu method and total flavonoids by aluminum chloride method.

**Results:** The study revealed the presence of several physiologically active phytochemicals such as alkaloids, phenols, flavonoids and tannins. The highest antibacterial activity of methanol extract of the leaf was observed against *Bacillus subtilis* with 14.33±0.57 mm inhibition zone and water extract of stem bark against *Proteus vulgaris* with 17.67±1.52 mm inhibition zone among other extracts. The lowest activity of leaf methanol and leaf water extract was observed against *Bacillus subtilis*. The IC<sub>50</sub> values of 58.18±2.04 µg/ml, 116.58±2.43 µg/ml, 134.78±3.64 µg/ml and 387.28±2.9 µg/ml for DPPH activity were observed in methanol stem bark, ethyl acetate stem bark, methanol leaf and ethyl acetate leaf extracts respectively. The reducing power of extracts was very potent which increased with increasing concentrations of the sample.

**Conclusion:** The results indicate that phytochemicals like alkaloids, phenols, flavonoids and tannins may be responsible for the antimicrobial and antioxidant properties. The results suggest that *P. fragrans* can be further researched to be used as a natural source of a potent drug by mankind.

**Keywords:** *Polyalthia fragrans*, Phytochemicals, Antibacterial activity, Antioxidant activity

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INTRODUCTION

Plants are usually known to make use of their diverse health benefits through the numerous phytochemicals they contain. Plant-derived substances have recently turned to be of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [1]. According to an estimation of world health organization, about 80% of world's populations rely on traditional medicine for their primary healthcare needs and the most of this therapy involves the use of plant extracts and their active compounds [2].

Annonaceae, a family of the flowering plant is also known as custard apple family which consists of trees, shrubs and rarely lianas. It is the largest family in Magnoliales which includes more than 130 genera and about 2300 to 2500 species [3]. The genus *Polyalthia* (Annonaceae) distributed in subtropical and tropical regions [4] which consist of about 120 species of shrubs and trees. The genus is considered to be of medicinal importance because of the presence of clerodane diterpenoids and alkaloids in various parts of the plant [5, 6]. The various phytochemicals like alkaloids, terpenes, benzopyrenes and flavonoids have been isolated from *Polyalthia* species [7-9]. *Polyalthia* has been used in folk medicine and also used as bitter tonic, febrifuge, and a cure for scorpion stings, high blood pressure and respiratory stimulant [10]. The plant *P. longifolia* is used to treat fever, skin diseases, gonorrhoea, uterus ailment, leucorrhoea, diabetes, hypertension and helminthiasis [11-14] and *P. simiarum* is known to exhibit antimicrobial and cytotoxic [15].

*Polyalthia fragrans* an endemic species, found in the evergreen to semi-evergreen forests of the Western Ghats-South and Central Sahyadris. It is commonly known in Karnataka as Chamada,

Gowri mara, Nedunar. The tree is economically important as timber, used in plywood industries, packing cases and boxes, stumps and racquet frames [16-18]. Although a lot of work was carried out with regard to the phytochemical and pharmacological evaluation of *Polyalthia longifolia*, *P. simiarum*, *P. cerasoides* but on the basis of ethnomedical claims, nothing has been explored with regard to *Polyalthia fragrans*. Therefore, the present study was aimed at the extraction of phytochemicals from *P. fragrans*, an endemic species of Western Ghats and evaluation of the antibacterial and antioxidant activity.

MATERIALS AND METHODS

Chemicals and reagents

DPPH (diphenyl-2-picrylhydrazyl), gallic acid, quercetin, ascorbic acid, Mueller-Hinton agar were obtained from Sigma-Aldrich Chemicals (St. Louis, MO, USA). All the other chemicals were of analytical grade purchased from Merck Limited (Mumbai, India).

Preparation of extracts

Healthy leaf and stem bark samples were collected during November 2013 from Sampaje forest, Kodagu district of Karnataka and the voucher specimens (MU/AB/AK-01) have been deposited in the herbarium of Department of Applied Botany, Mangalore University. The collected samples were washed and shade dried, grounded into fine powder and stored in air tight polythene bags until use. Fifty grams of the powdered leaf and bark samples were extracted with methanol, ethyl acetate and chloroform by soxhlet extraction at 40 °C for 48 h. The extracts obtained were evaporated to dryness. Aqueous extracts were prepared by soaking 50 g of powdered sample in water for 72 h in a water bath and the obtained extracts were filtered through Whatman No.1 filter paper, evaporated to dryness on a water bath. All the extracts were stored at 4 °C for further use.

### Phytochemical analysis

The extracts were used for preliminary screening of phytochemicals such as alkaloids (Hagers, Wagners, Mayers, Dragendorff's tests), flavonoids (Shinoda's test, lead acetate test), steroids (Liebermann-Burchard and Salkowski tests), phenols (FeCl<sub>3</sub> test), tannins (lead acetate test), saponins (foam test), glycosides (Molisch's, sodium hydroxide test) and resins (turbidity test) [19].

### Determination of total phenolic content

The total phenolic content present in the extract was estimated using the Folin-Ciocalteu method [20]. An aliquot of 100 µl stock sample (10 mg/ml) was mixed with 2.0 ml of 2% Na<sub>2</sub>CO<sub>3</sub> and allowed to stand for 2 min at room temperature. Then, 100 µl of 50% Folin Ciocalteu's phenol reagent was added. After incubation for 30 min at room temperature in darkness, the absorbance was read at 720 nm using the spectrophotometer. The total phenolic content was calculated as mean±SD (n= 3) and expressed as milligrams (mg) of gallic acid equivalents (GAE) per gram (g) of extract.

### Determination of flavonoid content

Total flavonoid content present in the extract was estimated by following the aluminium chloride method [21]. A known aliquot of each extract was made up to 4 ml using distilled water and 0.3 ml of NaNO<sub>2</sub> (1:20) was added. After 5 min, 0.3 ml of 10% AlCl<sub>3</sub>. H<sub>2</sub>O solution was added followed by the addition of 2 ml of 1 M NaOH solution after 6 min and then the total volume was made up with 2.4 ml of distilled water. The absorbance against blank was determined at 510 nm. The total flavonoid content was calculated as mean±SD (n=3) and results were expressed as milligrams (mg) of quercetin equivalents (QE) per gram (g) of extract.

### Antimicrobial activity

*In vitro* antimicrobial activity of different solvent extracts of *Polyalthia fragrans* were tested against four bacterial cultures, two Gram-positive (*Staphylococcus aureus*, MTCC 7443; *Bacillus subtilis*, MTCC 2274) and two Gram-negative (*Escherichia coli*, MTCC 8933 and *Proteus vulgaris* MTCC 426) obtained from National chemical laboratory, Pune, India and the strains were maintained on nutrient agar slants. Two hundred microliter of an overnight grown culture of each organism was dispensed into 20 ml of sterile nutrient broth and incubated for 4-5 h at 37 °C to standardize the culture to 10<sup>8</sup> CFU/ml. Fifty mg of dried crude extract was dissolved in 1 ml of 20% DMSO (Dimethyl sulphoxide) and from this stock solution, 10 µl of respective solvent extracts were added aseptically to the sterile empty discs purchased from Himedia Laboratories Mumbai. After drying, they were used for screening the antibacterial activity.

### Assay for antibacterial activity

Antibacterial assay was carried out by disc diffusion method. For this, 0.1 ml (10<sup>8</sup>CFU/ml) of 24 h old bacterial culture was placed on

Muller Hinton agar medium and spread throughout the plate by spread plate technique. The sterile discs (6 mm in diameter), soaked with plant extract was placed on the surface of the medium and incubated at 37 °C for 24 h. Antibacterial activity was recorded by measuring the diameter of the zone of inhibition. Streptomycin was used as positive reference standard.

### DPPH scavenging activity assay

The antioxidant activity of the extract was measured based on the scavenging activity of the stable DPPH free radical. The activity was determined by using the method of Liyana et al [22]. A solution of 0.135 mmol DPPH was prepared in methanol and 1 ml of this was mixed with 1 ml of varying concentrations of the extracts. The thoroughly vortexed reaction mixture was incubated for 30 min in dark at room temperature. 1 ml of distilled water was used as a control. The absorbance of the mixture was measured at 517 nm using ascorbic acid as standard. The ability to scavenge DPPH radical was calculated by using the formula;

$$\text{Scavenging activity (\% of inhibition)} = \left[ \frac{A_c - A_e}{A_c} \right] \times 100$$

Where, A<sub>c</sub>-absorbance of DPPH radical as control, A<sub>e</sub>-absorbance of DPPH radical in the presence of plant extracts.

### Reducing power assay

The reducing power of the extract was determined by the method of Oyaizu [23]. Varied concentrations of 100 µl of the extracts were mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. After incubation, 2.5 ml of 10% trichloroacetic acid was added to the mixture and centrifuged at 3000 rpm for 10 min. The supernatant (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of freshly prepared 0.1% FeCl<sub>3</sub> solution. Absorbance was measured at 700 nm. The increased absorbance of the reaction mixture was measured as the reducing power. Ascorbic acid was used as a standard.

### Statistical analysis

All the experiments were performed in triplicates. Statistical analysis was carried out using SPSS ver.20 software. Statistical differences between activities of extracts were determined using one way ANOVA with Duncun's multiple range test. Differences were considered statistically significant when p<0.05.

## RESULTS

### Phytochemical screening

The phytochemical screening of the extracts of *Polyalthia fragrans* revealed the presence of phenols, flavonoids and tannins in all the extracts assessed (table 1). Alkaloid was absent in ethyl acetate extract of leaf and bark. Glycosides, steroids and resins were not detected in any of the extracts.

Table 1: Qualitative phytochemical screening

Phytochemicals	Solvent				Extracts			
	LM	LE	LC	LW	SM	SE	SC	SW
Phenols	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+
Saponins	-	-	-	-	-	-	-	-
Alkaloids	+	-	+	+	+	-	+	+
Glycosides	-	-	-	-	-	-	-	-
Steroids	-	-	-	-	-	-	-	-
Resins	-	-	-	-	-	-	-	-

LM= leaf methanol, LE= ethyl acetate, LC= leaf chloroform, LW= leaf water, SM= stem bark methanol, SE= stem bark ethyl acetate, SC= stem bark chloroform, SW= stem bark water

### Total phenolics and flavonoid content

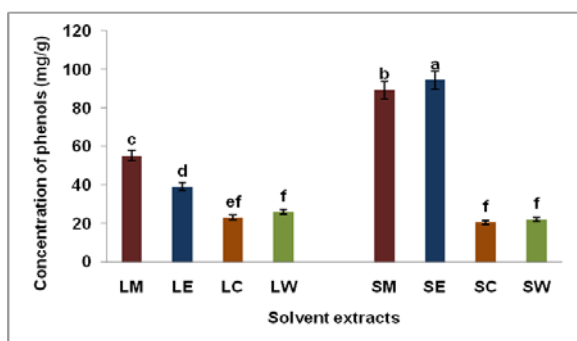
The amount of phenolics ranged from 20.47±0.25 mg to 94.38±0.75 mg GAE/g. (fig. 1a), the highest being 94.38±0.75 mg GAE/g in ethyl

acetate extract of stem bark followed by 89.30±3.94 mg GAE/g in the methanol extract of stem bark sample. Chloroform extract of both bark and leaf showed lower phenolic content. The flavonoid content in terms of quercetin equivalent was ranged between 34.29±1.41 to

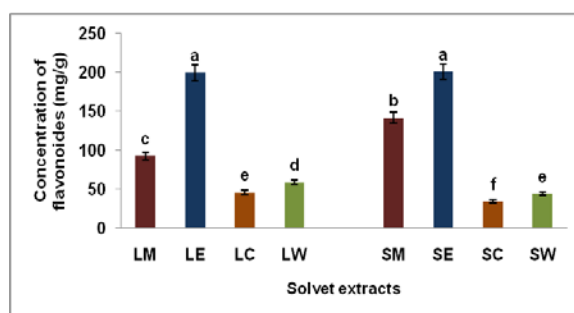
200.95±2.97 mg QE/g, the highest being 200.95±2.97 mg QE/g in ethyl acetate extract of stem bark followed by 141.42±0.00 mg QE/g in the methanol extract of stem bark.(fig. 1b).

#### Antimicrobial activity

The antibacterial activity of water extracts of stem bark was highest against *P. vulgaris* followed by *S. aureus* and least against *B. subtilis* (table 2). Leaf methanol extract showed the highest activity against *B. subtilis*. Leaf ethanol extract showed moderate activity against all the bacteria. Leaf water extract showed the least activity against *E. coli*, *B. subtilis* and *S. aureus* respectively. The antibacterial activity of all the extracts of *P. fragrans* was significantly lower than the standard Streptomycin.



**Fig. 1(a): Total phenolic content in different extracts of *Polyalthia fragrans*, LM= leaf methanol, LE= leaf ethyl acetate, LC= leaf ethyl chloroform, LW= leaf water, SM= stem bark methanol, SE= stem bark ethyl acetate, SC= stem bark chloroform, SW= stem bark water. Values represent the mean±SD of the three determinants. The values followed by different superscript differ significantly at p<0.05**



**Fig. 1(b): Total flavonoid content in different extracts of *Polyalthia fragrans*, LM= leaf methanol, LE= leaf ethyl acetate, LC= leaf chloroform, LW= leaf water, SM= stem bark methanol, SE= stem bark ethyl acetate, SC= stem bark chloroform, SW= stem bark water. Values represent the mean±SD of the three determinants. The values followed by different superscript differ significantly at p<0.05**

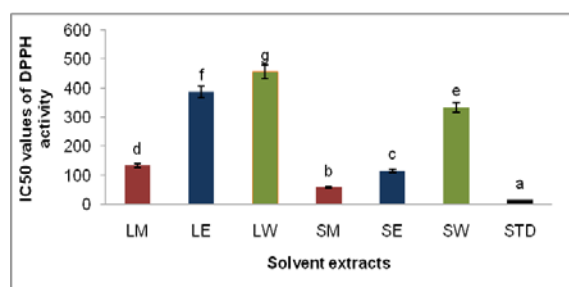
#### DPPH scavenging activity

The presence of phytochemicals such as phenols, alkaloids, terpenes and carotene contribute to antioxidant properties of plant extracts [24]. According to Cao [25] Phenolics and flavonoids present in the plant materials are important antioxidative components because of their scavenging ability. The leaf and stem bark extracts of *Polyalthia fragrans* showed a dose-dependent DPPH radical scavenging (fig. 2). The radical scavenging effect of leaf and stem bark extracts was in the order PSM>PSE>PLM>PSW>PLE>PLW. The IC<sub>50</sub> values of 58.18±2.04 µg/ml, 116.58±2.43 µg/ml and 134.78±3.64µg/ml for DPPH activity were observed in methanol stem bark, ethyl acetate stem bark and methanol leaf extracts respectively.

**Table 2: Antimicrobial activity of leaf and stem bark extracts of *Polyalthia fragrans***

Solvent extracts	Zone of inhibition (diameter in mm)			
	EC	BS	SA	PV
LM	11.00±1.00 <sup>c</sup>	14.33±0.57 <sup>b</sup>	12.67±0.57 <sup>c</sup>	10.33±0.57 <sup>d</sup>
LE	10.67±0.57 <sup>c</sup>	12.67±0.57 <sup>c</sup>	12.67±0.57 <sup>c</sup>	10.67±0.57 <sup>d</sup>
LC	10.67±0.57 <sup>c</sup>	8.33±0.57 <sup>d</sup>	9.33±0.57 <sup>f</sup>	10.33±0.57 <sup>d</sup>
LW	8.33±0.57 <sup>d</sup>	8.67±0.57 <sup>d</sup>	10.00±1.0 <sup>ef</sup>	11.67±0.57 <sup>cd</sup>
SM	10.67±1.15 <sup>c</sup>	8.67±0.57 <sup>d</sup>	12.33±0.57 <sup>cd</sup>	11.00±1.0 <sup>d</sup>
SE	8.67±0.57 <sup>d</sup>	8.00±0.0 <sup>d</sup>	12.67±0.57 <sup>c</sup>	8.67±0.57 <sup>e</sup>
SC	10.00±1.00 <sup>cd</sup>	13.33±1.52 <sup>bc</sup>	11.00±1.0 <sup>de</sup>	12.67±1.52 <sup>c</sup>
SW	13.33±1.15 <sup>b</sup>	8.67±0.57 <sup>d</sup>	15.00±1.0 <sup>b</sup>	17.67±1.52 <sup>b</sup>
STD	22.00±1.00 <sup>a</sup>	22.33±1.52 <sup>a</sup>	22.66±1.15 <sup>a</sup>	24.66±1.54 <sup>a</sup>
F value	52.660	89.774	65.045	101.714

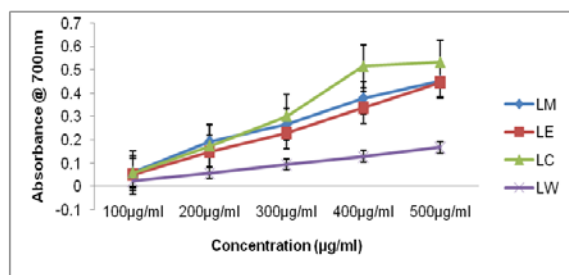
LM= leaf methanol, LE= leaf ethyl acetate, LC= leaf chloroform, LW= leaf water, SM= stem bark methanol, SE= stem bark ethyl acetate, SC= stem bark chloroform, SW= stem bark water, STD= standard. EC= *Escherichia coli*, BS= *Bacillus subtilis*, SA= *Staphylococcus aureus*, PV= *Proteus vulgaris*. Inhibitory zones in mm, represented as mean±SD values (n=3). The values followed by different superscript differ significantly at p<0.05.



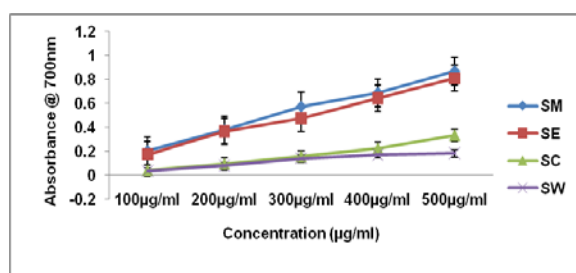
**Fig. 2: IC<sub>50</sub> values of DPPH scavenging activity of leaf and stem bark extracts of *Polyalthia fragrans*, LM= leaf methanol, LE= leaf ethyl acetate, LW= leaf water, SM= stem bark methanol, SE= stem bark ethyl acetate, SW= stem bark water. Values represent the mean±SD of the three determinants. The values followed by different superscript differ significantly at p<0.05**

#### Reducing power assay

Reducing capacity of the extract may serve as a significant indicator of its potential antioxidant activity [26]. In the reducing power assay, the presence of reductants in the extracts cause reduction of ferric to ferrous form which was monitored by measuring the absorbance at 700 nm [27]. The antioxidant activity exerted by the extract was by breaking the free radical chain by contributing a hydrogen atom. For the measurement of the reducing ability, the Fe<sup>3+</sup>-Fe<sup>2+</sup> transformation was investigated in the presence of *Polyalthia fragrans* extracts. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Fig. 3(a) and 3(b) show the reductive capabilities of the *Polyalthia fragrans* extracts. The reducing power of extracts was very potent which was increased with increasing concentration, the similar result was reported in leaf extracts of *Hopea ponga* [28]. The ferric reducing activity of leaf and stem bark extracts of *Polyalthia fragrans* was in the order PSM>PSE>PSC>PSW>PLM>PLC>PLE>PLW.



**Fig. 3(a):** Reducing power assay of leaf extracts of *Polyalthia fragrans*, LM= leaf methanol, LE= ethyl acetate, LC= leaf chloroform, LW= leaf water. Values represent the mean±SD of the three determinants



**Fig. 3(b):** Reducing power assay of stem bark extracts of *Polyalthia fragrans*, SM= stem bark methanol, SE= stem bark ethyl acetate, SC= stem bark chloroform, SW= stem bark water. Values represent the mean±SD of the three determinants

## DISCUSSION

Plants were of great importance to the health of individuals and communities. The preliminary phytochemical analysis of the plant extracts revealed the presence of secondary metabolites which are important for medicinal as well as physiological activities. Ghosh *et al.* [29] and Kavitha *et al.* [30] reported the presence of terpenoids, flavonoids, saponins, tannins and phenolic compounds in different solvent extracts of *Polyalthia longifolia*. *Polyalthia simiarum* also showed the presence of phenols, flavonoids, alkaloids, sterols, diterpenes and triterpenes in solvent extracts [31]. Methanol and water extracts of *Annona squamosa* leaf and seed showed the positive results for alkaloids, oils, tannins, phenols and flavonoids [32]. Leaf methanolic extract of *Holoptelea integrifolia* showed the presence of steroids, alkaloids and flavonoids [33].

Phenolics are widest spread secondary metabolites in the plant kingdom. These are very important plant constituents because of their scavenging ability on free radicals due to their hydroxyl groups [25]. The phenolic content of plants directly contributes to the antioxidant action. Kashalkar *et al.* [34] reported the phenolic and flavonoid content of *Polyalthia longifolia* ethanol extracts of leaf showed 255.0 mg/g, 339.2 mg/g respectively, which is quite high compared to the results of the present work. Mariod *et al.* [35] reported 167.9 mg/g of phenolic content in methanolic extracts of *Annona squamosa* bark which is quite high compared to the results of the present study. Total phenolic content of *Pisonia alba* ethanolic leaf extract showed 12.6 mg GAE/g) which is lower than the results of the present study [36]. However, the flavonoid content of *Annona squamosa* leaf and bark was 22.6 mg/g and 102.5 mg/g respectively, which is lower than the results of the present study. Saritha *et al.* [36] reported 7.5 mg QE/g of flavonoid content *Pisonia alba* ethanolic leaf extract is lower than the results of the present study.

The secondary metabolites like flavonoids, tannins and alkaloids are responsible for the antimicrobial activity [37-39]. The presence of flavonoids, tannins and alkaloids in different extracts of *Polyalthia fragrans* confirm the potential against selected microorganisms. The present study suggests a broad spectrum antibacterial activity of leaf and stem bark extracts of *Polyalthia fragrans*, although the degree of susceptibility differs between different organisms. Inhibition of

microorganisms by the leaf extracts of *Polyalthia longifolia* was in the order of methanol, ethanol, ethyl acetate, acetone and petroleum ether. Highest was against *B. subtilis* followed by *E. coli* by the leaf methanol extracts [40]. The seed and leaf, water, methanol and hexane extracts of *Annona squamosa* exhibited good antimicrobial activity against gram-ve bacterial strains [32].

Sivashanmugam *et al.* [41] reported the IC<sub>50</sub> values of 30.4±1.18 µg/ml and 118.6±7.28 µg/ml for DPPH radical scavenging activity of ethanol and chloroform bark extracts of *Polyalthia longifolia* respectively. Methanolic leaf and bark extracts of *Annona squamosa* showed the IC<sub>50</sub> values of 7.8 µg/ml and 125.0 µg/ml respectively [37], which is much lower than the values obtained in the present study for *P. fragrans*. Lalawmpui *et al.* [42] reported the IC<sub>50</sub> value of 6.86 µg/ml methanol bark extract of *Helicia nilagirica*, which is also much lower than the values obtained in the present study for *P. fragrans*. Goveas *et al.* [43] reported more DPPH radical scavenging activity of the *Coscinium fenestratum* methanolic stem extract than the methanolic leaf extract, the similar result was observed in *P. fragrans*. Sivashanmugam *et al.* [41] reported the concentration dependent reducing power activity of *Polyalthia longifolia* leaf ethanol and chloroform extracts.

## CONCLUSION

Phytochemical study of different solvent extracts of *Polyalthia fragrans* revealed the presence of alkaloids, phenols, flavonoids and tannins. In the present study, it was clear that methanol and ethyl acetate extract of *Polyalthia fragrans* leaf and stem bark contains an abundant amount of phenolic and flavonoid compounds, which possess high free radical scavenging and antioxidant activities. The antimicrobial activities of the plant might be due to the presence of large amounts of flavonoid compounds. Therefore, further research is needed to isolate, purify and characterize the bioactive compounds of the plant.

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## AUTHORS CONTRIBUTION

Design, experimental part of the work and writing of the manuscript was done by the first author Mr. Arunkumar. K. The design of the work and correction of the manuscript was done by the corresponding author Dr. K. R. Chandrashekar.

## CONFLICT OF INTERESTS

We declare that there is no conflict of interest

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