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Research Article

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Antimicrobial and antioxidant activity of leaf and flower of *Hypericum mysorense*

Anusha K. S., Ganashree N. K., Kruthika G. N., Poojashree R., Sushmitha K. K. and Prashith Kekuda T. R.*

Department of Microbiology, S. R. N. M. N. College of Applied Sciences, N.E.S Campus, Balraj Urs Road, Shivamogga, Karnataka, India

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ABSTRACT

The present study was conducted to determine antimicrobial and antioxidant activity of leaf and flower extract of Hypericum mysorense (Hypericaceae). Antibacterial and antifungal activity was determined by Agar well diffusion and Poisoned food technique respectively. Leaf extract showed high inhibitory activity against bacteria and fungi when compared to flower extract. Gram positive bacteria showed higher susceptibility to extracts when compared to Gram negative bacteria. Leaf extract inhibited C. capsici to high extent while flower extract inhibited B. sorokiniana to higher extent. Radical scavenging efficacy, as evaluated by DPPH scavenging assay, was high in case of flower extract when compared to leaf extract. The content of total phenolics was high in flower extract than leaf extract. A direct correlation was observed between phenolic content and radical scavenging efficacy of extracts. The plant appears to be promising source for development of agents with activity against pathogenic microorganisms and oxidative damage.

Key words: *Hypericum mysorense*, Agar well diffusion assay, Poisoned food technique, DPPH, Total phenolic

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INTRODUCTION

Western Ghats of India is considered to be one of the 34 biodiversity hotspots in the world. Western Ghats are known to harbor a large number of plant species, many of which are endemic. Various vegetation types *viz*., wet evergreen forests, moist and dry deciduous forests, montane forests, sholas, scrubs and savannas are found in Western Ghats. The mountain ranges of Western Ghats run through Gujarat, Karnataka, Tamil Nadu, Maharashtra, Goa and Kerala. The central Western Ghats of Karnataka (known as Sahyadri) encompass districts namely Chikmagalur, Shivamogga, Udupi, Dakshina Kannada, Uttara Kannada, Hassan and Coorg **[1,2]**. The genus *Hypericum* (Hypericaceae) is a large genus of herbs or shrubs with >450 species distributed worldwide. The plants grow widely in temperate regions and are used in folklore medicine in many parts of the world. The compounds isolated from this genus are shown to exhibit a wide range of bioactivities such as antimicrobial, antiviral, anticancer, antioxidant, antidepressant, anti-inflammatory etc. There are about 20 different species of *Hypericum* found in India, especially in Nilgiris. *H. mysorense* found primarily at high elevation in Western Ghats of India. The plant is well known in folklore medicine for its spasmolytic, hypotensive and antifungal activities. Wound healing, antiviral and nerve calming properties of *H. mysorense* are mentioned in traditional system of medicine **[3-8]**. The plant is shown to exhibit antiviral**[5]**, antioxidant **[3,8]**, hepatoprotective **[8]**, wound healing **[4]**, cytotoxic and antitumor **[9]** and antimicrobial activity **[10]**. In the present study, we determined antimicrobial and radical scavenging efficacy of leaf and flower extract of *H. mysorense*.

EXPERIMENTAL SECTION

Collection and identification of plant

The plants were collected during January 2015 at Seethalayyana giri, a stopover point on the way to Mullayana giri, Chikmagalur district, Karnataka. The plant was authenticated by Prof. D. Rudrappa, Department of Botany, S.R.N.M.N College of Applied Sciences, Shivamogga-01.

Extraction

The leaves and flowers were separated from the plant, washed well using clean water and dried under shade. The dried leaves and flowers were powdered separately in a blender. 25g of each powdered material was transferred into clean conical flasks containing 100ml of ethanol (HiMedia, Mumbai). The flasks were left for two days with occasional stirrings. The contents of the flasks were filtered through 4-fold muslin cloth followed by Whatman No. 1 filter paper. The filtrates were evaporated to dryness and stored in refrigerator until use **[2]**. The weight and color of leaf and flower extracts was noted.

Antibacterial activity of leaf and flower extracts

Three Gram positive namely *Staphylococcus aureus*, *Bacillus subtilis* and *B. coagulans* and three Gram negative bacteria namely *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* were used to assess their susceptibility to leaf and flower extracts by Agar well diffusion assay. The test bacteria were inoculated into sterile Nutrient broth (HiMedia, Mumbai) tubes and incubated for 24 hours at 37^{o}C. The broth cultures of test bacteria were inoculated on sterile Nutrient agar (HiMedia, Mumbai) plates by swabbing with the help of sterile cotton swabs. Using sterile cork borer, wells of 6mm diameter were punched in the inoculated plates. Leaf and flower extracts (20mg/ml of DMSO [Dimethyl sulfoxide, 25% in sterile water]), chloramphenicol (standard antibiotic, 1mg/ml of sterile distilled water) and DMSO (25%, in sterile water) were transferred aseptically into labeled wells. The plates were left for 30 minutes and then incubated in upright position for 24 hours at 37^{o}C. Using a ruler, the zone of inhibition formed around the wells were measured **[2]**.

Antifungal activity of leaf and flower extracts

Fungi namely *Bipolaris sorokiniana* (from root rot of wheat), *Fusarium oxysporum* f.sp. *zingiberi* (from rhizome rot of ginger), *Colletotrichum capsici* (from anthracnose of chilli) and *Alternaria* sp., and *Curvularia* sp. (from mouldy grains of sorghum) were tested for their susceptibility to leaf and flower extracts by Poisoned food technique **[11]**. Potato dextrose agar (HiMedia, Mumbai) medium was used. In brief, the well sporulated cultures of test fungi were aseptically inoculated at the centre of control (without extract) and poisoned plates (1mg extract/ml of medium) by point inoculation. The plates were incubated in upright position for 5 days at room temperature. The diameter of fungal colonies, in mutual perpendicular directions, was measured using a ruler. Antifungal activity of leaf and flower extracts, in terms of inhibition of mycelial growth (%) of test fungi, was determined using the formula:

Inhibition of mycelial growth (%) = (C – T / C) x 100, where C and T refers to diameter of fungal colonies on control and poisoned plates respectively.

Radical scavenging activity of leaf and flower extracts

DPPH (1,1-diphenyl-2-picryl hydrazyl) free radical scavenging assay was used to determine radical scavenging effect of leaf and flower extracts. In brief, 1ml of different concentrations of leaf and flower extract (6.25-100µg/ml of methanol) was mixed with 3ml of DPPH solution (0.004% in methanol), incubated at room temperature in dark for 30 minutes and the optical density was read at 517nm. The absorbance of DPPH control (1ml methanol+3ml DPPH) was noted. The scavenging activity (%) of each concentration was calculated using the formula:

Scavenging activity (%) = (Ac – At / Ac) x 100, where Ac and At refers to absorbance of DPPH control and DPPH and extract/standard combination respectively. Ascorbic acid was used as reference standard **[2]**.

Total phenolic content (TPC) of leaf and flower extracts

The TPC was estimated by Folin-Ciocalteau reagent (FCR) method. In brief, a dilute concentration of leaf and flower extract (0.5 ml) was mixed with 0.5 ml of FC reagent (1:1) and 2 ml of sodium carbonate (7%) in separate tubes. The tubes were incubated at room temperature for 30 minutes followed by measuring the absorbance at 765nm. A standard curve was plotted using different concentrations of Gallic acid (standard phenolic compound, 0-1000 µg/ml of sterile distilled water) and the TPC of extracts was estimated. The TPC was expressed as µg Gallic acid equivalents (GAE) from the graph **[2]**.

RESULTS

Yield and color or leaf and flower extract

The color of leaf and flower extract was brownish black and orange respectively. Yield of extract obtained was highest in case of leaf (39.93%) when compared to flower (29.60%).

Antibacterial activity of leaf and flower extract

The result of inhibitory activity of leaf and flower extract of *H. mysorense* against Gram positive and Gram negative bacteria is shown in Table 1 and Figure 1. Both extracts exhibited inhibitory activity against all test bacteria but to a varied extent. Overall, leaf extract displayed marked inhibitory activity against test bacteria when compared to flower extract. Inhibitory activity of reference antibiotic was higher when compared to leaf and flower extracts. Gram positive bacteria showed higher susceptibility to extracts as well as antibiotic when compared to Gram negative bacteria. DMSO did not cause inhibition of any of the test bacteria.

Table 1: Antibacterial activity of leaf and flower extract of *H. mysorense*

Test bacteria	Zone of inhibition in cm			
	Leaf extract	Flower extract	Antibiotic	DMSO
S. aureus	2.4	2.4	4.0	0.0
B. subtilis	3.0	2.0	3.9	0.0
B. coagulans	2.5	2.3	3.8	0.0
E. coli	2.0	1.9	3.2	0.0
P. aeruginosa	1.9	1.5	3.5	0.0
S. typhi	2.0	1.8	3.6	0.0

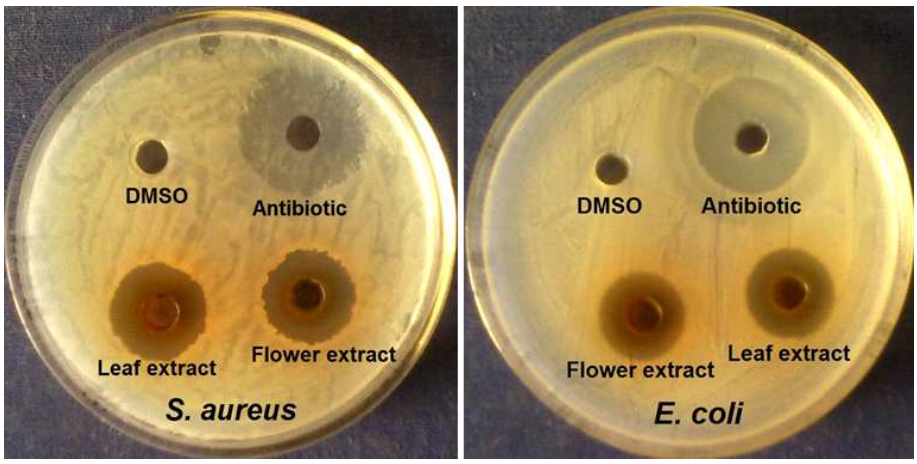

Figure 1: Inhibition of *S. aureus* and *E. coli* by leaf and flower extract

Antifungal activity of leaf and flower extract

Table 2 and Figure 2 and 3 depict the result of inhibitory potential of leaf and flower extract of *H. mysorense* against the mycelial growth of test fungi. Poisoning of medium with leaf and flower extract resulted in considerable suppression of growth of test fungi as revealed by lesser growth (colony diameter) when compared to growth on control plates. The leaf extract suppressed the growth of test fungi to high extent when compared to flower extract. Leaf extract inhibited *C. capsici* and *F. oxysporum* to high and least extent respectively. Flower extract suppressed the growth of *B. sorokiniana* and *Alternaria* sp. to high and less extent respectively.

Table 2: Colony diameter of test fungi on control and poisoned plates

Test fungi	Colony diameter in cm		
	Control	Leaf extract	Flower extract
Curvularia sp.	4.2	1.7	2.2
F. oxysporum	4.8	2.4	3.2
B. sorokiniana	3.9	1.5	1.8
C. capsici	3.1	1.0	1.5
Alternaria sp.	2.8	1.3	2.1

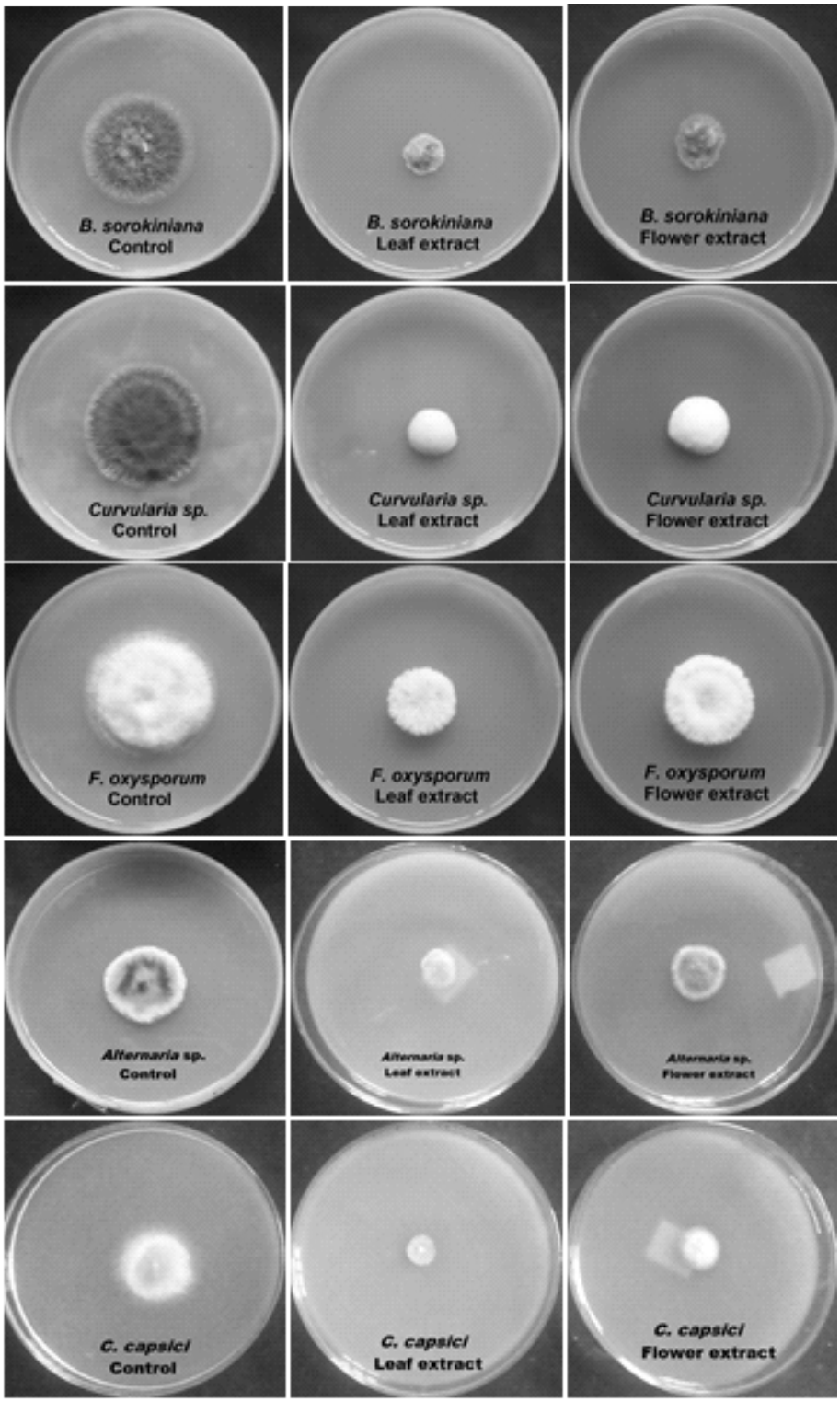

Figure 2: Growth of test fungi on control and poisoned plates (Left to right- Control, leaf extract, flower extract; Top to bottom- *B. sorokiniana*, *Curvularia* sp., *Fusarium* sp., *Alternaria* sp., *C. capsici*)

Radical scavenging activity of leaf and flower extract

The result of radical scavenging efficacy of leaf and flower extract of *H. mysorense* is depicted in Figure 4. The extracts were shown to exhibit dose dependent scavenging of DPPH free radicals as indicated by bleaching of radical solution color (purple to yellow) on increasing the concentration of extract. Among extracts, flower extract scavenged radicals more efficiently (IC_{50} value 28.06µg/ml) when compared to leaf extract (IC_{50} value 63.90µg/ml). However, the radical scavenging effect of reference antioxidant i.e., ascorbic acid was higher than that of both extracts as indicated by lower IC_{50} value ((2.91µg/ml).

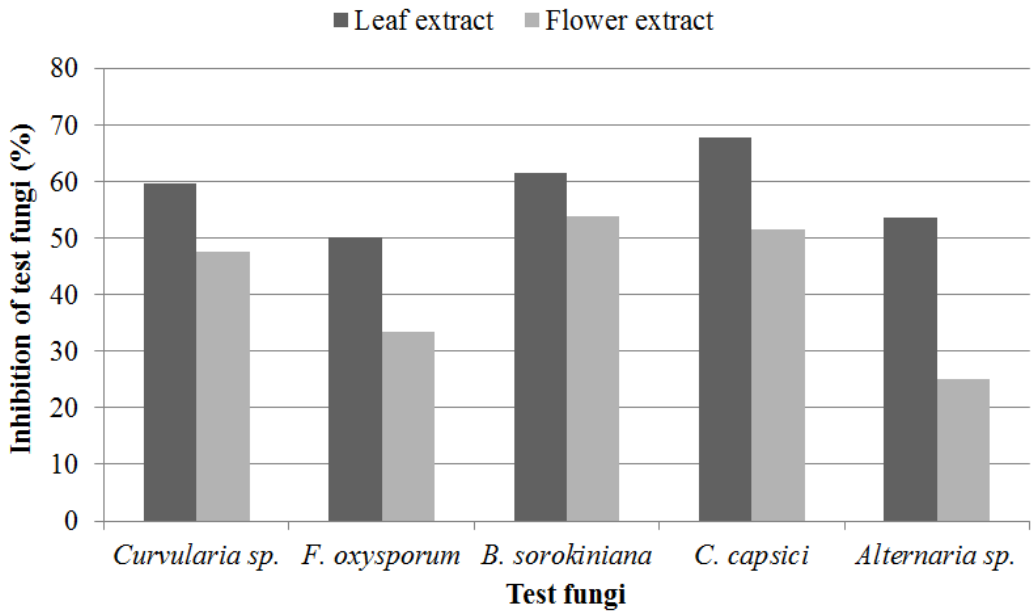

Figure 3: Inhibition of test fungi by leaf and flower extracts

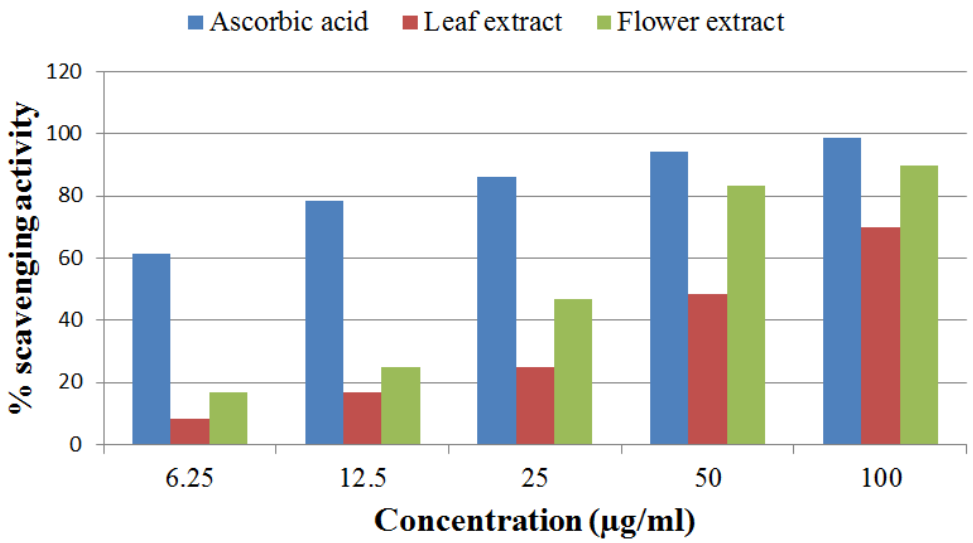

Figure 4: Scavenging of DPPH radicals (%) by leaf and flower extracts

Total phenolic content of leaf and flower extract

The content of total phenolics, as expressed in terms of mg GAE/g of extract, was found to be higher in flower extract (24.56mg/g) when compared to leaf extract (11.05mg/g).

DISCUSSION

One of the significant health related events of modern times was the discovery of antibiotics. The use of antibiotics has saved countless lives from most dreadful diseases of microbial origin. However, the successful use of any antibiotic is compromised by the potential development of tolerance or resistance. The overuse and abuse of these wonder drugs resulted in the potential development of resistance among pathogenic microorganisms **[12]**. Together with the resistance development problem, other problems such as high cost and side effects stimulated researchers to look for alternative sources of antimicrobial agents, especially plants and plant products **[2]**. In the present study, we evaluated antibacterial efficacy of leaf and flower extract of *H. mysorense* by Agar well diffusion assay which is widely used to determine antibacterial activity of various types of extracts/samples. The results revealed the potential of both extracts to inhibit test bacteria. Among extracts, marked inhibitory effect was shown by leaf extract when compared to flower extract. Both extracts inhibited all test bacteria except *B. subtilis* and *P. aeruginosa* to more or less similar extent. Among Gram positive bacteria, *B. subtilis* and *S. aureus* were inhibited to higher extent by leaf and flower extract respectively. In case of Gram negative bacteria, leaf extract inhibited *E. coli* and *S. typhi* to high extent whereas flower extract inhibited *E. coli* to higher extent. Among bacteria, Gram positive bacteria were

inhibited to higher extent than Gram negative bacteria by extracts and standard antibiotic. The lower susceptibility of Gram negative bacteria could be ascribed to the presence of outer membrane which may act as an additional barrier for the entry of extract/standard **[13,14]**. In an earlier study, the essential oil of leaf and flower extract were shown to exhibit inhibitory activity against several microorganisms **[10]**.

Among various phytopathogens, fungi represents an important group of pathogens causing a number of diseases in plants. Fungal infections leads to alterations in plants during developmental stages as well as post-harvest. Besides, fungi cause various quality problems related to aspect, nutritional value, organoleptic characteristics, and limited shelf life. On consumption of some contaminated plant commodities, allergic or toxic disorders may also be seen. Use of synthetic fungicides is one of the widely used strategies for controlling phytopathogenic fungi. However, the use of these fungicides is often associated with problems such as emergence of resistant pathogens, toxicity to non-target organisms and residual problem. Hence, search for antifungal agents from natural sources is much focused **[11,15,16,17]**. In the present study, the leaf and flower extract of *H. mysorense* displayed marked inhibitory effect against the mycelial growth of test fungi. Among extracts, potent inhibitory effect was shown by leaf extract when compared with flower extract. Among fungi, *C. capsici* (67.74% inhibition) and *B. sorokiniana* (53.84% inhibition) were inhibited to higher extent by leaf and flower extract respectively. *F. oxysporum* (50% inhibition) and *Alternaria* sp. (25% inhibition) were inhibited to least extent by leaf and flower extract respectively.

A number of *in vitro* assays are used to evaluate radical scavenging nature of various types of samples including plant extracts. DPPH free radical scavenging assay is one of the most widely used in vitro assays. DPPH is a stable, nitrogen centred, organic free radical having absorption maximum at 517nm in alcoholic solution. The radical becomes a stable diamagnetic molecule on accepting an electron or hydrogen atom from antioxidant. In the presence of a donor capable of donating hydrogen atom, the radical nature of DPPH is lost and its color (purple) changes to yellow (diphenylpicrylhydrazine). The solution loses color stoichometrically depending on the number of electrons taken up **[18-21]**. In the present study, the decrease in absorption of DPPH in the presence of varying concentrations of leaf and flower extract of *H. mysorense* was monitored at 517nm. The extracts showed dose dependent scavenging of DPPH radicals. Flower extract scavenged free radicals more effectively when compared to leaf extract as revealed by lower IC_{50} value. Similar results were observed in earlier study of Hariharapura *et al*. **[8]** where flower extract exhibited marked antioxidant activity than leaf extract. Ascorbic acid (reference antioxidant) displayed marked scavenging potential than leaf and flower extracts. Although the scavenging potential of extracts were lesser than that of ascorbic acid, it is clear from the study that the extracts possess hydrogen donating ability and hence, these extracts can act as free radical scavengers, acting possibly as primary antioxidants **[19]**. In another study, Chandrashekar *et al*. **[3]** evaluated antioxidant activity of *H.mysorense, H.perforatum, H.japonicum,* and *H. patulum*. Extract of *H.mysorense* was found to scavenge DPPH radicals more efficiently than other *Hypericum* species.

Phenolic compounds forms an important group of metabolites among various secondary metabolites produced by plants. Phenolic compounds exhibit a variety of bioactivities including antioxidant activity. The antioxidant property of phenolics is mainly due to their redox property. Phenolic compounds act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators. Hence, phenolic compounds are considered to be effective free radical scavengers and inhibitors of lipid peroxidation. The content of phenolics has been extensively studied for their contribution to antioxidant activity. There are many reports which correlate the total phenolic content of plants and their antioxidant activity **[22,23,24]**. In our study, the content of total phenolics, as estimated by FCR method and as expressed in terms of mg GAE/g of extract, was high in flower extract (24.56mg/g) than leaf extract (11.05mg/g). The flower extract shown high DPPH scavenging which may be attributed directly with the phenolic content of extract. The result is in justification with the earlier study of Hariharapura *et al*. **[8]** in which flower top extract containing high phenolic and flavonoid content displayed marked DPPH radical scavenging activity when compared to leaf extract which contained less phenolic and flavonoid content than flower top extract. Similarly, Chandrashekar *et al*. **[3]** observed a direct correlation between total phenolic content and DPPH radical scavenging activity of *Hypericum* species.

CONCLUSION

A marked antimicrobial and radical scavenging effect of leaf and flower extracts of *H. mysorense* was observed in the present study. The observed bioactivities of extracts could be ascribed to the presence of secondary metabolites present in the extracts in particular phenolic compounds. A direct correlation was observed between the content of total phenolics and radical scavenging activity of extracts. The plant appears to be promising resource for bioactive agents which can be exploited for the prevention and treatment of oxidative stress and dreadful microbial diseases. Further studies on isolation of phytochemicals and their bioactivity determinations are to be conducted.

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