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Research Article

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Antifungal and radical scavenging activity of leaf and bark of *Leea indica* (Burm. f.) Merr.

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ABSTRACT

Leea indica (Burm.f.) Merr. (Leeaceae) is an evergreen large shrub having traditional uses worldwide. In the present study, we investigated antifungal and radical scavenging potential of leaf and bark extract of L. indica. The leaf and bark powders were extracted using methanol. Antifungal activity of extracts was evaluated against Colletotrichum capsici, Helminthosporium sp., and Curvularia sp. by Poisoned food technique. Radical scavenging activity of extracts was assessed by DPPH free radical scavenging assay. The content of total phenolics in extracts was estimated by Folin-Ciocalteau reagent method. Leaf extract displayed marked antifungal effect when compared to bark extract. Among fungi, high and least susceptibility was shown by Helminthosporium sp. and Curvularia sp. respectively. Leaf extract scavenged DPPH radicals more efficiently than bark extract. Total phenolic content was also higher in leaf extract. A direct correlation was observed between total phenolic content and radical scavenging activity. Marked antifungal and radical scavenging potential of leaf extract might be attributed to the high phenolic content. The plant appears to be a promising source of bioactive principles with antifungal and antioxidant activity.

Key words: *Leea indica*, Poisoned food technique, DPPH, Folin-Ciocalteau

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INTRODUCTION

Leea indica (Burm.f.) Merr., belonging to the family Leeaceae is an evergreen large shrub growing up to 2–3 m in height. The plant is found in tropical and subtropical countries like India, Malaysia, Thailand, Bangladesh and China. It is a perennial shrub with stout, soft wooded, glabrous stems. The plant is traditionally used in various parts of the world for the treatment of several ailments such as diarrhea, dysentery, cough, diabetes, headache, snake bite, allergy, skin diseases, cancer etc. Several chemicals such as phthalic acid, palmitic acid, 1-eicosanol, solanesol, farnesol, three phthalic acid esters, gallic acid, lupeol, β-sitosterol, gallic acid and ursolic acid have been identified in the leaf **[1-5]**. The plant is reported to exhibit several bioactivities. The leaf was shown to exhibit bioactivities such as antiviral **[6]**, sedative and anxiolytic **[7]**, antioxidant **[5,8]**, cytotoxic **[5,8]**, hypoglycemic **[9]**, hypolipidemic **[9]**, analgesic **[10]** and antimicrobial activity **[5]**. The stem bark extract was shown to exhibit hepatoprotective activity against paracetamol induced liver toxicity in rats **[11]**. The essential oil from flowers showed moderate antimicrobial activity **[2]**. The ethanol extract of roots showed potent phosphodiesterase inhibitory activity **[12]**. The present study was carried out to investigate antifungal and radical scavenging efficacy of methanol extract of leaf and bark of *L. indica*.

EXPERIMENTAL SECTION

Collection and identification of plant

The plant was collected at Haniya, Hosanagara Taluk of Shivamogga district, Karnataka in the month of January 2014. The plant material was identified by Dr. Vinayaka K.S, Department of Botany, KFGC, Shikaripura, Karnataka.

Extraction

The leaves and barks were separated from the plant, washed well using clean water and dried under shade. The shade dried plant materials were powdered in a blender. The powdered plant materials were extracted using methanol. 25g of leaf and bark powder was taken in separate conical flasks, 100ml of methanol was added and the flasks were shaken regularly. After two days, the contents of the flaks were filtered through muslin cloth followed by Whatman No. 1 filter paper. The filtrates were evaporated to dryness at 50^{o}C **[13]**.

Phytochemical analysis

The methanol extract of leaf and bark was qualitatively tested for the presence of various phytochemical constituents such as alkaloids, flavonoids, tannins, saponins, glycosides, steroids and terpenoids by following standard phytochemical procedures **[5,14,15]**.

Antifungal activity of leaf and bark extract

Poisoned food technique was performed to investigate antifungal effect of leaf and bark extract of *L. indica* against three molds *viz*., *Colletotrichum capsici* (an isolate from chilli anthracnose), *Helminthosporium sp*. and *Curvularia sp* (from moldy sorghum grains). Potato dextrose agar medium was prepared, poisoned with extract (1mg/ml of medium), sterilized by autoclaving and dispensed into sterile petriplates. The test fungi were inoculated at the centre of control (without extract) and poisoned plates and the plates were incubated at 28^{o}C for five days in upright position. The colony diameters of fungi were measured in mutual perpendicular directions. The inhibition of mycelial growth of test fungi (%) was calculated using the formula:

Antifungal activity (%) = (C – T / C) x 100, where C and T refers to colony diameter of test fungi on control and poisoned plates respectively **[13]**.

Radical scavenging activity of leaf and bark extract

The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging method employed by Vivek *et al*. **[16]** was followed to assess radical scavenging efficacy of leaf and bark extract of *L. indica*. 1ml of different concentrations (3.125-100µg/ml of methanol) of extracts and ascorbic acid (reference antioxidant) were mixed with 3ml of DPPH solution (0.004% in methanol) in separate tubes. The tubes were left in dark for 30 minutes followed by measuring absorbance at 517nm in a UV-Visible spectrophotometer (ELICO, SL159). The absorbance of DPPH control (1ml methanol + 3ml DPPH solution) was also noted. The radical scavenging efficacy of each concentration of leaf and bark extract was calculated using the formula:

Scavenging activity (%) = [(C–T) /C] x100, Where 'C' and 'T' refer to absorbance of DPPH control and absorbance of DPPH in presence of extract/standard respectively. The IC_{50} (inhibitory concentration) value for the extract was calculated. IC_{50} represents the concentration of extract required to scavenge 50% of DPPH free radicals.

Total phenolic content of leaf and bark extract

Folin-Ciocalteu reagent (FCR) method was performed to estimate the content of total phenolic compounds in the leaf extract and bark extract **[16]**. In brief, a dilute concentration of each extract (0.5ml) was mixed separately with 0.5ml diluted FCR (1:1) and 2ml of sodium carbonate (7%). The tubes were incubated at room temperature for 30 minutes followed by measuring the absorbance at 765nm in a UV-Visible spectrophotometer (ELICO, SL159). Gallic acid was used as standard and a curve was plotted using different concentrations of gallic acid (0-1000µg/ml). The concentration of total phenolics in extracts was interpreted as µg gallic acid equivalents (GAE) from the graph.

RESULTS

Phytoconstituents detected in leaf and bark extract

The preliminary phytochemical analysis of leaf and bark extract of *L. indica* showed the presence of alkaloids, glycosides, flavonoids and tannins in both extracts. Terpenoids, steroids and saponins were detected only in leaf extract (Table 1).

Table 1: Phytochemicals detected in leaf and bark extract of *L. indica*

Phytochemical	Leaf extract	Bark extract
Alkaloids	+	+
Glycosides	+	+
Terpenoids	+	-
Flavonoids	+	+
Steroids	+	-
Tannins	+	+
saponins	+	-

'+' detected; '-' not detected

Antifungal activity of leaf and bark extract

The result of inhibitory activity of leaf and bark extract of *L. indica* against test fungi is shown in Table 2 and Figure 1. The diameters of fungal colonies on poisoned plates were lesser than that of diameters of fungal colonies on control plates indicating inhibitory potential of extracts. When compared to control plates, a drastic reduction in the mycelial growth of *C. capsici* and *Helminthosporium* sp. was observed on poisoned plates. Both extracts caused >50% inhibition of *C. capsici* and *Helminthosporium* sp. When compared to bark extract, leaf extract was more effective in inhibiting mycelial growth of test. The susceptibility of fungi to extracts was in the order: *Helminthosporium* sp.> *C. capsici* > *Curvularia* sp.

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

Test fungi	Colony diameter in cm*		
	Control	Leaf extract	Bark extract
C. capsici	3.2	1.2	1.6
Helminthosporium sp.	3.6	1.3	1.6
Curvularia sp.	3.4	2.1	3.1

**Result is mean of three trials*

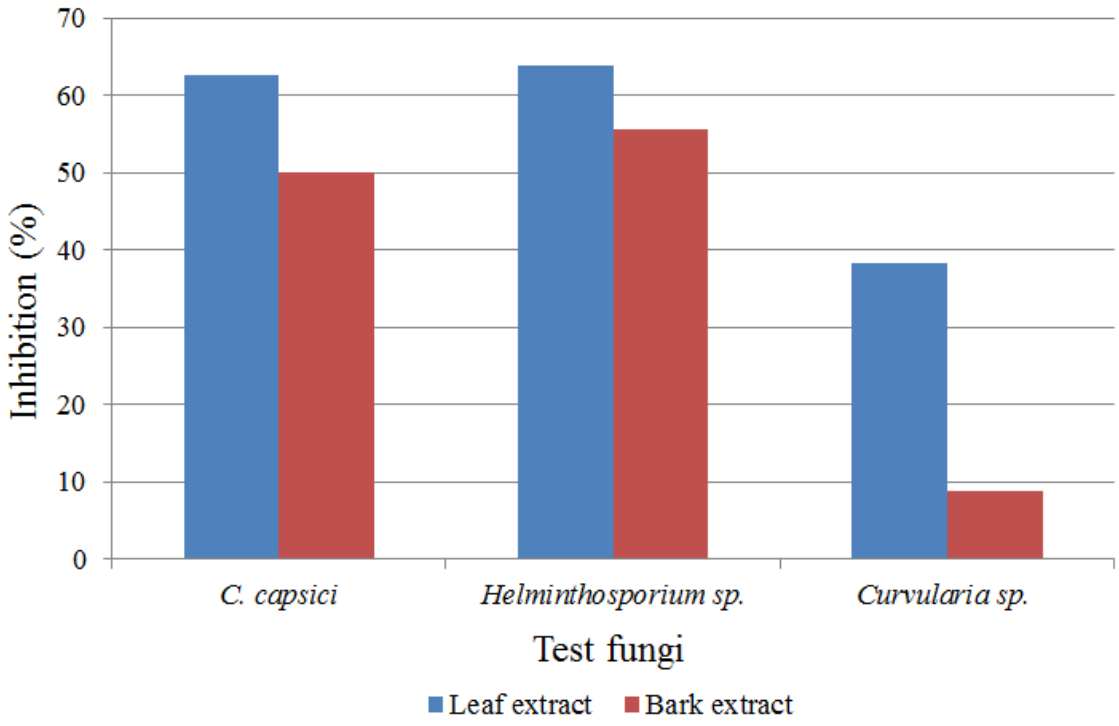

Figure 1: Inhibition of test fungi (%) by leaf and bark extract of *L. indica*

Radical scavenging activity of leaf and bark extract

Figure 2 shows the result of radical scavenging ability of extracts of *L. indica*. The extracts and ascorbic acid were shown to exhibit dose dependent scavenging of DPPH radicals. The radical scavenging effect of leaf and bark extract ranged from 13.33 to 97.77% and 6.66 to 95.55% respectively. Marked scavenging effect was observed in case of leaf extract (IC_{50} 11.55µg/ml) when compared to bark extract (IC_{50} 12.50µg/ml). Ascorbic acid (IC_{50} 1.57µg/ml) scavenged radicals more efficiently than leaf and bark extract. At lower concentrations, the extracts

exhibited lesser scavenging efficacy when compared to ascorbic acid. At concentrations 25 and 50µg/ml, the scavenging efficacy of extracts was higher than that of ascorbic acid.

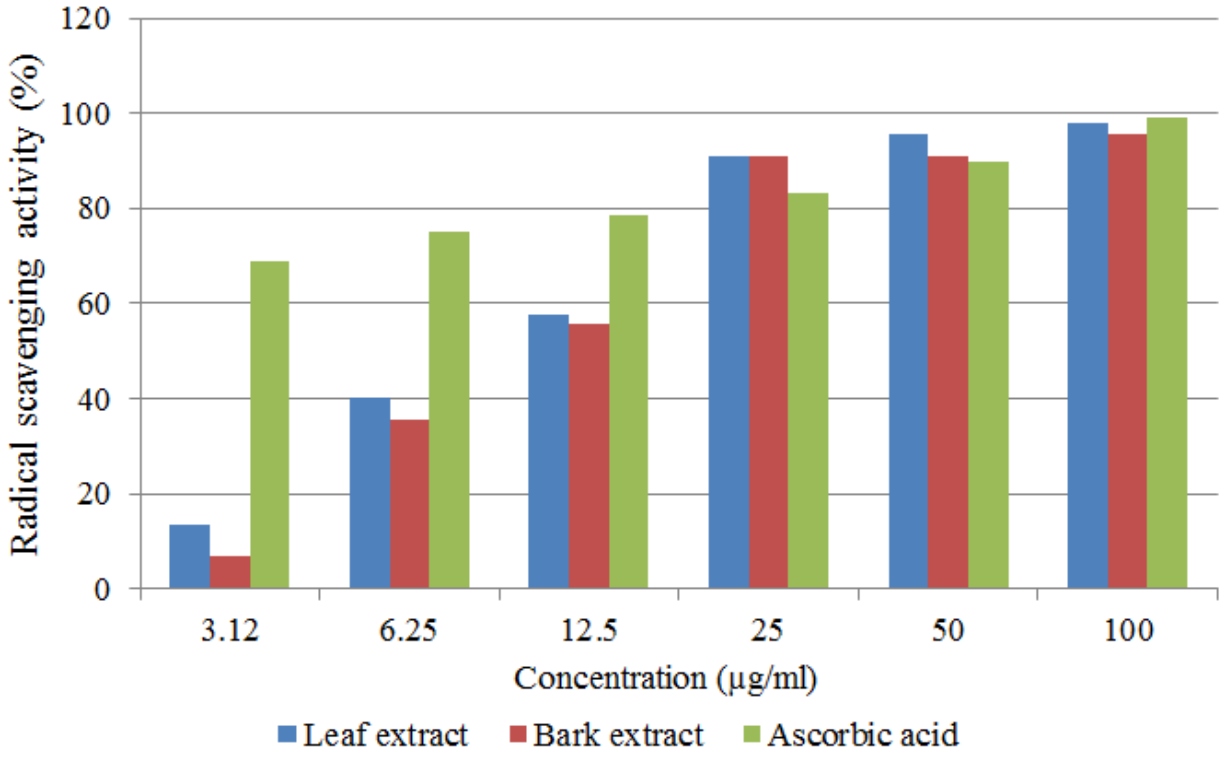

Figure 2: Radical scavenging efficacy of *L. indica* (Result is mean of three trials)

Total phenolic content of leaf and bark extract

The content of total phenolic compounds in leaf and bark extract was found to be 36.66 and 28.14µg GAE/mg of extract respectively.

DISCUSSION

Antifungal activity of leaf and bark extract

Pathogens such as bacteria, fungi, viruses and nematodes cause several diseases in agricultural and horticultural crops. Among these, fungi are more aggressive and cause a number of plant diseases leading to reduced crop yield and economic loss. The crop loss may account for >50% in severe cases. Several approaches are employed to prevent and control plant diseases caused by fungi. Among these, chemical control method is widely used. However, chemical approach for disease control has several drawbacks such as high cost, environmental problems, effect on non-target organisms and emergence of resistant fungal strains. Hence, an immense interest in search of natural products having antifungal activity has been triggered. Extracts, essential oils and purified components from plants are reported to exhibit marked antifungal activity against phytopathogenic fungi **[17-20]**. In the present study, we evaluated antifungal effect of leaf and bark extract of *L. indica* against three fungi (isolated from chilli anthracnose and moldy grains of sorghum) by poisoned food technique. A reduction in the colony size of fungi on poisoned plates when compared to control plates indicates antifungal effect of extracts. Leaf extract was more effective in inhibiting test fungi when compared to bark extract. Among fungi, *Helminthosporium* sp. was strongly inhibited while least inhibitory effect was observed in case of *Curvularia* sp. In an earlier study, Rahman *et al*. **[5]** showed inhibitory potential of leaf extract of *L. indica* against *Aspergillus flavus*, *Candida albicans*, and *Fusarium equisetii*. In another study, Srinivasan *et al*. **[2]** observed moderate antifungal effect of essential oil from flowers of *L. indica* against *Fusarium moliniformae*, *Penicillium notatum* and *Aspergilllus niger*.

Radical scavenging activity of leaf and bark extract

Among various *in vitro* free radical scavenging assays, DPPH radical scavenging assay is most popular and widely used as the method is simple, rapid and requires small concentration of samples. The assay is used to evaluate radical scavenging nature of various kinds of samples including plant extracts. DPPH is a stable, nitrogen centred, commercially available, organic free radical and has an absorption maxima at 515-517nm in methanol. On accepting

hydrogen from donor (antioxidant), the solution of DPPH loses the characteristic deep purple colour and becomes yellow coloured diphenylpicryl hydrazine. In this assay, a lower IC_{50} value indicates the potent ability of the sample to scavenge DPPH radicals. A high IC_{50} value indicates the low scavenging efficacy of sample which in turn indicates the requirement of high sample concentration to scavenge 50% of radicals **[5,21,22,23]**. In this study, the DPPH radical scavenging assay was used to screen the efficacy of leaf and bark extract of *L. indica* to scavenge radicals by donating proton. Both the extracts scavenged radicals dose dependently. Among extracts, leaf extract scavenged radicals more efficiently than bark extract as indicated by lower IC_{50} value. In previous studies, Reddy *et al*. **[8]** and Rahman *et al*. **[5]** showed antioxidant potential of leaf extract of *L. indica*. In the present study, the leaf and bark extracts showed hydrogen donating ability and could serve as free radical scavengers, acting possibly as primary antioxidants **[24]**.

Total phenolic content of leaf and bark extract

Polyphenolic compounds including flavonoids are a large and diverse group of plant metabolites having a broad range of bioactivities including antioxidant activity. These phenolic compounds exhibit strong antioxidant activity both *in vitro* and *in vivo*. Increased consumption of phenolic compounds is shown to be associated with decreased risk of several diseases/disorders such as cardiovascular diseases, neurodegenerative diseases and certain types of cancer. FCR method is an oldest and widely used method to estimate the content of total phenolic compounds in several kinds. The method was initially used for the analysis of proteins. Later, the method was employed to estimate phenolic content in other samples such as wine and plants. Despite the undefined chemical nature of FCR, the estimation of total phenolics by FCR method is simple, convenient and reproducible. The phenolic compounds react with FCR under basic conditions (pH~10, adjusted by Na_2CO_3). Dissociation of a phenolic proton leads to a phenolate anion, which is capable of reducing FCR and a blue colour is formed **[21,23]**. In the present study, we estimated the content of total phenolics in leaf and bark extract of *L. indica* by FCR method. Leaf extract was found to contain high phenolic content than bark extract. Studies have shown a direct correlation between the total phenolic content and antioxidant activity **[25,26,27]**. Similar observation was made in the present study as the leaf extract containing high phenolics displayed higher scavenging activity than bark extract.

CONCLUSION

Leaf and bark extract of *L. indica* displayed antifungal and radical scavenging activity *in vitro*. Leaf extract was more potent than bark extract. Higher activity of leaf extract could be ascribed to high phenolic content. The plant appears to be a promising source of antifungal and antioxidant agents.

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