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Original Research

Antimicrobial and Radical Scavenging Efficacy of Leaf and Flower of *Aristolochia indica* Linn.

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Abstract

The present study was performed to screen antibacterial, antifungal and radical scavenging efficacy of leaf and flower extract of *Aristolochia indica* Linn. Antibacterial and antifungal activity was evaluated by agar well diffusion and poisoned food technique respectively. Radical scavenging potential was determined by DPPH free radical scavenging assay. Overall, Leaf extract and flower extract displayed stronger antibacterial and antifungal activity respectively. Leaf extract was found to scavenge DPPH radicals more effectively when compared to flower extract. In suitable form, the plant can be used against microbial infections and oxidative stress.

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INTRODUCTION

The morbidity and mortality caused by infectious agents have been drastically reduced after the discovery and subsequent use of antibiotics. However, the traditional antibacterial therapy which uses antibiotics (natural or synthetic analogues) is facing a lot of problems among which the development of resistance in pathogenic microorganisms is the most important. Bacteria such as *Staphylococcus aureus*, enterococci, multidrug resistant tuberculosis, *Escherichia coli* and *Pseudomonas aeruginosa* are few among antibiotic resistant bacteria against which most of the antibiotics are not effective. Most importantly, these drug resistant pathogens have the potential to inherit the resistance gene to susceptible bacteria which makes the condition even worst. Hence, development of new antibacterials from natural sources is of much interest. Plants are considered as an important reservoir of various metabolites having a range of medicinal properties including antimicrobial activity. Several plants are used for treating various diseases caused by pathogenic bacteria (Ojala *et al*., 2000; Vaghasiya and Chanda, 2007; Hemaiswarya *et al*., 2008; Davies and Davies, 2010; Wright, 2010; Kekuda *et al*., 2013).

Among various pathogens causing plant diseases, fungi are considered as more aggressive. Fungal infections of plants results in considerable reduction of crop yield and economy. One of the most widely used strategies to control fungal diseases of plants is the use of chemical agents (fungicides) from synthetic origin. However, the use of these chemical agents resulted in several hazardous effects. These chemical agents have some drawbacks such as high cost, toxicity to nontarget organisms, residual problem and development of resistance in pathogens. This situation triggered interest in searching alternates for disease control. Natural products, in particular from plants, can be the potential candidates which can be used against phytopathogenic fungi. The use of these agents is risk-free when compared to synthetic chemicals (Gomathi and Kannabiran, 2000; Abou-Zeid *et al*., 2008; Yazdani *et al*., 2011; Rahman *et al*., 2011; de Barros *et al*., 2011; Bajpai and Kang, 2012; Dileep *et al*., 2013).

Free radicals such as superoxide radical, hydroxyl radical, peroxyl radical and nonradical species such as hydrogen peroxide that are produced by various means such as radiations, chemical reactions and several redox reactions involving various compounds results in oxidative stress. These radicals contribute to protein oxidation, DNA damage and lipid peroxidation in living systems and are involved in many diseases such as cancer, cardiovascular diseases, liver cirrhosis and neurological disorders. Cells have antioxidant defense mechanisms which include antioxidant enzymes *viz*., superoxide dismutase, catalase, glutathione oxidase and small molecules such as vitamin C and vitamin E. however, under pathological conditions, there is an extra requirement for antioxidants from exogenous sources. Strong restrictions have been placed on the use of synthetic antioxidants such as BHT, BHA and gallates due to their potential adverse effects. Plants are richer sources of antioxidant chemicals. Polyphenols

including flavonoids have shown to be excellent antioxidants (Dasgupta and De, 2004; Choi *et al*., 2007; Gulcin *et al*., 2011; Junaid *et al*., 2013; Kekuda *et al*., 2013).

Aristolochia indica Linn., belongs to the family Aristolochiaceae. Different parts of the plant are reported to possess several medicinal properties and are used in Indian system of medicine. It is commonly known by as Indian birthwort and snakeroot due its traditional use in postpartum infections and snakebite respectively. The fresh juice of the leaves is a popular antidote to snake poison. The leaves and barks are used in bowel complaints of children, diarrhoea and in intermittent fevers. The dried roots and rhizomes are used as a gastric stimulant and bitter tonic. The root is used in skin diseases. In traditional medicine, the underground parts of the plant are rubbed with honey and given to treat leprosy. The roots and stems are used in ethno veterinary aches and pains, rheumatism, anthrax, madness, antibacterial effect, antineoplastic effect, antiarthritic effect and snakebite (Wu *et al*., 2004; Ramachandran *et al*., 2008; Sati *et al*., 2011; Mathew *et al*., 2011).

It has been experimentally shown that the plant several various bioactivities such as antimicrobial (Shafi *et al*., 2002; Umamaheshwari & Muthy, 2012; Venkateswarlu *et al*., 2013), antioxidant (Thirugnanasampandan *et al*., 2008; Hossen *et al*., 2014), inhibition of drug induced hyperuricemia (Ramachandran *et al*., 2008), anthelmintic (Mini *et al*., 2013), antihyperglycemic (Shanmugam *et al*., 2014), antidiarrheal (Dharmalingam *et al*., 2014), anti-inflammatory (Mathew *et al*., 2011), antipruritic (Mathew *et al*., 2011), mast cell stabilizing (Mathew *et al*., 2011) and immunomodulatory (Mallaiah *et al*., 2015) activity. In the present study, we determined antimicrobial and radical scavenging efficacy of leaf and flower extract of *A. indica*.

MATERIALS AND METHODS

Collection and Extraction of Plant Material

The plant *A. indica* was collected at outskirts of Chitradurga, Karnata, India during September 2014. The plant was authenticated by Prof. D. Rudrappa, Lecturer and Head, Dept. of Botany, S.R.N.M.N College of Applied Sciences, Shivamogga, Karnataka. The leaves and flowers were separated from plants, washed well, dried under shade and powdered. 25g of leaf and flower powder was transferred into separate conical flasks containing 100ml ethyl alcohol (HiMedia, Mumbai) and stirred well. The flasks were left for 48 hours with occasional stirring. The content of each flask was filtered through Muslin cloth followed by Whatman No. 1 filter paper. The leaf and flower extracts were evaporated to dryness and used for bioassays (Dileep *et al*., 2014).

Antibacterial Activity of Leaf and Flower Extract of *A. indica*

Agar well diffusion assay was used to evaluate antibacterial activity against three Gram positive (*Staphylococcus aureus*, *Bacillus coagulans*, *B. subtilis*) and three Gram negative (*Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*) bacteria. In brief, 24 hours old Nutrient broth cultures of test bacteria were swab inoculated on sterile Nutrient agar plates followed by punching wells of 6mm diameter. Respective wells were filled with extracts (20mg/ml of 25% Dimethyl sulfoxide [DMSO]), antibiotic (Streptomycin, 1mg/ml of sterile distilled water) and DMSO (25%, in sterile water). The plates were then incubated at 37^{o}C for 24 hours. The zones of inhibition formed around the wells were recorded (Kekuda *et al*., 2013).

Antifungal Activity of Leaf and Flower Extract of *A. indica*

Poisoned food technique was conducted to evaluate antifungal activity of leaf and flower extract of *A. indica* against test 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 *Curvularia* sp. (from mouldy grains of sorghum). Potato dextrose agar was poisoned with the extracts (1mg/ml of medium). In brief, well sporulated cultures of test fungi were inoculated on control (without extract) and poisoned plates by point inoculation using sterile inoculation needle. The plates were incubated in upright position for 5 days at room temperature. The diameter of colonies was measured in mutual perpendicular directions. Antifungal activity of 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 (Vinayaka *et al*., 2014).

DPPH RADICAL SCAVENGING ACTIVITY of Leaf and Flower Extract of *A. indica*

DPPH (1,1-diphenyl-2-picryl hydrazyl) assay was used to evaluate free radical scavenging activity of leaf and flower extract of *A. indica*. In brief, 1ml of different concentrations of extract (6.25-100µg/ml) was added to 3ml of DPPH solution (0.004% in methanol) in separate tubes. The tubes were incubated at room temperature in dark for 30 minutes followed by measuring the absorbance at 517nm. The absorbance of DPPH control (1ml methanol+3ml DPPH) was noted. Ascorbic acid was used as reference standard. The scavenging activity (%) of each concentration of leaf and flower extract was calculated using the formula:

Scavenging activity (%) = $(Ao–A_1/A_1)x100$, where Ao and A_1 refers to absorbance of DPPH control and DPPH and extract/standard combination respectively (Kekuda *et al*., 2013).

RESULTS AND DISCUSSION

Antibacterial Activity of Leaf and Flower Extract of *A. indica*

The pharmacological treatment of disease began long ago with the use of plants. Plants, a key component of traditional medicine, are an important source of valuable medicines. Plants have been used to treat various types of ailments since time immemorial. In the present study, we determined antibacterial efficacy of leaf and flower extract of *A. indica* against Gram positive and Gram negative bacterial by agar well diffusion method which is commonly used to screen antibacterial activity of various kinds of samples including plant extracts. The result of antibacterial activity of leaf and flower extract of *A. indica* is shown in Table 1 and Figure 1. Both extracts revealed inhibitory efficacy against test bacteria but to a varied extent. Overall, leaf extract was more inhibitory to test bacteria when compared to flower extract. Leaf extract caused high inhibition of *B. coagulans* followed by *B. subtilis* and others. Least inhibition caused by leaf extract

was recorded against *S. aureus*. In case of flower extract, *S. typhi* and *B. coagulans* were inhibited to higher extent when compared to other bacteria. Flower extract did not inhibit *S. aureus*. Among bacteria, *P. aeruginosa* was least inhibited by flower extract. Reference antibiotic displayed stronger inhibitory activity against test bacteria when compared to both extracts. DMSO did not inhibit growth of any test bacteria. It has been proven that *A. indica* exhibit antibacterial activity. The essential oil (Shafi *et al*., 2002), leaf and stem extract (Vaghasiya and Chanda, 2007; Kumar *et al*., 2011; Murugan and Mohan, 2012), root extract (Umamaheshwari and Muthy, 2012; Gopinath and Prakash, 2013) have shown antimicrobial activity. Butanolic extract of *A. indica* exhibited marked inhibitory effect against *L. monocytogene*, a cattle pathogen (Ravikumar *et al*., 2005).

Table 1: Antibacterial activity of leaf and flower extract of *A. indica*

Test bacteria	Zone of inhibition in cm			
	Leaf extract	Flower extract	Antibiotic	DMSO
E. coli	1.5	1.3	3.0	0.0
P. aeruginosa	1.1	1.0	2.1	0.0
S. typhi	1.5	1.5	2.9	0.0
B. subtilis	1.6	1.4	2.9	0.0
B. coagulans	1.8	1.5	3.0	0.0
S. aureus	1.0	0.0	2.5	0.0

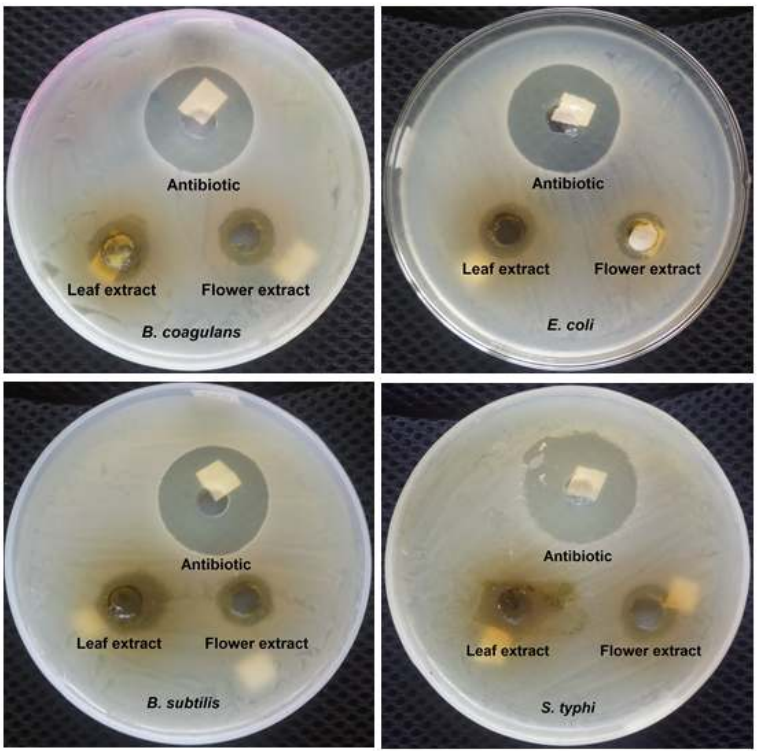

Figure 1: Inhibition of test bacteria by leaf and flower extracts

Antifungal Activity of Leaf and Flower Extract of *A. indica*

Various fungicides are employed in order to control fungal diseases of plants. The resistance to these fungicides has been noticed in most fungal pathogens. Moreover, the residues of these fungicides remain in the harvested produce and may result in toxic effect on consumption. Hence, search for alternative disease control strategies are of immense interest. Natural products are promising in terms of their low cost, potential efficacy as well as no or negligible side effects. Plants and their derivatives have been extensively studied for the control of phytopathogenic fungi. Several studies have been carried out on inhibitory potential of many botanical extracts against phytopathogenic fungi (Gomathi and Kannabiran, 2000; Rahman *et al*., 2011; Bajpai and Kang, 2012; Dileep *et al*., 2013). The result of antifungal potential of extracts of *A. indica* is shown in Table 2 and Figure 2 and 3. Poisoning of medium with the extracts resulted in reduction of mycelial growth of test fungi when compared to control plates (without extract). Both extracts inhibited test fungi to a varied extent. Among fungi, *F. oxysporum* displayed higher susceptibility to extracts followed by *Curvularia* sp., *B. sorokiniana* and *C. capsici*. Inhibition of *B. sorokiniana* by leaf and flower extracts was similar (8.57%). Flower extract inhibited *Curvularia* sp. and *F. oxysporum* to higher extent when compared to leaf extract. Inhibition of *C. capsici* by leaf extract was marked when compared to flower extract. It has been shown earlier that *A. indica* possess antifungal activity against molds. In an earlier study, Kumar *et al*. (2011) observed dose dependent inhibitory activity of leaf extract of *A. indica* against *Aspergillus niger*, *A. flavus* and *A. fumigatus*. Venkateswarlu *et al*. (2013), the leaf extract of *A. indica* exhibited dose dependent inhibition of mycelial growth of *Sclerotium oryzae*, causal agent of stem rot in paddy.

Table 2: Antifungal activity of leaf and flower extract of *A. indica*

Test fungi	Colony diameter in cm		
	Leaf extract	Flower extract	Control
B. sorokiniana	3.2	3.2	3.5
Curvularia sp.	3.7	3.6	4.3
C. capsici	3.6	3.7	3.8
F. oxysporum	3.8	2.8	4.4

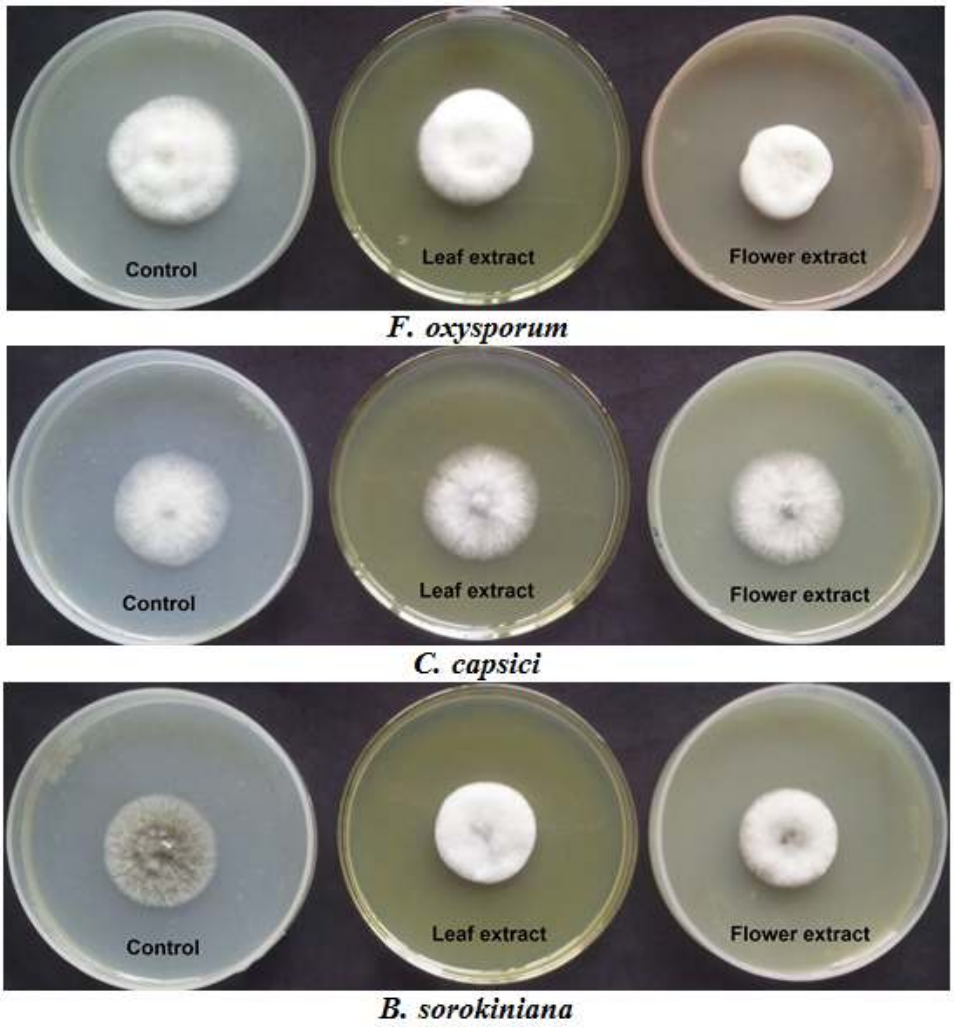

Figure 2: Growth of test fungi on control and poisoned plates

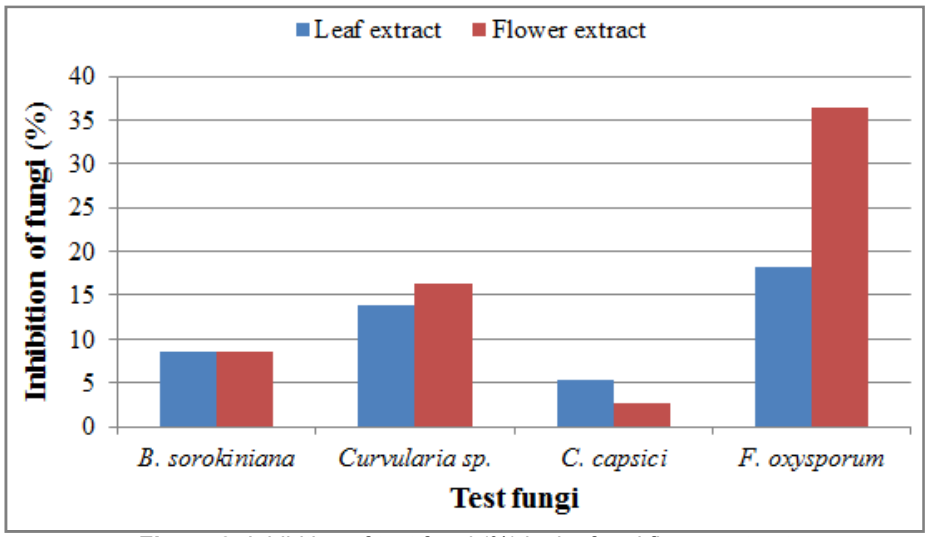

Figure 3: Inhibition of test fungi (%) by leaf and flower extract

Radical Scavenging Activity of Leaf and Flower Extract of *A. indica*

A number of *in vitro* assays are used to evaluate radical scavenging potential of plant extracts. Among these, DPPH assay is one of the widely used assays. DPPH is one of the stable, nitrogen centred, commercially available organic free radical having absorption maxima at 515-517 nm in alcoholic solution. On accepting hydrogen from a corresponding donor, the solution of DPPH loses the characteristic deep purple colour and becomes yellow coloured diphenylpicryl hydrazine (Huang *et al*., 2005; Conforti *et al*., 2008; Tirzitis and Bartosz, 2010; Kekuda *et al*., 2013). The result of scavenging potential of leaf and flower extract of *A. indica* is shown in Figure 4. The extracts exhibited dose dependent scavenging of DPPH radicals as evidenced by bleaching of color of DPPH solution (purple to yellow). Among extracts, marked scavenging nature was seen in leaf extract when compared to flower extract. At concentration 100µg/ml, radical scavenging activity of 48.68 and 10.52% was exhibited by leaf and flower extract respectively. Reference antioxidant i.e., ascorbic acid displayed high scavenging of radicals when compared to leaf and flower extract. Although the scavenging abilities of extracts were lesser than that of ascorbic acid, it was evident that the extracts showed hydrogen donating ability and could serve as free radical scavengers, acting possibly as primary antioxidants (Chung *et al*., 2006). Similar result was observed in an earlier study of Hossen *et al*. (2014) where extract from aerial parts of *A. indica* showed dose dependent scavenging of DPPH radicals and the scavenging potential of extract was lesser than that of ascorbic acid. In another study, Thirugnanasampandan *et al*. (2008) showed *in vitro* antioxidant efficacy of different solvent extracts of various *Aristolochia* species including *A. indica*.

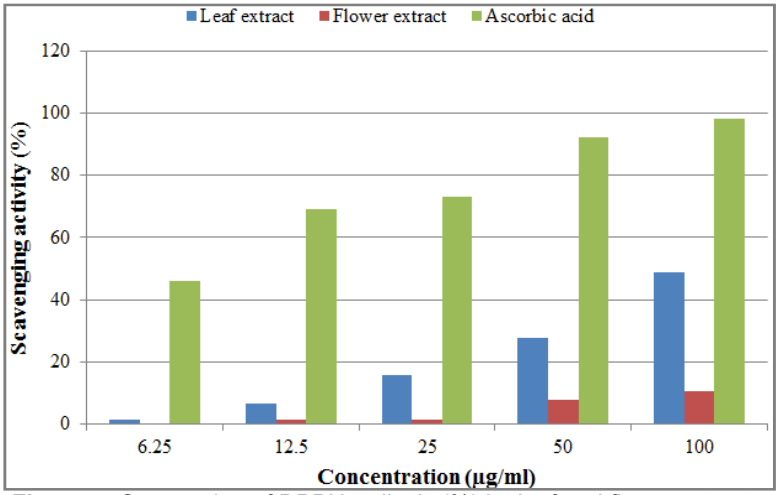

Figure 4: Scavenging of DPPH radicals (%) by leaf and flower extracts

CONCLUSIONS

In the present study, we observed antimicrobial and radical scavenging efficacy in leaf and flower extract of *A. indica*. The plant can be used to treat infections caused by pathogenic bacteria. The plant, in suitable formulation, can be used to control fungal agents which cause plant diseases. The plant can also be used to prevent and control oxidative stress which is induced due to free radicals. Further studies are to be conducted to isolate active principles from the plant and to investigate their bioactivities.

Conflict of Interest

All the authors declared no conflict of interest.

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