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Antibacterial, antifungal and antioxidant activity of *Dichapetalum gelonioides* (Roxb.) Engl. (Dichapetalaceae)

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ABSTRACT

Objectives: *Dichapetalum gelonioides* (Roxb.) Engl. belongs to the family Dichapetalaceae. In the present study, we investigated antibacterial, antifungal and antioxidant activity of methanolic extract of leaf and fruit of *D. gelonioides*.
Methods: Maceration process was carried out for extraction of leaf and fruit of *D. gelonioides*. Agar well diffusion method was employed to evaluate antibacterial activity of extracts against gram positive and gram negative bacteria. Poisoned food technique was performed to investigate antifungal activity of extracts against two seed-borne fungi. Antioxidant activity was evaluated by DPPH radical scavenging and ferric reducing assays.
Results: Both leaf and fruit extracts were effective in causing inhibition of all test bacteria. Highest and least inhibitory activity was observed against *Bacillus cereus* and *Escherichia coli* respectively. Both *Aspergillus niger* and *Bipolaris* sp. were inhibited to >50% by leaf and fruit extracts. Extent of inhibition of *Bipolaris* sp. was slightly higher when compared to *A. niger*. Both leaf and fruit extracts showed a dose dependent scavenging of DPPH radicals with high activity being showed by leaf extract. Leaf extract was shown to exhibit marked reducing potential than fruit extract.
Conclusions: Overall, leaf extract was shown to be more effective in displaying antioxidant activity and causing inhibition of bacteria and fungi when compared to fruit extract. The results indicate that the plant possess active principles which are to be purified, characterized and subjected for antimicrobial and antioxidant assays in further studies.

Keywords: *Dichapetalum gelonioides*, Maceration, Agar well diffusion, Poisoned food technique, DPPH, Ferric reducing

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INTRODUCTION

Plants are widely used as indispensable sources of food, medicine, dyes, flavoring agents and timber worldwide. Plants have been considered as an integral part of ethnomedicine and people from several parts of the world rely on plant based medicines (in the form of single or polyherbal formulations, decoctions, paste, infusions and others) for primary healthcare needs. Plants are key ingredients in some medicinal systems such as Ayurveda, Traditional Chinese Medicine, Siddha and Unani.[1-7] The genus *Dichapetalum* belonging to the family Dichapetalaceae includes shrubs or small trees. Species of *Dichapetalum* are rich source of triterpenoids. *Dichapetalum* species are used traditionally as medicine to treat certain illnesses.[8-10] Bioactive dichapetalins and several other compounds have been isolated from *Dichapetalum* species.[11-16]

Dichapetalum gelonioides (Roxb.) Engl (Figure 1) is a large shrub or small tree with pubescent branchlets and found distributed in evergreen and semi-evergreen forests as forest undergrowth. Leaves are elliptic or elliptic-lanceolate, 15x5cm, acuminate or caudate at

apex and glabrous. Flowering occurs more or less throughout year. Flowers are small, polygamo-monoecious, found in short-peduncled axillary clusters. Fruit is a 2-celled drupe, 1-2 seeded, densely tomentose, stone hard and enclosed in a scarlet mesocarp.[17] The plant is known to be a hyperaccumulator of nickel.[18] The fruits and leaves of *D. gelonioides* is used traditionally to treat amenorrhea, mouth ulcers, swelling, pit viper biting, polydipsia and wounds.[10] Isolated constituents, in particular dichapetalins, exhibit some biological activities.[11,14] The bark of the plant is reported to exhibit weaker muscarinic receptor activity.[19] The present study was conducted to evaluate antibacterial, antifungal and antioxidant activity of leaf and fruit of *D. gelonioides*.

Figure 1: *D. gelonioides* (Photograph by Prashith Kekuda)

MATERIALS AND METHODS

Collection and extraction of plant material

Plant was collected during December 2017 at Haniya, Hosanagara Taluk, Shivamogga district, Karnataka. The plant was identified by Dr. Vinayaka K.S, Principal, KFGC, Shikaripura, Karnataka. Leaves and fruits were separated, washed to remove adhering matter, dried under shade and powdered mechanically. The powdered leaf and fruit materials were extracted separately by maceration process using methanol as solvent.[6] The crude leaf and fruit extracts were screened for phytochemicals by standard tests.[20,21]

Antibacterial activity of leaf and fruit extracts

Agar well diffusion method was used to investigate antibacterial activity of leaf and fruit extracts of *D. gelonioides* (20mg/ml of DMSO) against 24 hours old broth cultures of test bacteria viz. *Bacillus cereus*, *Escherichia coli*, *Shigella flexneri* and *Salmonella typhimurium*. Streptomycin (1mg/ml of sterile distilled water) was used as standard antibiotic. Diameter of zones of inhibition formed around wells was measured.[6]

Antifungal activity of leaf and fruit extracts

Poisoned food technique was employed to evaluate antifungal potential of leaf and fruit extracts of *D. gelonioides* against two seed-borne fungi viz. *Aspergillus niger* and *Bipolaris* sp. Antifungal activity of leaf and fruit extracts, in terms of inhibition of mycelial growth of test fungi (%), was determined using the formula:

Inhibition of fungal growth (%) = [Dc – Dt / Dc] x 100, where 'Dc' and 'Dt' denotes the diameter of fungal colonies in control (without extract) and poisoned plates (1mg extract/ml of medium) respectively.[6]

DPPH free radical scavenging activity of leaf and fruit extracts

Various concentrations of ascorbic acid (reference antioxidant) leaf and fruit extracts of *D. gelonioides* (12.5-200µg/ml) were screened for antiradical potential by performing DPPH radical scavenging assay.[6] The absorbance was measured spectrophotometrically at 517nm. Radical scavenging activity (%) was determined using the formula:

Radical scavenging activity (%) = (Ac – At / Ac) x 100, where 'Ac' and 'At' represents absorbance of DPPH control and absorbance of DPPH in the presence of extract/ascorbic acid.

Ferric reducing activity of leaf and fruit extracts

Various concentrations of ascorbic acid (reference standard) and leaf and fruit extracts of *D. gelonioides* (12.5-200µg/ml) were screened for ferric reducing activity. The absorbance of reaction mixture in each tube was measured spectrophotometrically at 700nm. An increase in the absorbance with increase in concentration indicates ferric reducing potential.[6]

Statistical analysis

Antimicrobial studies were carried out in triplicates (n=3). Results are presented as Mean±Standard deviation (S.D).

RESULTS AND DISCUSSION

Plants produce a number of secondary metabolites by means of metabolic pathways such as mevalonic acid pathway, malonic acid pathway and shikimic acid pathway. These metabolites are distributed in various parts of the plant such as leaf, root, seed and flower. Secondary metabolites protect the plants from herbivores and pathogens and contribute to flavor and aroma of plants. Many of plant secondary metabolites exert definite physiological roles in human body and exhibit a range of biological activities including anticancer, antimicrobial and antioxidant activities.[22-26] Saponins, tannins, alkaloids, flavonoids, triterpenoids and glycosides were detected in leaf extract of *D. gelonioides*. Sterols were not detected in leaf extract. In case of fruit extract, phytochemicals viz. alkaloids, flavonoids, tannins and glycosides were detected.

Plants are considered as one of the promising alternates for modern drugs as the use of antibiotics is accompanied with some drawbacks such as high cost, emergence of resistant strains of pathogenic bacteria and possible side effects. Plants have been widely used worldwide to cure diseases caused by pathogenic bacteria. It is evident from several studies that crude extracts and isolated metabolites from plants exhibit antibacterial activity against several pathogenic bacteria including antibiotic resistant bacteria.[6,23,27-34] In this study, both leaf and fruit extracts of *D. gelonioides* were effective in causing inhibition of test bacteria as

evidenced by the zones of inhibition around wells. Among bacteria, marked susceptibility was shown by *B. cereus* while *E. coli* was inhibited to least extent. Leaf extract was found to inhibit test bacteria to greater extent than fruit extract. Susceptibility of gram negative bacteria to extracts was in the order: *S. flexneri* > *S. typhimurium* > *E. coli*. Inhibition of bacteria by reference antibiotic was high when compared to leaf and fruit extracts. DMSO was shown to display no inhibitory activity (Table 1).

Table 1: Inhibition of test bacteria by leaf and fruit extracts of *D. gelonioides*

Treatment	Zone of inhibition in cm (Mean ± S.D; n=3)			
	B. cereus	***E. coli***	***S. flexneri***	***S. typhimurium***
DMSO	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Antibiotic	3.23±0.05	2.93±0.05	3.10±0.00	3.13±0.05
Leaf extract	2.40±0.00	1.66±0.05	1.86±0.05	1.76±0.05
Fruit extract	2.13±0.05	1.40±0.00	1.60±0.00	1.50±0.00

Interest in botanicals with antifungal activity against phytopathogens is expanded due to several drawbacks of synthetic fungicides such as high cost, deleterious effects on soil flora and fauna, adverse effects on human health and onset of resistance in fungal pathogens. A number of studies have revealed the potential of botanical extracts to inhibit a range of phytopathogenic fungi including seed-borne fungi.[6,35-40] In the present study, both leaf and fruit extracts of *D. gelonioides* were effective in suppressing mycelial growth of both test fungi. A considerable reduction in the mycelial growth of test fungi was observed in plates poisoned with leaf and fruit extracts. Among extracts, marked antifungal activity was shown by leaf extract when compared to fruit extract. Both leaf and fruit extracts caused >50% inhibition of test fungi (Figure 2; Table 2). Inhibitory activity of extracts was slightly higher against *Bipolaris* sp. when compared to their inhibitory potential against *A. niger*. In an earlier study by Nagabhushan and Raveesha[10], the aqueous extract of *D. gelonioides* did not show antifungal activity against yeast, molds and dermatophytes.

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

Extract	Colony diameter of fungi in cm	
	A. niger	***Bipolaris* sp.**
Leaf extract	2.26±0.05	1.70±0.00
Fruit extract	2.50±0.00	2.03±0.05
Control	5.43±0.05	4.56±0.05

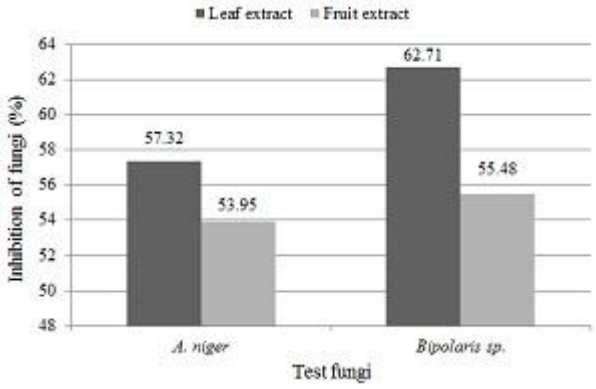

Figure 2: Extent of inhibition of test fungi by leaf and fruit extracts

Scavenging of stable, organic, nitrogen centred DPPH radical is one of the extensively used in vitro radical scavenging assays for evaluating antiradical nature of plant extracts.[6,41-45] In the present study, both leaf and fruit extracts of *D. gelonioides* showed a concentration dependent scavenging activity against DPPH free radicals. A scavenging activity of >50% was observed at extraction concentration of 25µg/ml and higher. At 200µg/ml concentration, only leaf extract displayed >90% scavenging of radicals. The scavenging activity observed was in the order: Ascorbic acid > leaf extract > fruit extract (Figure 3). Although the scavenging potential of ascorbic acid was higher, it is evident from the results obtained that both leaf and fruit extracts of *D. gelonioides* possess radical scavenging potential, hence, the extracts can act primary antioxidants.

The ferric reducing capability of extracts/compounds is considered as a significant indicator of antioxidant activity and the reducing ability is mainly due to the presence of reductones. Ferric reducing assay is widely used to evaluate antioxidant activity of plant extracts.[6,46-51] In the present study, we evaluated the reducing capacity of leaf and fruit extracts of *D. gelonioides* by the measurement of the absorbance due to the formation of Perl's Prussian blue complex on addition of excess of ferric ions. An increase in the absorbance of reaction mixture with an increase in the concentration of extracts was observed and indicated the reducing ability of leaf and fruit extracts (Figure 4). The reducing potential observed was in the order: Ascorbic acid > leaf extract > fruit extract.

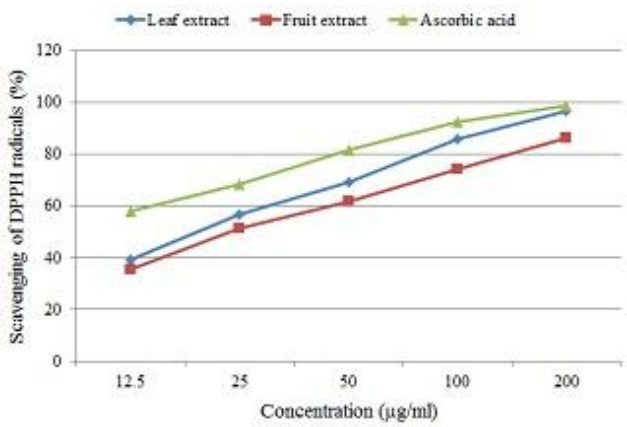

Figure 3: DPPH radical scavenging activity of leaf and fruit extracts

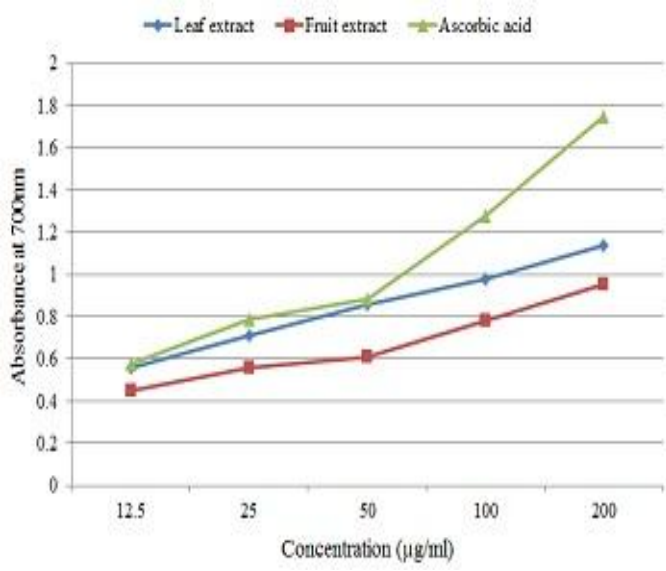

Figure 4: Ferric reducing activity of leaf and fruit extracts

CONCLUSIONS

Leaf extract was effective in displaying antibacterial, antifungal and antioxidant activity to higher extent than fruit extract. Based on the results obtained, it can be concluded that the plant *D. gelonioides* can be used in the treatment of infectious diseases, to manage seed-borne fungal pathogens and oxidative damage. The observed bioactivities could be attributed to the phytochemicals that have been detected in the plant. Further studies are to be undertaken to isolate and characterize bioactive principles from the plant.

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