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PHYTOCHEMICAL ANALYSIS AND ANTIFUNGAL POTENTIAL OF *DURANTA ERECTA* AGAINST SOME PHYTOPATOGENIC FUNGI

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ABSTRACT

The present study describes the antifungal activity of *Durenta erecta* L. against some phytopathogenic fungi: *Aspergillus niger*, *A. flavus*, *A. fumigatus* and *Penicillium sp*. For this purpose, methhanolic extract of leaf, stem and root were prepared and tested by "Disc Diffusion Method". The methanol extract was subjected to preliminary phytochemical analysis. Alkaloids, saponins, and polyphenols (tannins and flavonoids) were detected as phytoconstituents of the methanol extract. As a result of antifungal activity it was found that the extract of leaf generally revealed antifungal activity against all *Aspergillus spp.* but activity was highest against *A. fumigatus* (20±0.67 mm). Stem extract showed less activity against all test fungi but no activity against *A. flavus*. Root extract did not show any antifungal activity except *A. fumigatus* with less activity (9±0.98).

INTRODUCTION: Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs. Plant products still remain the principal source of pharmaceutical agents used in traditional medicine [1, 2]. Medicinal plants represent a rich source of antimicrobial agents [3]. Many of the plant materials used in traditional medicines are readily available in rural areas at relatively cheaper than modern medicines [4].

Duranta erecta Linn. (Syn. *Duranta plumieri* Jacq., *D. repens* Linn. and Eng: Goldendewdrop) is commonly known as pigeon berry and locally called 'Kata mehedi' belongs to the family Verbenaceae. It is shrubs, herbs or small tree usually 1 to 3 m. in height [5]. The plant is not browsed by cattle and is believed to be poisonous [6]. Ethyl acetate and aqueous extracts of leaves showed significant antimalarial activity when administered to mice [7]. The fruits are used in the treatment of malaria and intestinal worms [8]. The leaves are used in the treatment of abscess [9]. From the genus *Duranta* several iridoid glycosides as durantosides I, II, III, IV, and lamiide were isolated [10, 11]. Flavonoids and C-alkylated flavonoids [12, 13] and some alkaloids [14] were isolated.

The aim of the present study was phytochemical screening of plant and evaluation of the *in vitro* antifungal activity of the crude methanolic extracts (Leaf, stem and root), of *Duranta erecta* against some phytopathogenic fungi.

MATERIAL AND METHOD:

Plant Material: *Duranta erecta* leaf, stem and root were collected from local area of Jaipur city. The collected material was authenticated from Herbarium, Department of Botany, University of Rajasthan, Jaipur.

Extract Preparation: The collected materials were washed thoroughly in water, chopped, air dried for a week at 35-40°C and pulverized in electric grinder. For methanolic extraction, powdered dry plant material (50 gm) was extracted with 100 ml methanol for 24 hrs using Soxhlet apparatus. The extracts were filtered and concentrated under vacuum sounding apparatus for 30 min. and the extracts were stored at 4°C.

Phytochemical screening: Phytochemical screening was performed using standard procedures [15, 16].

1. **Test for terpenoids (Salkowski's test):** To 0.5 g each of the extract was added 2 ml of chloroform. Concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids.

2. **Test for flavonoids:** Three methods were used to test for flavonoids. First, dilute ammonia (5 ml) was added to a portion of an aqueous filtrate of the extract. Concentrated sulphuric acid (1 ml) was added. A yellow colouration that disappears on standing indicates the presence of flavonoids. Second, a few drops of 1% aluminium solution were added to a portion of the filtrate. A yellow colouration indicates the presence of flavonoids. Third, a portion of the extract was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration indicates the presence of flavonoids.

3. **Test for saponins:** To 0.5 g of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

4. **Test for tannins:** About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

5. **Test for alkaloids:** 0.5 g of extract was diluted to 10 ml with acid alcohol, boiled and filtered. To 5 ml of the filtrate was added 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Dragendroffs reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Dragendroffs reagent) was regarded as positive for the presence of alkaloids.

6. **Test for phenol:** To estimate total phenols in each of the test sample, the protocol of Bray and Thorpe [17]was followed, wherein a standard curve of caffeic acid (a phenol) was prepared A stock solution (100µg/ml) of caffeic acid was prepared in 80% ethanol, from which 0.1 to 0.9 ml was transferred into test-tubes separately and the volume in each case was raised to 1 ml with 80% ethanol. To each of these tubes, 1 ml of Folin-Ciocalteu reagent (prepared by diluting the reagent with distilled water in 1:2 ratio just before use) accompanied by 2 ml of 20% Na_2CO_3 solution was added and the mixture was shaken vigorously. Each of these were boiled on a water bath (1 min), cooled and diluted to 25 ml with distilled water. The OD was taken at 750 nm using a spectrophotometer against a blank.

7. **Test for steroids:** To 0.2 g of each portion, 2 ml of acetic acid was added; the solution was cooled well in ice followed by the addition of conc. H_2SO_4 carefully. Colour development from violet to blue or bluish-green indicated the presence of a steroidal ring i.e. aglycone portion of cardiac glycoside [16].

Antifungal activity:

1. **Test Organisms**: Four test organisms, *Aspergillus niger A. flavus*, *A. fumigatus* and *Penicillium sp.* were obtained from Plant Pathology Laboratory, University of Rajasthan, Jaipur, Rajasthan and maintained on Potato Dextrose Agar (PDA).

2. **Bioassay**: Disc diffusion bioassay was employed for testing antifungal activity of plant extracts [18]. The readymade PDA medium (Hi-media, 39g) was suspended in 1 lit. distilled water and autoclaved at pressure of 15lbs for 20min. Seven days old cultures of test organisms (0.5 ml) were seeded onto plate and uniformly spread with spreader.

Paper discs measuring 6mm diameter, that absorbs about 0.1ml of the test sample and a known quantity of standard reference antibiotic (Fluconazole) were used. The inoculated plates were kept at 5°C for 45-55min and then incubated at 25-27°C for 48hrs. The inhibition zone was measured and compared with those of the standard reference antibiotics. Three to four replicates were maintained for each treatment.

3. **Determination of activity index:** The activity index [19] of the crude plant extract was calculated as:

Activity index (A.I.) = Mean of zone of inhibition of the extract/Zone of inhibition obtained for standard antibiotic drug.

RESULTS AND DISCUSSION: The presence of alkaloids is interesting, as significant quantities are used as antimalarials, analgesics and stimulants [20]. The flavonoids are known to inhibit tumor growth and serve also to protect against gastrointestinal infections and are of Pharmacognostic importance thus giving evidence to the use of the plant in ethnomedicine.

Tannins are known to have antimicrobial and astringent activity and play a very important biochemical role in wound healing [21]. Such roles include binding to proteins of exposed tissues, thus precipitating the proteins, and forming antiseptic protective coat which enables the regeneration of new tissues to take place. Thus, the antibacterial activity of the extract will have a positive effect on wound healing. In the present work phytochemical screening of the crude extracts of leaf of *Duranta erecta* revealed the presence of excellent amount of alkaloids, flavonoids, terpenoids, saponins and phenolic compounds (**Table 1**).

TABLE 1: RESULT OF THE PHYTOCHEMICAL SCREENING OF CRUDE METHANOLIC EXTRACTS OF *DURANTA ERECTA*.

Phytochemical groups	Leaf	Stem	Root
Alkaloids	++++	+	+
Flavonoids	+++	++	++
Terpenoids	++++	+	+
Saponins	++++	-	-
Steroids	+	++	+
Tannins	+	+	+
Phenolics	++++	++	++

- Absent; + Present; ++ Low concentration; +++ Moderate concentration; ++++ High concentration.

The antimicrobial compounds may be found as alkaloids, flavonoids, phenolic compounds, saponins, steroids, tannins and triterpenoids, whose presence may be attributed to the medicinal properties of plants [22, 23, 24, 25].

In the present study, leaf extract was found to possess higher antifungal activity against all *Aspergillus spp.* but activity was highest against *A. fumigatus* (20±0.67 mm). Stem extract showed less activity against all test fungi except *A. flavus* (No activity). Root extract did not show any antifungal activity except *A. fumigatus* with less activity (9±0.98) (**figure 1, Table 2**).

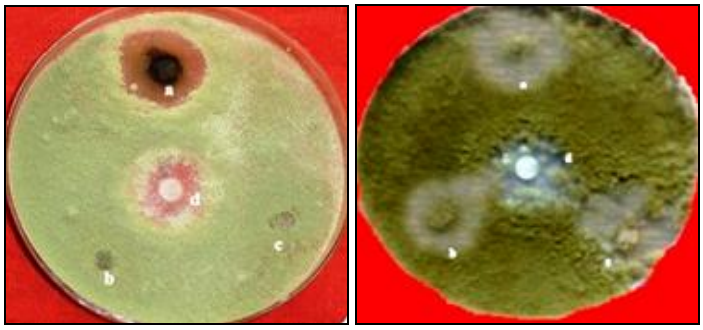

PLATE: A **PLATE: B**

a: Leaf; b: Stem; c: Root; d: Standard

FIG. 1: ANTIFUNGAL ACTIVITY OF *DURANTA ERECTA* AGAINST *A. FLAVUS* (PLATE: A) AND *A. FUMIGATUS* (PLATE: B)

TABLE 2: ANTIFUNGAL ACTIVITY OF *DURANTA ERECTA* CRUDE METHANOLIC EXTRACT ON DIFFERENT PHYTOPATHOGENIC FUNGI

Plant parts	*A. niger*		*A. flavus*		*A. fumigatus*		*Penicillum sp.*	
	DIZ (Mean ± SEM mm)	AI	DIZ (Mean ± SEM mm)	AI	DIZ (Mean ± SEM mm)	AI	DIZ (Mean ± SEM mm)	AI
Leaf	17±0.4	0.85	15±1.32	0.42	20±1.77	1.00	7±0.28	0.25
Stem	15±1.2	0.75	-	-	15±0.67	0.75	10±1.31	0.35
Root	-	-	-	-	9±0.98	0.45	-	-
Fluconazole (Standard)	20±0.44		17±1.64		20±0.54		28±1.54	

DIZ = diameter of inhibition zone; AI= activity index

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