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EFFICACY OF *DELONIX REGIA* RAFIN (SYN. *POINCIANA REGIA* BOJER EX. HOOK) FOR POTENTIAL ANTIFUNGAL ACTIVITY

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ABSTRACT
Dry powdered materials of flowers and seeds of *D. regia* were sequentially soxhlet extracted with various solvents to isolate metabolite-rich fractions such as flavonoids, anthraquinones and sterols and also evaluate for antifungal activity against *Aspargillus niger, A. flavus, Rhizopus bataticola and Fusarium auxisporum* by agar disk diffusion method. In both plant parts, flavonoids showed maximum antifungal activity against *A. flavus.*

INTRODUCTION

Now a days multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly use in the treatment of infectious diseases (Davis, 1994; Service, 1995). But sometimes antibiotics are associated with adverse effects on the host including hypersensitivity, immune suppression and allergic reactions (Ahmad *et al.,* 1998). This situation forced scientists to search for new antimicrobial substances. Given the alarming incidence of antibiotic resistance in bacteria of medical importance (Monroe and Polk, 2000), there is a constant need for new and effective therapeutic agents (Bhavanani and Ballow, 2000). Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants (Clark, 1996; Cordell, 2000). There are several reports on the antimicrobial activity of different herbal extracts in different regions of the world (Chung *et al.,* 2004; Nair and Chanda, 2004; De Boer *et al.,* 2005; Nair *et al.,* 2005).

Plant based antimicrobials represent a vast untapped source of medicines and further exploration of plant antimicrobials need to occur. Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side-effects that are often associated with synthetic antimicrobials (Iwu *et al*., 1999).

In *D. regia*, these compounds are mostly secondary metabolites such as flavonoids (Subramanian *et al.,* 1966) sterols (Guerere *et al.,* 1986) tannins and phenol compounds (El Sherbeing *et al.*, 1971). Some carotenoids like ²-carotene, zeaxanthein etc. (Jungalwala and Cama, 1962; Sun Gene and Co., 2004; Krishna and Grampurohit, 2005) also exhibits antimicrobial activities. In the present work *Delonix regia* Rafin (syn. *Poinciana regia* Bojer ex. Hook) was evaluated for its antifungal properties against some pathogenic fungi.

MATERIALS AND METHODS

Plant collection

The plant was collected from campus of University of Rajasthan, Jaipur, India, and authenticated from the herbarium, Department of Botany, University of Rajasthan, Jaipur, India. Fresh plant material was washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles.

Source of test organisms

The pure cultures of test fungi, namely *Aspargillus niger, A. flavus, Rhizopus bataticola and Fusarium auxisporum* were obtained from the Seed Pathology Laboratory, Department of Botany, University of Rajasthan, Jaipur, India, which were maintained on Potato Dextrose Agar medium.

Culture of test microbes

For the cultivation of fungi, Potato Dextrose Agar Medium (PDA) was prepared by mixing 1000 mL vpotato infusion + 20g agar + 20g glucose, followed by autoclaving and then test fungi were incubated at 27ºC ± 1ºC for 48h and the cultures were maintained on the same medium by regular subculturings.
To prepare the test plates, 10-15 mL of the respective medium was poured into the Petri dishes and used for screening. For assessing the fungicidal efficacy, a uniform spread of the test

Table 1: Fungicidial efficacy of extracts of *Delonix regia*

Test organism		Extracts of dried flower								Extracts of dried seed material							
		PE	C_6H_6	CH_3COH_3	$CHCl_3$	EtOH	AQ	FLAV.	STER.	ANTHR.	PE	C_6H_6	CH_3CH_3	$CHCl_3$	EtOH	AQ	FLA.	STE.	ANT.
A. flavus	IZ±	18.00	16.00	23.00	21.00	±	-	25.00	24.00	24.00	16.00	14.00	11.00	14.00	±	-	22.00	13.00	16.00
	AI*	0.90	0.80	1.15		1.05		1.25	1.20	1.20	0.80	0.70	0.55	0.70			1.10	0.65	0.80
A. nigar	IZ	16.00	18.00	22.00	10.00	18.00	-	22.00	20.00	18.00	14.00	15.00	±	16.00	-	-	16.00	21.00	19.00
	AI	0.76	0.85	1.04	0.47	0.85		1.04	0.95	0.85	0.66	0.71		0.76			0.76	1.00	0.90
R.bataticola	IZ	18.00	17.00	18.00	-	-	-	23.00	24.00	19.00	15.00	16.00	18.00	±	14.00	13.00	21.00	21.00	19.00
	AI	0.72	0.68	0.72				0.92	0.96	0.76	0.60	0.64	0.72		0.56	0.52	0.84	0.84	0.76
F.moniliforme	IZ	9.00	9.00	11.00	±	±	-	14.00	13.00	13.00	±	13.00	7.00	8.00	8.00	-	19.00	17.00	14.00
	AI	1.12	1.12	1.37				1.75	1.62	1.62		1.62	0.87	1.00	1.00		2.37	2.12	1.75

(±) Trace activity, IZ± = Inhibition zone (in mm) including the diameter of Disc (6mm); (-) Not measurable activi

Activity Index (AI) = $\frac{\text{Inhibition area of the test sample}}{\text{Inhibition area of the standard}}$

Abbreviations: PE = Petroleum ether; C_6H_6 = Benzene, CH_3COCH_3 = Acetone, $CHCl_3$ = Chloroform; EtOH = Ethanol; AQ = Aqueous; ANT. = Anthraquinones; FLA. = Flavonoids; STE = Sterols; Standard: Clotrimazole = 100 units/ disc

Plate 1: A. *Aspergillus niger;* B. *Aspergillus flavus;* C. *Rhizophus bataticola;* D. *Fusarium moniliforme*

a. Petroleum ether; b. Benzene, c. Acetone; d. Chloroform; e. Alcohol and f. Water.

Plate 2: E. *Aspergillus niger;* F. *Aspergillus flavus;* G. *Rhizophus bataticola;* H. *Fusarium moniliforme*

a. Petroleum ether; b. Benzene, c. Acetone; d. Chloroform; e. Alcohol and f. Water.

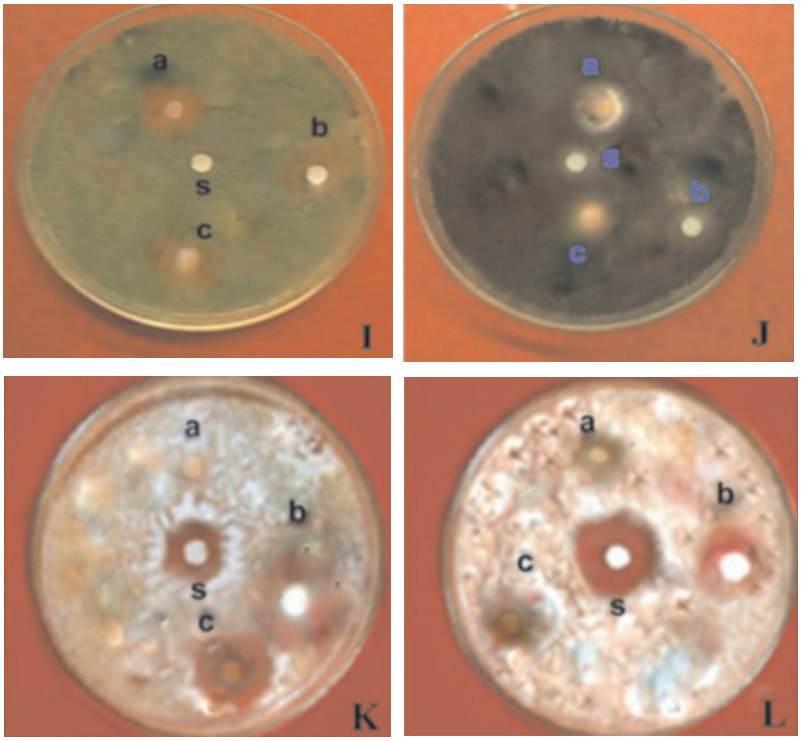

Plate 3: I. *Aspergillus niger;* J. *Aspergillus flavus;* K. *Rhizophus bataticola;* L. *Fusarium moniliforme*

a. Petroleum ether; b. Benzene, c. Acetone; d. Chloroform; e. Alcohol and f. Water.

fungi was made using the sterile swab.

Preparation of test extract

Dry and powdered materials of flowers and seeds (50 g each) of *D. regia* were sequentially soxhlet extracted with petroleum ether (600-800C), benzene, acetone, chloroform, alcohol and water successively, similarly, various metabolites-rich fractions, such as flavonoids, anthraquinones and sterols were also extracted separately by using established protocols.

All these extracts/fractions were stored at 90C in refrigerator until screened, when their final volume was raised to a known concentration (1g/mL) in their respective solvents before use.

Antifungal assay

The antimicrobial activity of different plant species was evaluated by agar disk diffusion method (Gould and Bowie, 1952; Bauer *et al.*, 1966; Salie *et al.*, 1996) The different test organisms were preceded separately using a sterile swab over previously sterilized culture medium plates and the zones of inhibition were measured around sterilized dried discs of Whatman No. 1 paper (6 mm in diameter), which were containing 4 mg per disc(or 0.4 mL) of the test extracts of control (0.4 g/mL of the respective solvent) or Clotrimazole (100 units/mL) as reference drugs separately. Such treated discs were air dried at room temperature to remove any residual solvent which might interfere with the determination. These plates were initially placed at low temperature for 1 h, so as to allow the maximum diffusion of the compounds from the test discs into the agar plates and later, incubated at 370C for 48 h, after which the zones of inhibition could be easily observed. Five replicates of each test extract were examined and the mean values were then referred.

RESULTS AND DISCUSSION

The result of antifungal activity of *D. regia,* are cited in the Table 1. The extracts of sequential extraction with various solvents namely - pt.ether, benzene, acetone, chloroform, ethanol, aqueous and flavonoids, anthraquinones and sterols were used against certain pathogenic fungi namely *F. moniliforme, Rhizophus bataticola, Aspergillus niger* and *A. flavus*. Although all the bioactive compounds - the secondary metabolites flavonoids, anthraquinones and sterols exhibits antifungal activity, but flavonoids extracted from flowers(IZ : 23mm; AI : 1.15) and seeds (IZ :22mm; AI : 1.10) exhibit maximum antifungal activity against *A. niger* .

In other metabolites rich fractions, acetone extracts of both plant parts were found to be more active against *A. niger* (flowers: IZ: 23 mm, AI: 1.15; seeds: IZ: 18mm, AI: 0.72) as compare to other extracts. Earlier, *D. regia* has been reported by a number of workers, with an aim to identify the active principle (s) and their bioefficacy (Thiribhuvanamala and Narasimhan, 1998; Aqil and Ahmad, 2003; Satish *et al*., 2007, 2009; Dutta *et al*., 2008).

It may, therefore be concluded that almost all the metabolite rich fractions, flavonoid fractions, anthraquinones fractions and sterol fractions exhibit antifungal activity except aqueous fraction that is rich in sugars. The above findings would prove useful in undertaking further the bioactivity oriented separation of compounds in *D. regia* and can be used as source of indigenous medicine.

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