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In Vitro Studies on Phytochemical Screening of Different Leaf Extracts and Their Antifungal Activity against Seed Borne Pathogen *Aspergillus niger*

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Abstract

In the present study, screening of aqueous extracts obtained from five medicinally active plants (*Melia azedarach, Cassia siamea, Murraya koenigii, Jatropha curcas* and *Delonix regia)* was performed against *Aspergillus niger*, a causative agent of different destructive diseases of plants. The extracts were also used for the phytochemical screening following *Horborne* method. The results obtained from this study demonstrated occurrence of alkaloids, tannins, saponins, flavonoids, phenolics, amino acids and terpenes, in the aqueous leaf extract. To obtain an optimum concentration of the extracts, for inhibition of *Aspergillus niger,* 10%, 15% and 20% concentrations of all the five plants were prepared. The three plants viz., *M. azedarach*, *C. siamea* and *M. koenigii* showed antifungal activity against *A. niger* in all the concentrations. Inhibition of mycelial growth was lowest at the concentration of 20% of *D. regia* followed by *J. curas*. The present protocol showed that the growth of *A. niger* was inhibited to greater extent at 20% than at lower concentrations of the extracts. It could be suggested that plant extracts could possibly be exploited for the management of seed-borne pathogenic fungi to prevent biodeterioration of seeds in an eco-friendly way.

Keywords: Phytochemical; Plant extract; Antifungal activity; *Aspergillus niger*; Seed borne pathogen

Introduction

The medicinal plants are rich sources of a wide variety of chemical compounds and have been used as a constant source of medicaments for a variety of diseases. The use of herbs to treat disease is almost universal among non-industrialized societies, and is often more affordable than purchasing expensive modern pharmaceutical products. In the recent years, efforts have been made to develop antimicrobial compounds to put a check on the damages caused by the microorganisms [1].

Some medicinal plants are the rich sources of antimicrobial agents [2], these have been found to possess fungicidal properties against various phytopathogenic fungi [3-5]. Secondary metabolites produced by the plants constitute an important source of microbicides, pesticides and pharmaceutical drugs [6]. A large number of plant derived compounds have been exploited against pathogenic microorganisms [7].

Plant pathogens cause great damages to agricultural products leading to reduction in food resources [8]. According to Food and Agriculture Organization (FAO) about 25% food crops of the world are affected by mycotoxins during growth period and storage phase. *Aspergillus* and *Penicillium* are the major reported genera having ability to produce mycotoxins [9,10]. The plant extracts to be used as antifungal agents have a bright future in modern plant protection and alternative of synthetic fungicides which are hazardous to the environment as well as human health.

Melia azedarach Linn. (Meliaceae), an indigenous plant, possesses several medicinally important compounds. From preliminary phytochemical screening of *M. azedarach*, it was found that the extracts possessed a number of organic compounds i.e., terpenoids, flavonoids, steroids, acids, anthraquinones, alkaloids, saponins and tannins [11,12]. The leaf extract of *M. azedarach* was reported to have antiviral and antifertility characteristics [13,14]. Fungicidal properties of *M. azedarach* were attributed to hydroxycoumarin scopoletin, vanillin, 4-hydroxy-3- methoxycinnamaldehyde and (±) pinoresinol [15,16]. Amyrin, Ursolic acid, Benzoic acid, 3, 5-Dimethoxybenzoic acid isolated from *M. azedarach* leaves had antifungal properties [17] (Table 1).

Cassia siamea (Caesalpinaceae) is an important medicinal plant, its leaf extracts exhibited antimicrobial activity [18]. *Morraya koenigii* (Rutaceae) is commonly known as curry leaf tree. It is used as condiment to enhance the flavor of certain dishes. It is reported to promote appetite and enhance digestion process [19]. Antibacterial and antimicrobiological characteristics reported by Manfred et al. [20] and Shrinivasan [21]. The plant possesses antioxidative and cytotoxic properties [22]. The chemical compounds 1- formyl-3 methoxy-6-methyl carbazole and 6,7-dimethoxy-1- hydroxy-3- methyl carbazole present in aqueous leaf extract of *M. koenigii* are fungicidal in nature [23] (Table 2).

Jatropha curcas (Euphorbiaceae) is used because of its medicinal properties in many subtropical and semi-arid regions. It has been reported to be a source of bio-fuel as its seed kernels contain high amount (58-60%) of oil [24]. The components in the extracts of *Jatropha* species especially *J. curcas* displayed potent cytotoxic, anti-tumor and anti-microbial activities in different assays [25].

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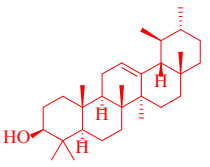
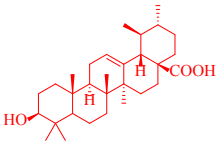
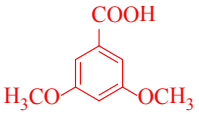
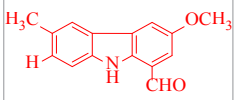
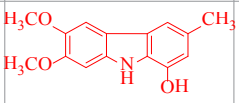
S.N.	Plant	Compounds	Structure	Reference
1	*Melia Azedarach*	Amyrin		[17]
2	*Melia Azedarach*	Ursolic acid		//
3	*Melia Azedarach*	Benzoic acid		//
4	*Melia Azedarach*	3,5Dimethoxybenzoic acid		//
5	*Murraya koinegii*	1- formyl-3 methoxy-6- methyl carbazole		[23]
6	*Murraya koinegii*	6,7-dimethoxy-1-hydroxy-3- methyl carbazole		//

Table 1: Compounds isolated from plants showing anti-fungal activity.

Plant Name	Alkaloid	Tannin	Saponin	Phenolics	Flavonoid	Aminoacid	Terpene
Melia azedarach	+	+	+	+	+	+	+
Cassia siamea	+	+	+	+	-	-	+
Murraya koenigii	+	+	+	+	-	+	+
Jetropha curcas	-	-	+	+	+	+	-
Delonix regia	-	+	+	-	+	-	-

(+)=Peresence, (-)=Absence

Table 2: Phytochemical screening of aqueous leaf extracts of *Melia azedarach, Cassia siamea, Murraya koenigii, Jatropha curcas* and *Delonix regia.*

Delonix regia (Caesalpinaceae) is grown as ornamental tree in many tropical areas. The medicinal properties of extracts of *D. regia* were reported by several authors [26-28].

Traditionally, the fungal diseases of plants are controlled by using different synthetic fungicides. The use of fungicides is not only expensive, but also hazardous to the environment and human health. On the other hand, the indiscriminate use of pesticides may result into development of resistance in the pathogens. To overcome these problems, some alternative approaches must be employed. Several studies have revealed the plant extracts as source of natural pesticides that make excellent efforts for new pesticide development [29,30].

The antifungal action of plant extracts has gained much attention and therefore, the plants are being used against many plant pathogenic fungi. The plants serve as eco-friendly and economical bio-control agents. To keep in mind the medicinal values of plants and negative role of fungicides, this experiment was carried out to investigate the antifungal activity of five medicinal plants on *A. niger* under *in vitro* conditions.

Material and Methods

Collection of leaves

The leaves of *M. azedarach*, *C. siamea*, *M. koenigii J. curcas* and *D. regia* were collected from the campus of Aligarh Muslim University, Aligarh and immediately brought to the laboratory to study their antifungal effect on the growth of *A. niger*.

Collection and maintenance of pathogen

The tested pathogen *A. niger* was obtained from Indian Agriculture Research Institute (IARI) New Delhi and maintained on Potato Dextrose Agar medium (PDA) in BOD incubator at 25 ±2°C. The well grown fungal colony was sub cultured for further studies.

Extraction of plant materials

For the preparation of aqueous extracts, 25 g fresh leaves of each plant were weighed and taken separately, washed 2-3 times with running tap water followed by distilled water. These were surface sterilized with 90% alcohol. Subsequently the leaves were ground in 100 ml distilled water for the preparation of aqueous extracts. The macerates were strained through double layered muslin cloth and filtered through filter paper. The aliquots so obtained were centrifuged at 5,000 rpm for 20 minutes. The supernatants were filtered through Whatmann No. 1 filter paper and sterilized by passing through 0.2 micron disposable filters. This formed the standard aqueous extract solution (100%). From these extracts 10%, 15%, 20% concentrations were prepared by adding required volume of distilled water.

Preparation of IR sample

For IR analysis a small amount of powdered sample was mixed with KBr (about 0.1 to 2%) powder and ground for 3-5 minutes. The mixture was die-set and pressed for 5 minutes to form thin and transparent KBr pellet. IR spectrum was recorded with a Shimadzu IR-408 Perkin-Elmer1800 instrument (FTIR), and the values were recorded in cm^{-1}.

Phytochemical screening

Qualitative chemical tests using aqueous extracts for alkaloids, flavonoids, saponins, tannins, phenolic, amino acids and terpenoids, were performed according to the procedure described by Harborne [31]. Mayers test, Wagner's test for alkaloids, lead acetate test for tannins, froth test for saponins, Shinodas test for flavonoids, Ninhydrin test for amino acids and Salkowski test for terpenoids were performed.

Fungi-toxic assay

Fungitoxicity of plant extracts was determined by food-poison technique. Standard extracts of each different concentration were mixed with 45 ml of sterilized potato dextrose agar (PDA) medium and transferred equally into Petri plates. The media were allowed to solidify. Seven day old, well grown fungal culture tube was taken and inoculated into the center of Petri plates containing plant extracts in aseptic condition. Only PDA medium, without plant extracts served as control. All plates were incubated at 28 ± 2°C and radial growth of colony was observed after 5 days of incubation. Each test was performed in triplicate. The antifungal effect of different concentration of extracts over control was evaluated by measuring zones of inhibition of fungal growth surrounding the plant extracts.

S. No.	Botanical Name of Plant	Concentration (%)	Antifungal activitay aginst *A.niger*
1	*Melia Azedarach*	10%	+
		15%	++
		20%	++
2	*Cassia siamea*	10%	+
		15%	+
		20%	++
3	*Murraya koenigii*	10%	*
		15%	+
		20%	++
4	*Jetropha curcas*	10%	-
		15%	*
		20%	+
5	*Delonix regia*	10%	-
		15%	-
		20%	*

(++) =highly effective, (+) =moderate effective, (*) =least effective, (-) =no effect,

Table 3: Antifungal activity of aqueous leaf extracts *Melia azedarach, Cassia siamea, Murraya koenigii, Jetropha curcas* and *Delonix regia* against *Aspergillus niger.*

Results and Discussion

In the present study, fungitoxic effect of aqueous leaf extracts of the five plants *Melia azedarach*, *Cassia siamia*, *Murraya koenigii, Jatropha curcas* and *Delonix regia* were screened against *Aspergillus niger* and the results obtained are shown in Table 3 and Figure 1.

For the phytochemical screening of different aqueous plant extracts used in the present study, the results are given in Table 2**.** The FT-IR spectrum of aqueous leaf extracts (Figure 2) showed strong absorption bands at 3435, 3436, 3384 and 3438 cm^{-1} attributed to phenolic hydroxyl (OH) in *M. azedarach, C. siamea, M. koenigii* and *J. curcas*, respectively. Whereas, there was no peak for phenolic group in *D. regia*. Similarly, sharp peaks at 1635, 1639 and 1641 cm^{-1} were assigned to CO-(NH) (bending vibration) in proteins and amino acids in *M. azedarach, M. koenigii* and *J. curcas*, respectively. Furthermore there were no peaks for CO-(NH) (bending vibration) in *C. siamia* and *D. regia* that confirmed absence of amino acids in the extract. Bands at 680, 665, 687, 711 and 668 cm^{-1} were attributed to =C-H (bending vibration) in *M. azedarach*, *C. siamia*, *M. koenigii*, *J. curcas* and *D. regia*, respectively. The above FTIR data showed the presence of phenolics, proteins, amino acids residues aromatics and aliphatic hydrocarbons in the aqueous leaf extracts.

Aqueous leaf extract of *M. azedarach*, *C. siamea* and *M. koenigii* were found to be more effective in reducing growth of the pathogen than *J. curcas* and *D. regia*. *M. azedarach* and *C. siamia* aqueous leaf extracts that were more potent for all the concentrations used in the study ie. 10%, 15% and 25% (Table 2, entries 1 and 2), while 10% aqueous leaf extract of *M. koenigii* was least effective than *M. azedarach and C. siamea.* Strong antifungal activity of crude aqueous and organic solvent extracts of leaves of *M. azedarach* against *Aschochyta robiei* was reported by Jabeen and Javaid [32]. Antifungl activity of aqueous and organic solvent extract of *M. koenigii* leaves were tested against different phytopathogenic fungi [33]. In case of *J. curcas,* 10% extract had no effect on the growth of pathogen but as the concentration of extract increased by 5% there was a slight positive result. Further increase in the concentration by 5% (Table 2 entry 4) a moderate effect was obtained. Crude extract of *Jatropha* scpecies proved to be inhibitory against plant pathogenic fungi by Gaikwad et al. [34]. In *D. regia*, 10% and 15% aqueous extract had no effects on the growth inhibition of *A. niger* while 20% has least effect. Antifungal activity of the extracts was enhanced by increase in the concentration of the extracts [35]. Thus the inhibition activity of the extracts was concentration dependent. This minimum inhibition is due to absence of phenolic compounds and alkaloids in the extract. The potent inhibition of aqueous extract of these plants is due to presence of phenolics, alkaloids, flavonoids and tannins**.** Phenolic compounds serve as potent antifungal agents as compared to other compounds because these compounds can move

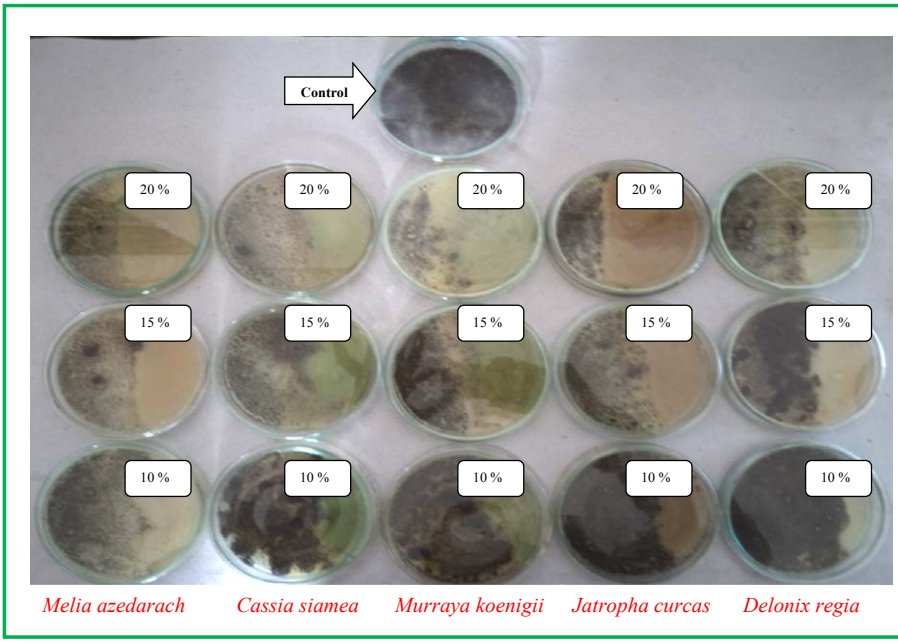


Figure 1: Antifungal activity of aqueous leaf extracts of *Melia azedarach*, *Cassia siamea*, *Murraya koenigii*, *Jatropha curcas* and *Delonix regia* against *Aspergillus niger* at 10%, 15% and 20% concentration.

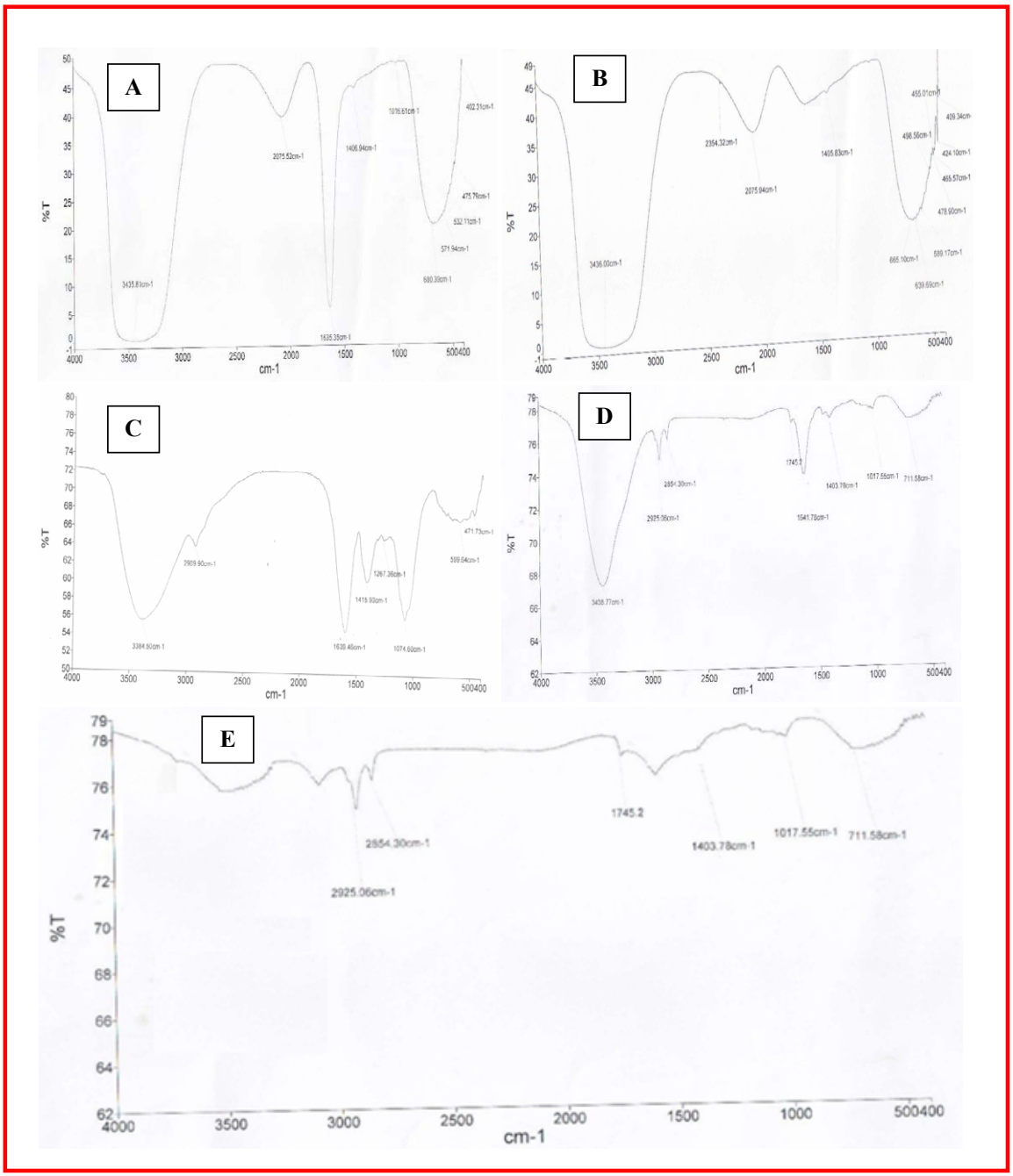


Figure 2: FT-IR spectrum of aqueous leaf extracts of **(A)** *Melia Azedarach* **(B)** *Cassia siamea* **(C)** *Murraya koenigii* **(D)** *Jetropha curcas* and **(E)** *Delonix regia*.

across the microbial membrane penetrating into the cell cytoplasm and affect the synthesis of ergosterol, glucan, chitin, proteins and glucosamine and interfere in the metabolic pathways [36].

This variation in antifungal activity of plant materials might be due to the difference in their chemical compositions, and secondly their solubility in water. This finding was found to be in agreement with the reports of Qasem and Abu-Blan [37] and Amadioha [38]. Therefore, this result suggests that aqueous extracts of *C. siamia, M. azedarach* and *M. koenigii* would be very beneficial for controlling fungal diseases in plants caused by *A. niger*.

Conclusion

This finding is important from the view of controlling pathogenic fungus *A. niger* without the use of synthetic fungicide in view of the environmental pollution likely to be caused. Phytochemical studies indicated that alkaloids and phenolics were prominent in the aqueous leaf extract of *M. azedarach*, *C. siamia*, and *M. koenigii* along with other compounds which might have an important role in antifungal activity. The efficacy of the leaf extracts could be enhanced by increasing the concentration of the extract. However, studies are to be continued to find out the active principal involved in them.

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