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Antifungal activity of a known medicinal plant *Mimusops elengi* L. against grain moulds

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The aqueous and different solvent extracts *viz*., petroleum ether, benzene, chloroform, methanol and ethanol extracts and isolated constituents of *Mimusops elengi* L. (Sapotaceae) was screened *in vitro* for antifungal activity, by poisoned food technique against wide array of seed borne phytopathogenic fungi. The test organisms included *Alternaria alternata*, two species of *Drechslera*, eight species of *Fusarium*, ten species of *Aspergillus* and three species of *Penicillium*, which are frequently associated with sorghum [*Sorghum bicolor* (L.) Moench], maize (*Zea mays* L.) and paddy (*Oryza sativa* L.) seeds. Aqueous, methanol and ethanol extract recorded highly significant antifungal activity against all the tested fungi. Methanol extract was subsequently fractioned and monitored by antifungal activity guided assay leading to the isolation of an active fraction and confirmed as alkaloids by further phytochemical analysis. The results indicated that the antifungal activity of alkaloid fraction is highly significant compared to Dithane M-45 and other fungicides. *Mimusops elengi* L. has significant medicinal value, hence the results of the present investigation indicate that, it could be exploited in the management of seed borne pathogenic fungi and prevented biodeterioration of grains and mytoxin elaboration during storage.

Key words: *Mimusops elengi*, antifungal activity, poisoned food technique, sorghum

Introduction

Plants have been formed the basis of natural pesticides, that make excellent leads for new pesticide development (Newman *et al.,* 2000). The potential of higher plants as a source of new drugs is still largely unexplored. Hence, last decade witnessed an increase in the investigation on plants as a source of new biomolecules for human disease management (Grierson and Afolayan, 1999). Traditionally plants have been well exploited by man for the treatment of human diseases, Ayurveda is a good example, but not much

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information is available on the exploitation of plant wealth for the management of plant diseases, especially against phytopathogenic fungi.

Fungi cause severe damage to stored food commodities. Among different species of fungi *Aspergillus* sp., *Fusarium* sp. and *Penicillium* sp. are associated with heavy loss of grains, fruits, vegetables and other plant products during picking, transit and storage rendering them unfit for human consumption by producing mycotoxins and affecting their nutritive value (Miller, 1995; Janardhana *et al*., 1999; Galvano *et al*., 2001). Many seed borne fungi, which cause severe damage to stored food commodities, were generally managed by synthetic chemicals, which were considered both efficient and effective. The continuous use of these synthetic fungicides started unraveling nonbiodegradability and known to have residual toxicity to cause pollution (Pimentel and Levitan, 1986). Pesticide pollution of soil and water bodies is well documented (Nostro *et al.,* 2000). Hence in recent time application of plant metabolites for plant disease management has become important viable component of Integrated Pest Management, as plant metabolites are eco-friendly.

Mimusops elengi is a wild plant distributed in tropical and subtropical region belonging to the family sapotaceae. Earlier report reveals that the fruits are used in chronic dysentery, constipations; flowers are used as snuff to relive headache, lotion for wounds and ulcers. Barks are used to increase fertility in women and known to have antiulcer activity (Shah *et al.,* 2003). They are rich source of tannin, saponin, alkaloids, glucoside, ursolic acid (Anonymous, 1969). A pentacylcic triterpene 3β, 6β, 19α, 23-tetrahydroxy-urs-12-ene reported from bark recorded moderate inhibiting activity against β–glucuronidase enzyme associated with gastric ulcers (Jahan *et al*., 2001)**.** Seeds of *Mimusops* is known to contain several saponins such as mimusin Mi-saponin A and 16α-hydroxy Mi-saponin A (Sahu *et al.,* 1997), taxifolin, α-spinasterol glucoside, Mi-glycoside 1, mimusopside A and B (Sahu, 1996). Seed kernel also reported to have saponins (Lavaud *et al*., 1996). A scientific and systematic phytochemical investigation of leaves with regard to the various biological activities in general and antifungal activity against phytopathogenic fungi in particular of this plant is lacking, hence the present study. Considering these, higher plants are routinely screened in our laboratory; during this routine screening *Mimusops elengi* recorded highly significant antifungal activity. Thus detailed investigations were conducted to test the efficacy of *M. elengi* against important wide array of seed borne phytopathogenic fungi.

Materials and Methods

Collection of plant materials

Fresh leaves of Mimusops elengi free from diseases were collected from Mysore (India), washed thoroughly 2-3 times with running tap water and once with sterile water, shade-dried, powdered and used for extraction. A voucher specimen of the plant is deposited in the herbarium of Department of Studies in Botany, University of Mysore, Mysore. Karnataka, India.

Preparation of extracts

Aqueous extract

Samples (50 g) of shade dried, powder of leaves of *M. elengi* was macerated with 100 ml of sterile distilled water in a Waring blender (Waring International, new Hartford, CT, USA) for 10 min. The macerate was first filtered through double layer muslin cloth and then centrifuged at 4000 *g* for 30 min. The supernatant was filtered through Whatman No. 1 filter paper and heat sterilized at 120 ºC for 30 min (Satish *et al.,* 1999). The extract was preserved aseptically in a brown bottle at 5 ºC until further use.

Solvent extracts

Fifty gram of shade dried, powder of *M. elengi* was filled in the thimble and extracted successively with petroleum ether, benzene, chloroform, methanol and ethanol using a Soxhlet extractor for 48 h. (Mohana and Raveesha, 2006). All the extracts were concentrated using rotary flash evaporator and preserved at 5 ºC in airtight bottles until further use. All the extracts were subjected to antifungal activity assay.

Phytochemical analysis

Phytochemical analysis of the evaporated methanol extract was conducted following the procedure of Anonymous (1985) and Harborne (1998). Methanol extract was fractioned in to different fractions as acidic (fraction 1), phenolic (fraction 2), alkaloid (fraction 3) and neutral (fraction 4) following the procedure of Roberts *et al.* (1981). All the fractions were again subjected to antifungal activity.

Isolation of important phytopathogenic fungi

Seed samples of sorghum, paddy rice and maize were collected directly from farmer fields, regulated markets, warehouse and retail shops to isolate the important pathogenic fungi associated with these seeds. The collected seed samples were subjected to standard blotter method (ISTA, 1996) and incubated in alternative cycles of dark and light. On the seventh day of incubation, samples were screened for seed mycoflora with the help of stereobinocular microscope and compound microscope. Associated fungi were identified based on growth characteristic, mycelial morphology, spore morphology and other important characters using standard manuals. The fungi, which were frequently associated in higher percentage in sorghum were selected which served as tested fungi.

Anti-fungal activity assay

Different concentrations (10, 20, 30, 40 and 50%) of aqueous extract, all the solvent extracts and isolated constituents (Fraction I to IV) of *Mimusops elengi* were subjected to antifungal activity assay by poisoned food technique. In Czepak Dox Agar (CDA) medium, the extracts were added to the medium to achieve the desired concentrations in the medium, autoclaved and poured into Petridishes (20 ml each) and allowed to cool. After complete solidification of the medium, 5 mm disc of 7-day-old culture of the tested fungi were transferred. Four replicates were maintained for each concentration. The CDA media devoid of the extract served as control. The plates were incubated at 26±1ºC for seven days. The fungitoxicity of the extract in terms of percentage inhibition of mycelial growth was calculated by using the formula:

$$\%\text{inhibition} = dc - dt \times 100/dc$$

Where dc=Average increase in mycelial growth in control, dt=Average increase in mycelial growth in treatment (Singh and Tripathi, 1999).

Synthetic fungicides, viz., Blitox, Captan, Dithane M-45 and Thiram were also tested at their recommended dosage (2000 ppm) for antifungal activity by poisoned food technique for comparative studies (Zehavi *et al.*, 1986).

Results

Antifungal activity

Aqueous extract

It is interesting to note that all the fungi recorded 50% of mycelial growth inhibition at 50% concentration of the aqueous extract, except *A. tamari*. Among twenty-four fungi tested *Drechslera* sp. recorded high susceptibility with both *D. halodes* and *D. tetramera* which showed complete inhibition at 50% concentration. *F. graminearum* showed maximum susceptibility among *Fusarium* species, which showed 100% susceptibility. *A flavus* and *A. versicolor* recorded more than 90% of inhibition compared to other *Aspergillus* species tested and *P. chrysogenum* showed maximum susceptibility among *Penicillium* species (Table 1).

Solvent extracts

Among different solvent extracts tested, methanol and ethanol extracts recorded highly significant antifungal activities. Whereas, activity was not observed in petroleum ether, benzene and chloroform extracts (Table 2). Even for the methanol extract, all tested fungi showed over 50% of susceptibility among which, *D. halodes, D. tetramera, F. equiseti, F. graminearum, F. moniliformae, F. proliferatum, F. solani, A. flavus, A. fumigtus, A. versicolor, P. chrysogenum* and *P. griseofulvum* which recorded over 80% of mycelial growth inhibition at 2 mg/ml concentration. *A. niger* was the least susceptible and *A. flavus* was the most susceptible among the 24 tested fungi. Antifungal activities were varied among different tested pathogenic fungi against ethanol extract, which recorded significant activities next to methanol extract.

Phytochemical analysis

Phytochemical analysis of methanol extract revealed the presence of carbohydrates and glycosides, proteins and amino acids, alkaloids, phenolic compounds and tannin. Phytosterols, saponins, oils, gum and mucilage were found absent in methanol extract (Table 3).

Comparative efficacy of different fractions with fungicides

Fraction III (Alkaloids) recorded highly significant antifungal activity, where as activity was not observed in fraction I, II and IV indicating the nature of active principle. The susceptibility of tested fungi to alkaloid fractions varied, *D. halodes* recorded high susceptibility and *F. oxysporum* was least susceptible. Comparative efficacy of alkaloid fraction with fungicides is presented in Table 4. Among four tested fungicides, Thiram recorded significant antifungal activity and Dithane M-45 with least antifungal activities. The activity of alkaloid fraction is highly significant compared to Dithane M-45 and other tested fungicides at recommended dosage of 2000 ppm.

Table 3. Preliminary phytochemical analysis of methanol extract of *Mimusops elengi*.

	Tests	**Methanol extract**
1.	Carbohydrates/Glycosides	+ +
2.	Proteins/Aminoacids	+ +
3.	Alkaloids	+ +
4.	Phytosterols	- -
5.	Phenolic compounds	+ +
6.	Saponin	--
7.	Tannin	++
8.	Oils	--
9.	Gums and mucilage	--

++ Present, -- Absent.

Discussion

Higher plants are much more important in the production of economically important organic compounds, pharmaceuticals and pesticides (Hostettman and Wolfender, 1997). The plant world is a rich storehouse of natural chemicals that could be exploited for use as pesticides. The total number of plant chemicals may exceed 400,000 and of these 10,000 are secondary metabolites whose major role in the plants is reportedly defensive (Grayer and Harborne, 1994). Many species of higher plants have not been described, much less surveyed for chemical or biologically active constituent and new sources of commercially valuable pesticides (Varma and Dubey, 1999; Gottlieb *et al.,* 2002). This is mainly due to lack of information on the screening and evaluation of diverse plants for their antimicrobial potential.

Biologically active plant derived pesticides are expected to play an increasingly significant role in crop protection strategies. Several plants are regularly screened for antifungal activity in our laboratory (Satish *et al.,* 1999; Mohana and Raveesha, 2006). The screening revealed that *M. elengi* was effective for inhibition of mycelial growth tested by poisoned food technique at different concentrations. Among different extracts of *M. elengi* tested, significant antifungal activity was observed in aqueous and methanol extract, suggesting that the active compound is better extracted with water and methanol than the other solvents. Since, the present study aimed at management of seed borne fungal pathogens, this result was highly promising as the active principle is more polar. The results of the present investigation draws conclusion that the active principle is of alkaloid in nature.

The occurrence of pyrrolizidine alkaloids within the family sapotaceae including *Mimusops elengi* was reported by Hart *et al*. (1968). Further work needs to be carried out to confirm whether the same previously reported pyrrolizidine alkaloids widely distributed in sapotaceae is responsible for broad-spectrum antifungal activity of leaf extracts. This paper for the first time reporting the broad spectrum antifungal activities of aqueous extracts, different solvent extracts and isolated constituents and also laid foundation for further isolation and characterization of active principle responsible for the desired activity. Since a single plant is known to contain several metabolites with diverse biological activity. Alkaloid fraction is highly effective against *Drechslera*, *Aspergillus* and *Penicillium* species with more than 85% inhibition, where as *Fusarium* recorded varied response. This result indicates the broad-spectrum antifungal activities of the active principles. The comparative efficacy against synthetic fungicides revealed that maximum inhibition was observed at 50 ppm of alkaloid fraction compared to tested synthetic fungicides at the recommended dosage (2000 ppm). This result was

also encouraged, where low concentration of alkaloid fraction was enough to obtain significant antifungal activity which compared to synthetic fungicides.

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Table 1. Antifungal activity of aqueous extract of *Mimusops elengi* at different concentrations against phytopathogenic fungi.

Phytopathogenic field fungi	Percent mycelial inhibition				
	Concentrations (%)				
	10	**20**	**30**	**40**	**50**
Alternaria alternata	33.40±0.49	47.50±0.49	59.92±0.44	73.50±0.49	88.93±0.75
Drechslera halodes	31.40±0.49	56.40±0.49	87.48±0.49	100.00±0.00	100.00±0.00
D. tetramera	27.91±0.43	47.61±0.43	59.73±0.43	92.31±0.43	100.00±0.00
Fusarium equiseti	25.57±0.64	34.87±0.64	44.58±0.49	55.77±0.64	91.12±0.75
F. graminearum	42.73±0.48	62.65±0.48	85.01±0.59	94.75±0.46	100.00±0.00
F. lateritium	20.52±0.42	29.82±0.42	34.27±0.46	45.87±0.42	50.98±0.52
F. moniliforme	57.75±0.34	65.79±0.34	75.03±0.34	86.76±0.34	95.70±0.48
F. oxysporum	43.28±0.60	55.27±0.60	62.68±0.48	74.28±0.60	87.55±0.50
F. proliferatum	45.89±0.47	58.89±0.77	73.71±0.45	86.82±0.37	91.92±0.72
F. semitectum	39.10±0.39	47.10±0.39	57.89±0.44	68.10±0.79	77.39±0.48
F. solani	34.20±0.52	46.20±0.32	65.20±0.62	89.20±0.62	98.41±1.00

Table 1 continued

Table 1 continued**.**

Phytopathogenic storage fungi	Percent mycelial inhibition				
	Concentrations (%)				
	10	**20**	**30**	**40**	**50**
Aspergillus candidus	36.73±0.60[1/]	48.67±0.60	59.78±0.30	75.56±1.00	87.58±0.50
A. columnaris	22.20±0.43	34.30±0.43	46.50±0.43	54.63±0.39	69.56±0.67
A. flavipes	35.80±0.72	46.90±0.72	54.60±0.72	62.18±0.7	75.16±0.81
A. flavus	38.24±0.41	52.24±0.41	74.24±0.41	87.02±0.53	96.45±0.58
A. fumigatus	44.26±0.52	56.56±0.58	64.96±0.55	78.56±1.00	89.56±1.00
A. niger	18.00±0.52	22.04±0.52	34.00±0.52	44.21±0.42	56.83±0.60
A. ochraceus	21.42±0.60	34.82±0.60	45.82±0.60	56.75±0.56	86.76±0.56
A. tamari	19.40±0.21	24.70±0.61	29.60±0.61	39.35±0.30	42.36±0.50
A. terreus	22.65±0.34	31.62±0.04	45.67±0.0.45	58.88±0.34	68.38±0.87
A. versicolor	28.47±0.40	47.74±0.86	65.24±0.40	85.12±0.55	96.10±0.40
Penicillium chrysogenum	37.54±0.56	49.24±0.32	67.64±0.78	91.16±0.65	96.45±0.35
P. griseofulvum	21.67±0.56	26.45±0.56	36.34 ±0.67	74.89±0.72	87.23±0.45
P. oxalicum	21.06±0.67	27.67±0.32	33.45±0.25	56.63±0.78	74.45±0.78

[1/]Data given are means of four replicates ± standard error.

Table 2. Antifungal activity of different solvent extracts of *Mimusops elengi* at 2000 mg/ml concentration against phytopathogenic fungi.

Phytopathogenic field fungi	Percent mycelial inhibition				
	Petroleum ether extract	Benzene extract	Chloroform extract	Methanol extract	Ethanol extract
Alternaria alternata	-	-	-	75.46±0.47	54.54± 0.50
Drechslera halodes	-	-	-	85.78±0.32	61.45± 0.56
D. tetramera	-	-	-	87.53±0.56	63.67 ±0.33
Fusarium equiseti	-	-	-	88.75±0.19	62.00± 0.78
F. graminearum	-	-	-	86.53±0.29	60.00± 0.36
F. lateritium	-	-	-	56.86±0.67	32.57± 0.67
F. moniliforme	-	-	-	87.34±0.25	64.67± 0.56
F. oxysporum	-	-	-	79.08±0.48	62.00± 0.45
F. proliferatum	-	-	-	85.43±0.56	68.00± 0.56
F. semitectum	-	-	-	79.34±0.99	61.33± 0.57
F. solani	-	-	-	87.04±0.56	69.45 ±0.78

Table 2 continued

Table 2. continued.

Phytopathogenic storage fungi	Percent mycelial inhibition				
	Petroleum ether extract	**Benzene extract**	**Chloroform extract**	**Methanol extract**	**Ethanol extract**
Aspergillus candidus	-	-	-	75.45±0.56[1/]	51.45±0.34
A. columnaris	-	-	-	59.45±0.23	45.35± 0.45
A. flavipes	-	-	-	68.56±0.56	44.34±0. 20
A. flavus	-	-	-	89.67±0.42	52.36± 0.55
A. fumigatus	-	-	-	88.45±0.56	56.56± 0.30
A. niger	-	-	-	56.46±0.76	32.46± 0.27
A. ochraceus	-	-	-	79.49±0.35	45.70± 0.89
A. tamari	-	-	-	57.66±0.53	33.39± 0.20
A. terreus	-	-	-	73.49±0.56	45.46± 0.29
A. versicolor	-	-	-	77.81±0.59	45.80± 0.38
Penicillium chrysogenum	-	-	-	86.79±0.92	52.36± 0.67
P. griseofulvum	-	-	-	82.29±0.36	52.56±0.34
P. oxalicum	-	-	-	83.76±0.67	54.32±0.34

[1/]Data given are mean of four replicates ± standard error.

Table 4. Antifungal activity assay of different fractions of *Mimusops elengi* and some synthetic fungicides against seed borne phytopathogenic fungi.

Phytopathogenic field fungi	Percent mycelial inhibition							
	Active fractions of *M. elengi* (50 µg/ml)				Synthetic fungicide (2000 ppm)			
	Acidic	Phenolic	Alkaloid	Neutral	Blitox	Captan	Dithane M-45	Thiram
Alternaria alternata	-	-	95.48±0.75	-	72.46±0.58	91.48±0.35	77.19±0.21	100±0.00
Drechslera halodes	-	-	98.45±0.25	-	83.25±0.46	100±0.00	97.15±0.39	100±0.00
D. tetramera	-	-	96.43±0.56	-	84.37±0.33	98.63±0.42	97.76±0.60	100±0.00
Fusarium equiseti	-	-	82.34±0.35	-	95.55±0.36	81.85±0.32	76.07±0.61	100±0.00
F. graminearum	-	-	75.45±0.67	-	88.66±0.26	73.35±0.26	73.91±0.74	100±0.00
F. lateritium	-	-	68.45±0.67	-	42.88±0.25	63.48±0.27	48.90±0.37	100±0.00
F. moniliforme	-	-	76.45±0.56	-	97.24±0.45	78.45±0.72	65.84±0.85	100±0.00
F. oxysporum	-	-	68.18±0.78	-	85.69±0.64	68.68±0.48	65.18±0.18	100±0.00
F. proliferatum	-	-	76.66±0.56	-	74.38±0.27	68.66±0.25	69.09±0.45	100±0.00
F. semitectum	-	-	93.55±0.45	-	78.38±0.12	95.59±0.03	91.47±0.18	100±0.00
F. solani	-	-	70.70±0.35	-	72.80±0.25	72.70±0.26	65.32±0.30	100±0.00

Table 4 continued

Table 4 continued.

Phytopathogenic storage fungi	Percent mycelial inhibition							
	Active fractions of *M. elengi*				Synthetic fungicide			
	Acidic	**Phenolic**	**Alkaloid**	**Neutral**	**Blitox**	**Captan**	**Dithane M-45**	**Thiram**
Aspergillus candidus	-	-	95.45±0.56[1/]	-	100±0.00	100±0.00	47.89±0.31	100±0.00
A. columnaris	-	-	88.45±0.35	-	85.90±0.22	87.67±0.31	44.90±0.40	100±0.00
A. flavipes	-	-	87.45±0.34	-	91.35±0.30	88.87±0.19	24.68±0.13	100±0.00
A. flavus	-	-	92.98±0.43	-	96.03±0.37	91.98±0.14	54.17±0.15	100±0.00
A. fumigatus	-	-	93.48±0.45	-	100±0.00	92.98±0.37	16.52±0.40	100±0.00
A. niger	-	-	93.78±0.45	-	100±0.00	96.45±0.14	92.75±0.31	100±0.00
A. ochraceus	-	-	96.85±0.56	-	93.64±0.14	93.85±0.66	86.82±0.44	100±0.00
A. tamari	-	-	88.79±0.56	-	73.53±0.28	87.69±0.13	22.45±0.50	100±0.00
A. terreus	-	-	88.45±0.56	-	95.73±0.27	87.16±0.51	45.87±0.35	100±0.00
A. versicolor	-	-	85.86±0.45	-	93.40±0.20	83.86±0.43	13.41±0.36	100±0.00
Penicillium chrysogenum	-	-	95.56±0.34	-	100±0.00	100±0.00	53.45±0.39	100±0.00
P. griseofulvum	-	-	94.45±0.89	-	92.91±0.38	92.25±0.15	51.98±0.38	100±0.00
P. oxalicum	-	-	86.67±0.67	-	65.94±0.32	82.60±0.85	53.85±0.20	100±0.00

[1/] Data given are mean of four replicates ± standard error.