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In vitro antimicrobial activity of certain plant products / seed extracts against multidrug resistant *Propionibacterium acnes*, *Malassezia furfur*, and aflatoxin producing *Aspergillus flavus*

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Seed extracts of five different plants viz. *Phoenix sylvestris, Tamarindus indica, Syzygium cumini, Manilkara zapota,* and *Annona squamosa*, prepared by microwave assisted extraction (MAE) method were screened for their antimicrobial activity against various human pathogenic microbes. Extraction efficiency ranged from 6-24%. Antimicrobial activity of seed extracts was investigated against three yeasts (*Candida albicans, Saccharomyces cerevisae, Malassezia furfur*), one mold (*Aspergillus flavus*) and one anaerobic bacterium (*Propionibacterium acnes*) by broth dilution assay and minimum inhibitory concentration (MIC) was determined. Methanolic extract of *T. indica* and *S. cumini* inhibited 60% of test organisms. Amongst the microorganisms tested, the most resistant was found to be *C. albicans* and the most susceptible was *M. furfur*. Effect of eight seed extracts on aflatoxin production by *A. flavus* was also investigated. Ethanolic extract of *A. squamosa* caused 100% inhibition of aflatoxin production at 500 μg/mL.

Keywords: Aflatoxin; Microwave Assisted Extraction (MAE); Post Extract Effect (PEE); Antimicrobial; Minimum Inhibitory Concentration (MIC).

Several fungi have been known to cause infection in humans as well as plants. Particularly immunocompromised humans are at increased risk of fungal attack. Because the cells of fungi are eukaryotic, they present special problems in chemotherapy. Majority of chemotherapeutic drugs are designed to act on bacteria and are generally ineffective in combating fungal infections (Talaro, 2008). Screening of natural sources for novel antifungal compounds can help to control fungal pathogens of both humans and plants. There has been a renewed interest in natural antimicrobial compounds, particularly those derived from plants, which can be used in the control of mycotoxigenic fungi (Marin et al., 2011).

In past decades many publications have reported antimicrobial activity in a large number of plant products (Rukayadi and Hwang, 2006; Rahmah et al., 2011). Present study was aimed at screening of seed extracts of *Annona squamosa* L (Annonaceae), *Manilkara zapota* L (Sapotaceae), *Tamarindus indica* L (Leguminosae), *Syzygium cumini* (Linn.) skeels (Myrtaceae), and *Phoenix sylvestris* Roxb (Palmae) for their activity against *Malassezia furfur*, *Saccharomyces cerevisiae*, *Candida albicans*, *Aspergillus flavus*, and *Propionibacterium acnes*. Additionally these organisms were also challenged with certain pure phytocompounds i.e. quercetin, curcumin,

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gallic acid, and lycopene. Earlier we had reported antibacterial (Kothari et al., 2011; Kothari, 2011a) and antioxidant (Kothari et al., 2010; Kothari and Seshadri, 2010a) activity of above seed extracts. *M. furfur* is believed to be responsible for dandruff in 45-50% of global population (Dawber, 1994). *P. acnes*, a common skin organism, is most notably recognized for its role in acne vulgaris. It produces a number of virulence factors and is well known for its inflammatory and immunomodulatory properties (Perry and Lambert, 2006). *Aspergillus flavus* is known to infect and contaminate preharvest (Setamou et al., 1997) and postharvest seed crops with the carcinogenic secondary metabolite aflatoxin (Amaike and Keller, 2011).

Materials and Methods

Plant material: Seeds of all the five plants were procured from their fruits available in the local market of Ahmedabad. They were authenticated for their unambiguous identity by Prof. Y. T. Jasrai, Head of Botany Dept., Gujarat University, Ahmedabad.

Extraction: Seeds were extracted in three different solvents (Merck, Mumbai, India)- acetone, methanol, and ethanol (50%), with minor modification in microwave assisted extraction (MAE) method (Kothari et al., 2009) reported earlier by us. One gram of dry seed powder was soaked into 50 mL of solvent, and subjected to microwave heating (Electrolux EM30EC90SS) at 720 W. Total heating time was kept 90, 120 and 70 second for methanol, acetone, and ethanol respectively, with intermittent cooling (reheating duration with methanol was kept 5 sec instead of 10 sec described in our earlier publication). This was followed by centrifugation (10,000 rpm for 15 min), and filtration with Whatman paper # 1 (Whatman International Ltd., Maidstone, England). Solvent was evaporated from the filtered extract and then the dried extracts were reconstituted in dimethyl sulfoxide (DMSO; Merck) for broth dilution assay. Reconstituted extracts were stored under refrigeration for further use. Extraction efficiency was calculated as percentage weight of the starting dried plant material.

Microbial strains:

Test microorganisms (table 1) were procured from Microbial Type Culture Collection (MTCC), Chandigarh.

Table 1. Test organisms

Organism	MTCC no.	Remarks[a]
Candida albicans	3017	Resistant to amphotericin-B
Saccharomyces cerevisiae	170	Resistant to amphotericin-B, clotrimazole, and itroconazole
Malassezia furfur	1374	Resistant to amphotericin-B, clotrimazole, itroconazole, ketoconazole, and nystatin
Aspergillus flavus	2799	Resistant to amphotericin-B, clotrimazole, fluconazole, itroconazole, ketoconazole, and nystatin
Propionibacterium acnes	1951	Resistant to norfloxacin, cefuroxime, ciprofloxacin, ceftazidime, roxithromycin, and sparfloxacin

[a]Antibiotic resistance pattern as determined by disc diffusion assay in our lab.

MIC determination. MIC (minimum inhibitory concentration) determination was carried out using microbroth dilution method as per NCCLS guidelines (Jorgensen and Turnidge, 2003). Assay was performed in a 96-well microtitre plate. Total volume of the assay system in each well was kept 200 µL. Thioglycolate broth (HiMedia, Mumbai) with hemin and vitamin K was

used as growth medium for *P. acnes*, and RPMI-1640 (HiMedia) for all fungi except *M. furfur*. Later was grown in Sabouraud broth supplemented with 1% v/v olive oil, as the standardized RPMI-1640 medium does not support the growth of the *Malassezia* yeast cells, because it lacks the specific lipids they require (Rukayadi and Hwang, 2006). Methanolic extract of *T. indica* and ethanolic extract of *P. sylvestris* were tested in minimal media to avoid precipitation. Composition of minimal media for yeast (per liter) (Atlas, 2010): K_2HPo_4 7 g, KH_2Po_4 2 g, $(NH_4)_2So_4$ 1 g, glucose 1 g, sodium citrate 0.5 g, $MgSo_4.7H_2O$ 0.1 g. Composition of minimal media for *A. flavus* (http://www.fgsc.net/methods/anidmed.html): $NaNo_3$ 6 g, KCl 0.52 g, $MgSo_4.$ 7 H_2O 0.52 g, KH_2PO_4 1.52 g, glucose 10 g, 2 ml Hutner's reagent pH-6.5, distilled water 1000 ml.

Inoculum density of the bacterial and yeast suspension was adjusted to that of 0.5 McFarland standard. Turbidity of the *A. flavus* inoculum was adjusted between 0.09-0.11 at 530 nm (Ingroff and Pfaller, 2003). Broth was dispensed into wells of microtitre plate followed by addition of test extract and inoculum. Extracts (reconstituted in DMSO) were serially diluted into each of the wells. A DMSO control was included in all assays (Wadhwani et al., 2009). Streptomycin (HiMedia) served as positive control for bacteria, and amphotericin B (HiMedia) for fungi. *P. acnes* was incubated at 35ºC for 48 h in anaerobic jar (HiMedia), before being read at 655 nm in a plate reader (BIORAD 680). Anaerobic condition in the jar was created using anaerogas pack (HiMedia). *A. flavus* plates were incubated at 30ºC for 46-50 h. MIC was recorded as the lowest concentration at which no growth was observed. All MICs were determined on three independent occasions. Concentration at which growth was inhibited by 50% was recorded as IC_{50} value. After reading the plates for MIC, subculturing was made on appropriate nutrient media (YPD agar for *C. albicans* and *S. cerevisiae*, Sabouraud agar with and without olive oil for *M. furfur* and *A. flavus* respectively) from the wells showing no growth, so as to determine whether the extract is having cidal or static mode of action. Incubation was continued for 72 h, as the agents exhibiting a post antibacterial effect (PAE)/post antifungal effect (PAFE) require extended incubation following subculture in either time-kill or minimum lethal concentration (MLC) determinations in order to ensure the detection of slow-growing but not dead organisms (Pfaller et al., 2004). Growth on the plate indicated bacteriostatic action, absence of growth was interpreted as bactericidal action. Delayed growth as compared to control was interpreted as Post Extract Effect (PEE).

***Aflatoxin estimation*:** *A. flavus* was grown in Czepek dox medium with and without test extract, contained in 25 ml flasks; incubation was continued for 5 days at 30ºC under static condition with intermittent shaking. This was followed by separation of mycelia from the broth; mycelia was subjected to dry weight determination after drying at 55ºC till constant weight was achieved. Aflatoxin was extracted in a mixture of toluene:acetonitrile (9:1) (Nesheim and Stack, 2001), which was mixed with the broth remaining after removal of mycelia, and kept on shaker for 12 h at room temperature. This was followed by separation of solvent layer and measurement of aflatoxin at 350 nm. Amount of aflatoxin was calculated as:

$$\text{Aflatoxin } (\mu g/mL) = A \times mw \times 1000/\varepsilon.$$

Where, A: absorbance at 350 nm; mw: molecular weight of aflatoxin; ε: molar absorptivity

Total activity. Total activity (mL/g) was calculated as (Eloff, 2004): Amount extracted from 1 g (mg) / MIC (mg/mL).

Results and Discussion

Extraction: Extraction efficiency of all the extracts is listed in table 2. Highest extraction yield (24.33%) was obtained with ethanolic extract of *S. cumini* seeds.

***Antimicrobial activity of crude extracts*:** Results of antimicrobial assay are presented in table 3. *M. furfur* was susceptible to seven different extracts, of which acetone extract of *A. squamosa* was most effective against it with an MIC of 150 µg/mL. Among pure phytocompounds quercetin was most effective against *M. furfur* with an MIC value of 70 µg/mL. *A. flavus* was inhibited by no extract other than methanolic extract of *A. squamosa.* Highest average total activity (729 mL/g) was recorded for ethanolic extract of *S. cumini.* Total activity is a measure of the amount of material extracted from a plant in relation to the MIC of the extract, fraction or isolated compound. It indicates the degree to which the active fractions or compounds present in 1 g can be diluted and still inhibit growth of the test organism (Eloff, 2004). A total activity of 1216.5 mL/g of ethanolic extract of *S. cumini* against *M. furfur* means that one gram of this extract can be diluted up to 1216.5 mL, and still will inhibit growth of the test organism. In this study total activity was found to have a relatively weak linear correlation (r = 0.718) with extraction efficiency. In our earlier work with the same extracts against human pathogenic bacteria, these two quantities were found to have a strong positive linear correlation (Kothari, 2011b) indicating the importance of applying an efficient extraction method while screening crude extracts for their bioactivity. MAE has been reported to be suitable for extraction of antimicrobial and/or antioxidant compounds from plant materials (Kothari et al., 2010; Kothari and Seshadri, 2010a; Kothari, 2011a; Upadhyay et al., 2011; Pasquet et al., 2011) while simultaneously maintaining denaturation of heat-labile phytocompounds at a minimum.

Table 2. Extraction efficiency of all the seed extracts

Seed	Solvent	Extraction efficiency (%)
Annona squamosa	Ethanol (50%)	6.04
	Acetone	7.76
	Methanol	6.93
Manilkara zapota	Acetone	6.86
	Methanol	7.02
Tamarindus indica	Methanol	13.3
Phoenix sylvestris	Ethanol (50%)	6.99
Syzygium cumini	Methanol	17.34
	Ethanol (50%)	24.33

None of the test organisms got inhibited by gallic acid, with Ic_{50} >100 µg/mL for *S. cerevisiae, C. albicans,* and *A. flavus.* Curcumin was able to inhibit growth of *S. cerevisiae* by 50 % at a concentration of 90 µg/mL, but showed no significant effect against *M. furfur* and *C. albicans.*

Different plant products included in this study registered MIC against *M. furfur* in the range of 70-550 µg/mL. Marcon et al. (1987) have reported MIC value of flucytosine being in excess of 100 µg/mL, and that of amphotericin B between 0.3-2.5 µg/mL against various systemic and superficial *M. furfur* isolates. However *M. furfur* strain employed in our investigation was resistant (as determined by disc diffusion assay using Hexa Antimyco-01; HiMedia) to amphotericin B (100 units/disc), clotrimazole (10 µg/disc), itroconazole (10

µg/disc), ketoconazole (10 µg/disc), and nystatin (100 units/disc). Prevelance of drug resistance among pathogenic microorganisms makes it even more important to screen natural products for novel antimicrobials. Over the years the potential antifungal effects of certain bioactive compounds from plants have attracted serious attention within the scientific community, largely as a result of the growing problem of multidrug resistance among pathogenic fungi (Cowan 1999; Cushnie and Lamb, 2005). Rukayadi and Hwang (2006) reported xanthorrhizol isolated from *Curcuma xanthorrhiza* Roxb as an effective anti-*Malassezia* agent with MIC and MFC value of 1.25 and 5 µg/mL respectively. Celery essential oil (at 1%) was reported to inhibit *M. furfur* (Chee and Lee, 2009). Lee et al. (2010) reported MIC of plant essential oils against *M. furfur* to be 2 mg/mL; our extracts were able to inhibit *M. furfur* at much lower concentration (Table 3).

Table 3. Results of antimicrobial assay

No.	Extract/ Phytochemical	Organism	IC_{50} (µg/mL)	MIC (µg/mL)	Total activity (ml/g)	Average total activity (ml/g)	Type of effect
1.	Acetone extract of *A. squamosa*	*M. furfur*	<100	150	517.33	517.33	Static
2.	Methanol extract of *A. squamosa*	*M. furfur*	<150	240	288.75	182.81	Static
		A. flavus	601	901.5	76.87		Static
3.	Acetone extract of *M. zapota*	*M. furfur*	<200	238.73	298.26	298.26	Static
4.	Methanol extract of *S. cumini*	*M. furfur*	<400	400	433.5	330.45	Post extract effect (30 h)[b]
		S. cerevisae	400	500	314.55		Static
		P. acnes	<400	400	243.3		Post extract effect (72 h) [b]
5.	Ethanol extract of *S. cumini*	*M. furfur*	<200	200	1216.5	729	Static
		P. acnes	700	1000	243.3		Static
6.	Methanol extract of *T. indica*	*M. furfur*	500	500	266	605.89	Post extract effect (53 h) [b]
		S.cerevisae	400	600	221.67		Static
		P.acnes	200	300	1330		Static
7.	Ethanol extract of *P.sylvestris*	*M. furfur*	<400	550	126	126	Static
8.	Quercetin[c]	*C. albicans*	<70	80	-	-	Static
		M. furfur	<70	70	-	-	Static
		S. cerevisiae	100	200	-	-	Static
9.	Curcumin[c]	*S. cerevisiae*	90	>100	-	-	-
10.	Lycopene[c]	*P. acnes*	>40	>40	-	-	-
11.	Gallic acid[c]	*A. flavus*	80	>100	-	-	-

[b]Figure in parenthesis indicate duration of PEE.
[c]Gallic acid was prepared in water; all other phytochemicals were prepared in DMSO.

All the extracts active against *M. furfur* were fungistatic in their action, except methanolic extracts of *S. cumini* and *T. indica*. Latter exhibited a post extract effect (PEE), which refers to the persistent suppression of microbial growth after exposure to antimicrobial agents, and may last for several hours depending on the concentration of test agent and the susceptibility of the target organism. Similar effect of commonly used antibiotics termed as post antibiotic effect (PAE) (Ramadan et al., 1995) or post antibacterial/post antifungal effect (Pfaller et al., 2004) has been reported earlier in literature. Duration of PEE exerted by methanolic extract of *S. cumini* and *T. indica* was 30 h and 53 h respectively. This is to say that when *M. furfur* was subcultured

from experimental wells containing test extracts (at MIC) onto extract-free growth media, it took a time of 30 or 53 h before appearance of visible growth, as compared to 24 h taken for visible growth to appear in control plate (inoculated with content from negative control). Thus while determining type of action of any antimicrobial agent, possibility of PAE/PEE should also be considered in addition to conventional static and cidal action. Particularly the importance of extended incubation following subculture should be emphasized, otherwise a PEE/PAE may be mistaken as cidal action owing to insufficient length of incubation. Extended incubation can ensure the detection of slow-growing but not dead organisms (Pfaller et al., 2004). PAE can affect test organism in many ways e.g. changes in growth kinetic morphology, inhibition of enzyme and toxin production, loss of adhesive properties and susceptibility to host humoral and cellular immunity. These effects are usually observed when organisms are incubated with antimicrobials at levels below the MIC (sub-MIC). During PAE, microbial physiology may also be affected by exposure to the antimicrobial agents (Ramadan et al., 1995).

Malassezia species have been reported as significant causes of fungal infections in patients with cancer and AIDS. Interest in this organism has increased considerably in recent years, as this yeast has been implicated as the primary cause of the scalp disease known as seborrhoeic dermatitis or dandruff. It can become an opportunistic pathogen owing to the alterations on the skin surface environment and host defence (Rukayadi and Hwang, 2006). Seborrhoeic dermatitis and dandruff are perhaps the most common diseases associated with *Malassezia* species, with seborrhoeic dermatitis occurring in 1–3% and dandruff in greater than 50% of the general population. The incidence of seborrhoeic dermatitis is much higher in immunocompromised patients, especially AIDS patients, ranging from 30% to 33%. The vast majority of more recent data supports a direct causal link between *Malassezia* and seborrhoeic dermatitis or dandruff. Antifungal treatment is found to be effective in treating these diseases, and improvement in seborrhoeic dermatitis or dandruff is accompanied by a reduction in *Malassezia* levels on the scalp (Batra et al., 2005).

P. acnes proved susceptible to methanolic extract of *T. indica*, and ethanolic and methanolic extracts of *S. cumini* seeds. Former two exerted a bacteriostatic action whereas latter exerted a PEE of 72 h duration (time required for visible growth to appear in control plate was 24 h). Bacteriostatic action of methanolic extract of *S. cumini* (Kothari et al., 2011), and bactericidal action of methanolic extract of *T. indica* seeds (Kothari and Seshadri, 2010b) against few human pathogenic bacteria has earlier been reported by us. Later extract tested positive for alkaloids when mixed with dragendorff reagent. Presence of phenols and flavones in this extract is previously known (Kothari and Seshadri, 2010b). Methanolic extracts of *Camellia sinensis, Glycyrrhiza glabra,* and *Calendula officinalis* have been reported to be active against *P. acnes* (Nand et al., 2012). Emergence of antimicrobial resistance amongst *P. acnes* isolates from systemic infections has been demonstrated by Oprica and Nord (2005). The *P. acnes* strain employed in our study was resistant (determined using Icosa universal 1™, HiMedia) to norfloxacin (10 µg/disc), cefuroxime (30 µg/disc), ciprofloxacin (5 µg/disc), ceftazidime (30 µg/disc), roxithromycin (30 µg/disc), and sparfloxacin (5 µg/disc). *P. acnes* is a widespread opportunist most noted for its potential involvement in the inflammatory condition acne. It is also a cause of biofilm associated infections of indwelling medical devices. In addition to its role in human infection, *P. acnes* has recently been identified as the cause of necrosuppurative placentitis and abortion in an adult Holstein cow. An emerging number of studies have reported the association between the phylotypes of *P. acnes* and different infections. The strong

proinflammatory nature of *P. acnes* has been known for some time. There is a growing body of evidence to suggest that its involvement in various infections and diseases has been significantly underestimated, especially in relation to infections of indwelling medical devices where it will persist as an adherent biofilm. With the increase in our understanding of this organism, the need for effective antimicrobials against it may become more evident (McDowell and Patrick, 2011). The importance of the acne treatment in the adult should be enhanced as it can also lead to symptoms of serious depression and anxiety (Singh et al., 2011).

S. cerevisiae was also susceptible to methanolic extracts *of S. cumini* and *T. indica.* Both these extracts exerted a fungistatic effect against it. Quercetin was able to inhibit its growth at 80 µg/mL. As quercetin has been one of the constituents in *S. cumini* extract (Bhatia and Bajaj, 1972; Karthic et al., 2008; Kothari et al., 2011), and its MIC is less than that of crude extract, it may be considered to be significantly responsible for anti-*Saccharomyces* activity of methanolic extract of *S. cumini* seeds. Same can be said for anti-*Malassezia* activity of this extract. Quercetin in lotus leaves was indicated as potential antibacterial agent by Mingyu and Zhuting (2008).

We found both the extracts of *S. cumini* not capable of inhibiting *C. albicans*. Similar lack of activity of *S. cumini* extracts against *C. albicans* was previously reported by Duraipandiyan et al. (2006), Portillo et al. (2001), and Dulger and Gonuz (2004). However Hofling et al. (2010) found methanolic extract of *S. cumini* seeds to be effective against *C. albicans*. Nascimento et al. (2000), Chandrasekaran and Venkatesalu (2004) also found *C. albicans* to be susceptible to *S. cumini* extracts. These variations in results may be due to several factors such as seasonal variation among different batches of the plant, difference in the exraction method, etc.

Table 4. Effect of plant extracts on mycelial growth and aflatoxin production by *A. flavus*

Extract	Control mycelial weight (g)	Experimental mycelial weight (g)	% Difference as compareed to control	Aflatoxin B_1 in control (µg/ml)	Aflatoxin B_1 in Experimental (µg/ml)	% Difference as compared to control	Total Aflatoxin in control (µg/ml)	Total Aflatoxin in experimental (µg/ml)	% Difference as compared to control
A.squamosa (MeOH)[a]	0.103±0.00	0.106±0.00	-2.91%	7.27±0.00	10.90±1.12	+49.93 *	31.09±0.00	46.60±4.79	+49.88 *
A.squamosa (EtOH)	0.082±0.002	0.022±0.002	-73.17 *	1.87±0.084	0.00±0.00	100 % inhibition*	8.01±0.38	0.00±0.00	100 % inhibition*
S.cumini (MeOH)	0.102±0.00	0.100±0.004	-1.96	4.35±0.438	3.18±0.155	-26.89	18.60±1.85	13.51±0.53	-27.36
S. cumini (EtOH)	0.053±0.005	0.060±0.00	+13.20	0.40±0.346	0.00±0.00	100 % inhibition	1.73±1.47	0.00±0.00	100 % inhibition
M.zapota (Acetone)	0.099±0.007	0.082±0.002	-17.17	7.11±0.870	3.50±0.02	-50.77 *	30.42±3.73	14.91±0.06	-50.98 *
M. zapota (MeOH)	0.047±0.00	0.065±0.002	+38.29 *	0.90±0.049	1.81±0.028	+101 *	3.86±0.20	7.74±0.10	+100% *
T. indica (MeOH)	0.088±0.001	0.103±0.00	-17.04 *	2.60±0.077	1.93±0.176	-25.76 *	11.14±0.33	8.27±0.77	-25.76 *
P. sylvestris (EtOH)	0.086±0.00	0.070±0.013	-18.60	3.94±1.435	3.41±0.141	-13.45	16.87±6.15	14.58±0.58	-13.57

[a]This extract was tested at 600 µg/mL. All other extracts were tested at 500 µg/mL.
'+ 'or '–' sign indicates increase or decrease over control; * p <0.05; Aflatoxin concentration and mycelial weight are expressed as mean±SD of three independent replicates.

Effect of our extracts on growth and aflatoxin production by *A. flavus* was also investigated. Methanolic and ethanolic extract of *A. squamosa* were able to inhibit growth of *A. flavus* at 901.5 and 500 µg/ml respectively (Table 4). Ethanolic extract was also able to completely inhibit aflatoxin production by this organism, whereas methanolic extract caused an increase in aflatoxin production by 50%. Anti-aflatoxigenic activity was also exhibited by acetone extract of *M. zapota*, but it was not able to inhibit growth significantly. Interestingly methanolic extract of

the same seed stimulated both the growth and aflatoxin production by *A. flavus.* Overall no direct correlation seemed to exist between mycelia growth and aflatoxin production. Lack of such correlation was earlier reported by Reddy et al. (2011), whereas Rahmah et al. (2011) reported a direct correlation between fungal growth and aflatoxin B1 production. Extracts investigated by these researchers were able to inhibit aflatoxin production at much higher concentration (few mg/mL) than that required by our active extracts. Potent anti-aflatoxigenic activity in *S. aromaticum* extract was reported by Reddy et al. (2009), however they found a relatively low anti-aflatoxigenic activity in *A. squamosa* extract. Better activity in *A. squamosa* extracts found in our study may be attributed to difference in extraction methodology among other factors. Aqueous leaf extract of *A. squamosa* (at 100-300 mg/ml) was reported for its inhibitory effect on aflatoxin production by Reddy et al. (2011). Inhibitory effect of plant extracts on fungal growth and aflatoxin production may be due to their hydrophobicity characters enabling them to partition in the lipids of the fungal cell wall membranes and mitochondria, disturbing their structure and rendering them to more permeable (Rahmah et al., 2011). *A. flavus* has been considered as a noteworthy aflatoxin producing species in India. In north-west India in 1974, 25% of the exposed population died after ingestion of the molded maize with aflatoxin levels ranging from 6250 to 15600 mg/kg. (http://www.icrisat.org/ aflatoxin/health.asp). Some 106 indigeneous people (whose staple food was maize) died in western India during 1974 due to aflatoxicosis. Heavy mortality in chicks in chittor district of Andhra Pradesh was reported in 1982 due to aflatoxicosis (Reddy and Raghavender, 2007). Among the mycotoxins produced by this species aflatoxin B_1 is the most toxic form for mammals, and has been classified as a class 1 human carcinogen. Efforts need to be made towards search for new antifungal materials from natural sources for food preservation.

Our present study has identified certain plant seed extracts to possess antifungal and/or antibacterial activity. Extracts effective against *M. furfur* and *P. acnes* may further be investigated to search for potential lead compounds with both clinical and aesthetic applications. Plant products which are effective against *A. flavus* can be exploited in the management of mold infestation and mycotoxin contamination of foods, feeds and fodders. Most in vitro studies use high concentrations of plant products while evaluating their anti-aflatoxigenic activity (Marin et al., 2011), such high concentrations may actually not be possible to be applied without affecting sensorial quality of food products. Thus identifying plant products which can inhibit aflatoxin production at reasonably low concentrations is of particular importance.

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