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Inhibitory effect of alkaloids of *Albizia amara* and *Albizia saman* on growth and fumonisin B_1 production by *Fusarium verticillioides*

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Abstract

The investigation was aimed to evaluate the antifungal and antifumonisin activities of budmunchiamine A and pithecolobine against *Fusarium verticillioides*. The budmunchiamine A was isolated from *Albizia amara* and pithecolobine from *Albizia saman.* The results demonstrated that both budmunchiamine A and pithecolobine significantly inhibited the growth and fumonisin B_1 production by *F. verticillioides* in a dose dependent manner. The MIC and MFC values ranged from 0.125 to 0.25 mg/ml and 0.25 to 0.5 mg/ml, respectively. *In vitro* evaluation showed that the fumonisin B_1 production was completely inhibited by budmunchiamine A and pithecolobine at 0.25 mg/ml and 0.5 mg/ml, while *in vivo* evaluation showed complete inhibition at 0.25 g/kg and 0.5 g/kg, respectively. The present findings indicate the possible use of budmunchiamine A and pithecolobine as alternative agents to control the fungal and mycotoxin contaminations in food grains.

Introduction

Mycotoxins are toxic secondary metabolites produced by some species of filamentous fungi such as *Fusarium*, *Aspergillus* and *Penicillium*, which invade crops in the field and may grows on food commodities during harvest and storage under favourable conditions (Kumar *et al.*, 2008). Among the mycotoxins, fumonisins are the most toxic secondary metabolites mainly produced by *F. verticillioides* and *F. proliferatum*. Fumonisins production may occur in the field, during post-harvest, storage, and processing under appropriate environmental conditions favouring fungal growth (Jouany, 2007). More than ten types of fumonisins have been isolated and characterized. Of these, fumonisin B_1 (FB_1), fumonisin B_2 (FB_2), and fumonisin B_3 (FB_3) are the major fumonisins produced in nature. These toxins are of great concern due to their widespread occurrence in maize and their adverse effects on human and animal health viz., esophageal cancer, equine leukoencephalomalacia (ELEM), neurotoxicity, hepatotoxicity, nephrotoxicity, modulation of immune responses, developmental abnormalities, liver and kidney tumours, and other abnormalities (Fandohan *et al*., 2003; Domijan *et al*., 2008; Yazar and Omurtag, 2008).

The fumonisin B_1 produced by *F. verticillioides* is the most common contaminant of corn during pre- and post-harvest conditions (Shim and Woloshuk, 2001; Bankole and Adebanjo, 2003; Covarelli *et al.*, 2011). Chemical treatments and usage of food preservatives are the commonly employed strategies to control the growth of *F. verticillioides* and FB_1 biosynthesis in grains and food/feedstuffs. Chemical fungicides have the disadvantage of inflicting damage to the environment, ecosystem and causes ill effects on human health (Reddy *et al*., 2010). Use of natural compounds of plant origin with potential bioactivity would be an alternative strategy to combat against *F. verticillioides* and fumonisin contamination in maize (Yassin *et al*., 2012).

The *Albizia amara* and *Albizia saman* belong to the *Leguminosae* family, are rich in alkaloids, and their extracts have been reported to possess various bioactivities (Kareru *et al*., 2008; Raghavendra *et al*., 2008; Prasad *et al.*, 2008; Azhar *et al*., 2009; Nnamdi *et al*., 2010; Arulpriya *et al*., 2010; Ferdous *et al*., 2010; Karmegam *et al.*, 2012; Ajam *et al.*, 2012). Previous reports from the laboratory indicate the antimicrobial and antiaflatoxigenic activities of crude extracts of *A. amara*, *A. saman* and their active biomolecules (Praveen *et al*., 2011; Thippeswamy *et al*., 2011 and 2013). Till date, there are no reports on the antifungal and antifumonisin activities of budmunchiamine A (BUA) and antifumonisin activity of pithecolobine (PI) against *F. verticillioides*. Hence, in this study, an attempt has been made to analyse the antifungal and antifumonisin activities of BUA and PI.

Materials and Methods

Chemicals and culture media

Sabouraud dextrose agar/broth (SDA/SDB), dimethyl sulfoxide (DMSO), iodonitro tetrazolium

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(INT) and all analytical grade solvents were purchased from Hi-Media, Mumbai (India). Mancozeb (Dithane M-45) was purchased from Indofil Chemicals, Mumbai. Carbendazim (Bavistin) was procured from Saraswathi Agro Chemicals, Jammu, India. Microtiter-plates (96-well) were purchased from Axiva, New Delhi (India). The standard FB_1 was obtained from Sigma, Germany. Silica gel 60 F_{254} coated preparative thin layer chromatography (TLC) plates were obtained from Merck, Germany.

Collection of plant samples, and isolation and identification of bioactive alkaloids

Fresh leaves of *Albizia amara* (Roxb.), *B. boivin* and *Albizia saman* (Jacq.) Merr. were collected from the southern part of Karnataka (India) during 2010-12. The plant samples were authenticated by Dr. Sankara Rao, Professor, JCB National Herbarium and authenticated voucher specimens were deposited in JCB National Herbarium, Indian Institute of Science, Bangalore (India) (Voucher numbers: BUB/MB-BT/DCM/JU10/23 for *A. amara* and BUB/MB-BT/DCM/JU10/33 for *A. saman*). Leaves were shade-dried, powdered and used for alkaloid extraction following the procedure of Harborne (1998). The bioactive alkaloids budmunchiamine A from *A. amara* and pithecolobine from *A. saman* were isolated and characterised as reported earlier (Thippeswamy *et al*., 2013). The IR spectrum of active crystalline compounds of *A. amara* and *A. saman* showed characteristic absorption peaks at 1649.61 and 1646.47 (strong C=O stretch), 3359.77 and 3353.94 (N-H stretch) and 2945.54 and 2944.94 (alkane C-H stretch), respectively. In the positive mode ESI-MS, active compounds of *A. amara* and *A. saman* showed molecular ion peak (m/z) at 453.88 $[M]^+$ and 383.53 $[M]^+$ corresponding to the molecular formula $C_{27}H_{56}N4O$ (MW. 452.76) and $C_{22}H_{46}N4O$ (MW. 382.63), respectively. Further, based on NMR spectroscopic analysis and cited literature data, the isolated compounds were identified as budmunchiamine A (Figure 1a) from *A. amara* and pithecolobine (Figure 1b) from *A. saman* (Wiesner *et al.*, 1952 and 1968; Pezzuto *et al*., 1991 and 1992).

Antifungal activities of BUA and PI

Microbial strain

The FB_1 producing *F. verticillioides* was isolated from freshly harvested maize and the isolated fungus was identified using fungal key manuals (Watanabe, 2002; Nagamani *et al*., 2006) and authenticated by Prof. K.A. Raveesha, Department of Microbiology and Botany, University of Mysore, Mysore (India).

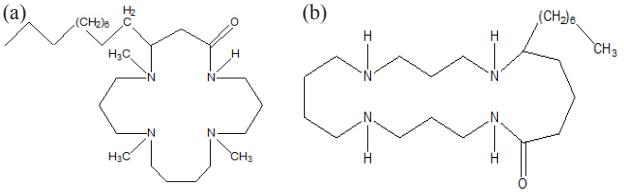

Figure 1. Chemical structures of the bioactive compounds: (a) budmunchiamine A and (b) pithecolobine

FB_1 production was confirmed by comparing with standard FB_1 on TLC plate. The isolated cultures were maintained on SDA and the seven-day-old culture was used for the assays.

Disc diffusion method

The disc diffusion method was employed for the determination of zone of inhibition (ZOI) according to the method described by Ebrahimabadi *et al.* (2010) with slight modifications. Briefly, sterile filter paper discs (6 mm in diameter) were individually impregnated with 20 µl of two-fold diluted BUA and PI (0.0095 to 1.0 mg/disc), placed onto the pre-inoculated plates (inoculum size: 100 µl of 10^4 spores/ml) and incubated at 30°C for 72 hrs. DMSO served as a negative control, and two-fold diluted carbendazim and mancozeb served as positive controls. Four replicates were maintained for each treatment. The ZOI diameters were measured in millimetres (mm).

Determination of minimum inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFCs)

The broth microdilution method was used to determine the MICs and MFCs of BUA and PI following the standard procedures with some modifications (Dung *et al*., 2008; Hajji *et al*., 2010). Briefly, 200 µl of two-fold serially diluted BUA and PI in SDB (0.0095 to 1 mg/ml) was added separately to the wells of a sterile 96-well microtiter plate and inoculated with 15 µl of fungal spore suspension containing 10^4 spores/ml and incubated at 30°C for 72 hrs. DMSO served as a negative control, and two-fold diluted carbendazim and mancozeb served as positive controls. After incubation, the MIC values of the compounds were determined by the addition of 50 µl of INT (2 mg/ml) according to the procedure of Hajji *et al.* (2010), and the MFC values were determined following the procedure of Dung *et al.* (2008). The complete absence of growth on the agar surface at the lowest concentration was defined as the MFC.

Effect of BUA and PI on the growth of F. verticillioides and FB_1 production - In vitro and In vivo

The efficacy of BUA and PI on mycelial dry

weight (MDW) losses and FB_1 production was determined *in vitro* following the method of Bailly *et al.* (2005) with some modifications. Briefly, 100 µl of a spore suspension (10^4 spores/ml) of *F. verticillioides* was inoculated into SDB/SDA, containing the requisite amount of BUA and PI (0.0312 to 2.0 mg/ml) and incubated at 28±2°C for 10 days. The culture of *F. verticillioides* along with SDA medium was used to estimate FB_1. The mycelial mat of *F. verticillioides* obtained after the filtration of the SDB media was used for the estimation of MDW losses. The efficacy of BUA and PI towards inhibition of FB_1 production was determined by TLC method. The FB_1 was visualised on eluted TLC plates by spraying with 0.5% p-anisaldehyde solution followed by heating at 110°C for 10 min. The amount of FB_1 was estimated qualitatively and quantitatively using spectrophotodensitometer (Biorad, Universal Hood II, 720BR/02170, USA) at 600 nm by comparing with different concentrations of standard FB_1.

The efficacy of BUA and PI on FB_1 production in maize seeds was determined *in vivo* following the procedures of Garcia *et al.* (2012) with minor modifications. Briefly, freshly harvested maize samples were collected and the water activity (a_w 0.95) was adjusted. The maize samples were treated with different concentrations of BUA and PI separately (0.0312 to 2.0 g/kg) and inoculated with 100 µl of a spore suspension (10^4 spores/ml) of *F. verticillioides*. All treatments were separately stored in plastic containers (200 g/pack) and incubated at 25°C up to 15 days. After incubation, the seed samples were used for FB_1 extraction and quantification following the procedure of Bailly *et al.* (2005).

Statistical analysis

Values were expressed as Mean ± standard error. Analysis of variance (ANOVA) was performed, and the differences between values were tested for significance by Tukey's multiple comparison tests employing the SPSS 20 (IBM, USA) programme. Differences at $P \leq 0.05$ were considered as statistically significant.

Results and Discussion

Mycotoxins are natural contaminants of cereals and other food commodities throughout the world and they have significant impact on human and animal health (Reddy *et al*., 2010). Fumonisins are common mycotoxins in maize, produced by *Fusarium* spp. in the field and their levels may increase during post-harvest handling and storage. Therefore to alleviate this problem, early control of fungal growth and mycotoxin production is desirable in the field (Garcia *et al*., 2012). The use of chemicals has been very effective in decreasing the incidences of yield losses in the field and during storage. However, the biggest challenge and limitations to the use of chemical fungicides are a) the toxic effects of these chemicals on human and animal health and b) acquired resistance by fungi to these chemicals in due course of time (Marei *et al.*, 2012). Hence, search for a safe but efficacious to chemical preservatives has gained attention and considerable research significance in the recent times (Reddy and Raghavender, 2007). Hence, the present study was initiated to evaluate the BUA and PI for their inhibitory activities against growth and FB_1 production by *F. verticillioides.*

Table 1. Determination of ZOI, MIC and MFC values of Budmunchiamine A, Pithecolobine, Carbendazim and Mancozeb against FB_1 producing *F. verticillioides*

Samples	ZOI (0.5 mg/disc)	MIC (mg/ml)	MFC (mg/ml)
Budmunchiamine A	10.8±0.4	0.125	0.25
Pithecolobine	10.3±0.3	0.25	0.5
Carbendazim	12.6±0.3	0.003	0.015
Mancozeb	14.3±0.3	0.5	>1.0

Data given are the mean of four replicates ± standard error ($P \leq 0.05$).

Table 2. *In-vitro* and *in-vivo* efficacies of budmunchiamine A (BUA) and pithecolobine (PI) on mycelial dry weight (MDW) and FB_1 production from *F. verticillioides*

Concentrations[a&b]	*In vitro*				*In vivo*	
	BUA		PI		BUA	PI
	MDW[c]	FB_1[d]	MDW[c]	FB_1[d]	FB_1[e]	FB_1[e]
Control	122.6±5.8	80.0	122.6±5.8	80.0	44.48	44.48
0.062	95.0±2.8	56.0	101.0±2.0	72.0	24.0	40.0
0.125	84.0±2.3	20.4	89.6±1.2	40.0	9.69	21.6
0.25	32.3±1.2	0	45.3±1.4	14.8	0	8.8
0.5	0	0	10.6±0.6	0	0	0
1.0	0	0	0	0	0	0

[a] *in vitro* treatment concentration (mg/ml); [b] *in vivo* treatment concentration (g/kg); [c] MDW (mg); [d] FB_1 (mg/l); [e] FB_1 (mg/kg); aqueous methanol (1:0.01 v/v) served as a negative control; Data given are the mean of four replicates ± standard error ($P \leq 0.05$).

The results of the present study implicate the strong inhibitory effect of both BUA and PI against *F. verticillioides* (Table 1). It was observed that the ZOI, MIC and MFC values ranged from 10.3−10.8 mm, 0.125−0.25 mg/ml and 0.25−0.5 mg/ml, respectively. The negative control, DMSO, did not show any inhibitory activity. The synthetic fungicide mancozeb exhibited the lowest MIC, but there was no corresponding MFC value, suggesting that it has only fungistatic activity, whereas BUA and PI showed concentration-dependent fungistatic as well as fungicidal activities which are comparable to synthetic fungicide carbendazim. The order of inhibitory activity was carbendazim>budmunchiamine A>pithecolobine>mancozeb.

The MDW of *F. verticillioides* and FB_1 production was strongly inhibited by BUA and PI both *in vitro* and *in vivo*. The decline in mycelial growth and FB_1 production was found to be a dose dependent (Table 2). The growth of *F. verticillioides* was completely

inhibited by BUA and PI at 1.0 mg/ml both *in vitro* and *in vivo*. It was observed that, in the BUA-treated group, there was a complete inhibition of FB_1 production at 0.25 mg/ml (*in vitro*) and 0.25 g/kg (*in vivo*). Similarly, PI completely inhibited FB_1 production at 0.5 mg/ml (*in vitro*) and 0.5 g/kg (*in vivo*). Of the two compounds studied, BUA showed highest FB_1 inhibitory activity than PI.

The *A. amara* and *A. saman* species are globally distributed throughout the tropical regions, and are widely used as folk remedy for curing various diseases (Ayyanar and Ignacimuthu, 2005; Prasad *et al*., 2008; Kareru *et al*., 2008). The antimicrobial activities of crude aqueous and solvent extracts of *A. amara* and *A. saman* have been reported against human and plant pathogenic microbes (Kareru *et al.*, 2008; Raghavendra *et al*., 2008; Prasad *et al*., 2008; Azhar *et al*., 2009; Nnamdi *et al*., 2010; Arulpriya *et al.*, 2010; Ferdous *et al*., 2010; Karmegam *et al*., 2012; Ajam *et al*., 2012). Previous reports from the laboratory indicated the antimicrobial efficacies of crude extracts of *A. amara* and *A. saman* against pathogenic bacteria and fungi (Thippeswamy *et al.*, 2011; Praveen *et al.*, 2011). *Samanea saman* (synonym - *Albizia saman*) has been reported to have cytotoxic, antioxidant, weedicidal, insecticidal and antiulcer activities (Azhar *et al.*, 2009; Ferdous *et al.*, 2010; Arumugam *et al*., 2011). The antioxidant, anti-dandruff, anti-inflammatory and analgesic activities have been reported from *A. amara* (Mar *et al.*, 1991; Muchuweti *et al*., 2006; Kumar *et al*., 2008; Kumar *et al*., 2010; Khan *et al*., 2010). Other earlier reports on *A. amara* and *A. saman* revealed the presence of a group of budmunchiamines in *A. amara* and pithecolobine in *A. saman* as the main alkaloid constituents (Wiesner *et al.*, 1952 and 1968; Pezzuto *et al*., 1991 and 1992; Rajkumar and Sinha, 2010; Ajam *et al.*, 2012). Ajam *et al*. (2012) reported the antimicrobial activity of pithecolobine of *S. saman* against a Gram positive *B. subtilis* and four phytopathogenic fungi viz., *Aspergillus flavus*, *A. niger*, *Cladosporium oxysporum* and *Penicillium oxalicum*. However, there are no reports pertaining to the inhibitory effects of BUA and PI against growth and FB_1 production by *F. verticillioides*. To the best of knowledge, the current investigation is the first of its kind which reports the inhibitory effects of BUA and PI against growth and FB_1 production by *F. verticillioides*.

Conclusion

The results of the present study showed that the budmunchiamine A and pithecolobine are potential natural compounds with strong inhibitory activity against *F. verticillioides* growth and FB_1 production. Hence, these findings indicate the possible use of BUA and PI as potential alternatives to chemical preservatives for the management of pre- and post-harvest fungal infestations and fumonisin B_1 contaminations in food grains. However, detailed studies are required to investigate the safety and toxicity of these compounds on suitable model system.

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References

Ajam, S.M.S., Salleh, B., Al-khalil, S. and Sulaiman, S.F. 2012. Antimicrobial activity of spermine alkaloids from Samanea saman against microbes associated with sick buildings. International Conference on Environment, Chemistry and Biology 49: 150-155.

Arulpriya, P., Lalitha, P. and Hemalatha, S. 2010. *In vitro* antioxidant testing of the extracts of *Samanea saman* (Jacq.) Merr. Der Chemica Sinica 1: 73-79.

Arumugam, S., Selvaraj, S.V., Velayutham, S., Natesan, S.K. and Palaniswamy, K. 2011. Evaluation of anti-ulcer activity of *Samanea saman* (Jacq) merr. bark on ethanol and stress induced gastric lesions in albino rats. Indian Journal of Pharmacology 43: 586-590.

Ayyanar, M. and Ignacimuthu, S. 2005. Medicinal plants used by the tribals of Tirunelveli hills, Tamil Nadu to treat poisonous bites and skin diseases. Indian Journal of Traditional Knowledge 4: 229-236

Azhar, I., Hasan, M.M., Mazhar, F. and Ali, M.S. 2009. Some biological evaluations on *Samanea saman*. Pakistan Journal of Pharmacology 26: 47-53.

Bailly, J.D., Querin, A., Tardieu, D. and Guerre P. 2005. Production and purification of fumonisins from a highly toxigenic *Fusarium verticillioides* strain. Revue De Medicine Veterinaire 156 (11): 547-554.

Bankole, S.A. and Adebanjo, A. 2003. Mycotoxins in food in West Africa: current situation and possibilities of controlling it. African Journal of Biotechnology 2 (9): 254-263.

Covarelli, L., Beccari, G. and Salvi, S. 2011. Infection by mycotoxigenic fungal species and mycotoxin contamination of maize grain in Umbria, central Italy. Food and Chemical Toxicology 49: 2365-2369.

Domijan, A.M., Zeljezic, D., Peraica, M., Kovacevic, G., Gregorovic, G., Krstanac, Z., Horvatin, K. and

Kalafatic, M. 2008. Early toxic effects of fumonisin B1 in rat liver. Human and Experimental Toxicology 27: 895-900.

Dung, N.T., Kim, J.M. and Kang, S.C. 2008. Chemical composition, antimicrobial and antioxidant activities of the essential oil and the ethanol extract of *Cleistocalyx operculatus* (Roxb.) Merr and Perry buds. Food and Chemical Toxicology 46: 3632-3639.

Ebrahimabadi, A.H., Ebrahimabadi, E.H., Bidgoli, Z.D., Kashi, F.J., Mazoochi, A. and Batooli, H. 2010. Composition and antioxidant and antimicrobial activity of the essential oil and extracts of Stachys inflata Benth from Iran. Food Chemistry 119: 452-458.

Fandohan, P., Hell, K., Marasas, W.F.O. and Wingfield, M.J. 2003. Infection of maize by *Fusarium* species and contamination with fumonisin in Africa. African Journal of Biotechnology 2 (12): 570-579.

Ferdous, A., Imam, M.Z. and Ahmed, T. 2010. Antioxidant, antimicrobial and cytotoxic activities of *Samanea saman* (Jacq.) Merr. Stamford Journal of Pharmaceutical Sciences 3: 11-17

Garcia, D., Ramos, A.J, Sanchis, V. and Marin, S. 2012. Effect of *Equisetum arvense* and *Stevia rebaudiana* extracts on growth and mycotoxin production by *Aspergillus flavus* and *Fusarium verticillioides* in maize seeds as affected by water activity. International Journal of Food Microbiology 153: 21-27

Hajji, M., Masmoudi, O., Souissi, N., Triki, Y., Kammoun, S. and Nasri, M. 2010. Chemical composition, angiotensin I-converting enzyme (ACE) inhibitory, antioxidant and antimicrobial activities of the essential oil from *Periploca laevigata* root barks. Food Chemistry 121: 724-731.

Harborne, J.B. 1998. Phytochemical methods: a guide to modern techniques of plant analysis (3rd ed.). Chapman & Hall Publication, London, UK.

Jouany, J.P. 2007. Methods for preventing, decontaminating and minimizing the toxicity of mycotoxins in feeds. Animal Feed Science and Technology 137: 342-362.

Kareru, P.G., Gachanja, A.N., Keriko, J.M. and Kenji, G.M. 2008. Antimicrobial activity of some medicinal plants used by herbalists in eastern province, Kenya. African Journal of Traditional, Complementary and Alternative Medicines 5: 51-55.

Karmegam, N., Jayakumar, M. and Karuppuswamy, S. 2012. Synergistic antibacterial activity of four medicinal plants collected from Dharapuram Taluk of Tiruppur District, south India. Journal of Plant Sciences 7: 32-38.

Kumar, P.S., Sucheta, S., Deepa, V.S., Selvamani, P. and Latha, S. 2008. Antioxidant activity in some selected Indian medicinal plants. African Journal of Biotechnology 7: 1826-1828.

Kumar, P.S., Sucheta, S., Umamaheswari, A. and Deepa, V.S. 2010. *In vitro* and *in vivo* evaluation of anti-dandruff activity of formulated polyherbal hair oil. Journal of Pharmacy Research 3: 2956-2958.

Mar, W., Tan, G.T., Cordell, G.A. and Pezzuto, J.M. 1991. Biological activity of novel macrocyclic alkaloids (Budmunchiamines) from *Albizia amara* detected on the basis of interaction with DNA. Journal of Natural Products 54: 1531-42.

Marei, G., Rasoul, M. and Abdelgaleil, S., 2012. Comparative antifungal activities and biochemical effects of monoterpenes on plant pathogenic fungi. Pesticide Biochemistry and Physiology 103: 56-61.

Muchuweti, M., Nyamukonda, L., Chagonda, S., Ndhlala, A.R., Mupure, C. and Benhura, M. 2006. Total phenolic content and antioxidant activity in selected medicinal plants of Zimbabwe. International Journal of Food Science and Technology 41: 33-38.

Nagamani, A., Kunwar, I.K. and Manoharachary, C. 2006. Handbook of soil fungi (1st ed.). I.K International Pvt. Ltd., New Delhi.

Nnamdi, L.O., Anthony, C.C.E., Pius, O.U. and Paul, M.E. 2010. Comparative phytochemical and antimicrobial screening of some solvent extracts of *Samanea saman* (*Fabaceae* or *Mimosaceae*) pods. African Journal of Pure and Applied Chemistry 4: 206-212.

Pezzuto, J.M., Mar, W., Lin, L.Z. and Cordell, G.A. 1991. DNA-based isolation and the structure elucidation of Budmunchiamines, novel macrocyclic alkaloids from *Albizia amara.* Heterocycles 32: 1961-1967.

Pezzuto, J.M., Mar, W., Lin, L.Z., Cordell, G.A., Neszmelyi, A. and Wagner, H. 1992. Budmunchiamines D-I from *Albizia amara.* Phytochemistry 31: 1795-1800.

Prasad, R.N., Viswanathan, S., Devi, J.R., Nayak, V., Swetha, V.C., Archana, B.R., Parathasarathy, N. and Rajkumar, J. 2008. Preliminary phytochemical screening and antimicrobial activity of *Samanea saman*. Journal of Medicinal Plants Research 2: 268-270.

Praveen, P., Thippeswamy, S., Mohana, D.C. and Manjunath, K. 2011. Antimicrobial efficacy and phytochemical analysis of *Albizia amara* (Roxb.) Boiv. an indigenous medicinal plant against some human and plant pathogenic bacteria and fungi. Journal of Pharmacy Research 4: 832-835.

Raghavendra, M.P., Satish, S. and Raveesha, K.A. 2008. *In vitro* antibacterial potential of alkaloids of *Samanea saman* (Jacq.) Merr. against *Xanthomonas* and human pathogenic bacteria. World Journal of Agricultural Sciences 4: 100-105.

Rajkumar, T. and Sinha, B.N. 2010. Chromatographic finger print analysis of Budmunchiamines in *Albizia amara* by HPTLC technique. International Journal of Research in Pharmaceutical Sciences 1: 313-316.

Reddy, B.N. and Raghavender, C.R. 2007. Outbreaks of aflatoxicoses in India. African Journal of Food, Agriculture, Nutrition and Development 7 (5): 1-15.

Reddy, K.R.N., Raghavender, C.R., Reddy, B.N. and Salleh, B. 2010. Biological control of *Aspergillus flavus* growth and subsequent aflatoxin B_1 production in sorghum grains. African Journal of Biotechnology 9 (27): 4247-4250.

Shim W. and Woloshuk, C.P. 2001. Regulation of Fumonisin B1 Biosynthesis and Conidiation in *Fusarium verticillioides* by a Cyclin-Like (C-Type) Gene, FCC1. Applied and Environmental Microbiology,

1607-1612.

Thippeswamy, S., Mohana, D.C., Abhishek, R.U. and Manjunath, K. 2013. Efficacy of bioactive compounds isolated from *Albizia amara* and *Albizia saman* as source of antifungal and antiaflatoxigenic agents. Journal of Consumer Protection and Food Safety, 297-305.

Thippeswamy, S., Praveen, P., Mohana, D.C. and Manjunath, K. 2011. Antimicrobial evaluation and phytochemical analysis of known medicinal plant *Samanea saman* (Jacq.) Merr. against some human and plant pathogenic bacteria and fungi. International Journal of Pharma and Bio Sciences 2: 443-452

Watanabe, T. 2002. Pictorial atlas of soil and seed fungi: morphologies of cultured fungi and key to species (2nd ed.). CRC Press LLC, 2000 N. W. Corporate Blvd., Boca Raton, Florida.

Wiesner, K., MacDonald, D.M., Valenta, Z. and Armstrong, R. 1952. Pithecolobine, the alkaloid of *Pithecolobium saman* Benth. I. Canadian Journal of Chemistry 30: 761-772

Wiesner, K., Valenta, Z., Orr, D.E., Liede, V. and Kohan, G. 1968. Structure of Pithecolobine. III. The synthesis of the 1,5- and 1,3-desoxypithecolobines. Canadian Journal of Chemistry 46: 3617-3624.

Yassin, M.A., Moslem, M.A., El-Samawaty, A.E.M.A. 2012. Mycotoxins and non-fungicidal control of corn grain rotting fungi. Journal of plant sciences 7(3): 96-104.

Yazar, S. and Omurtag, G.Z. 2008. Fumonisins, Trichothecenes and Zearalenone in Cereals. International Journal of Molecular Sciences 9: 2062-2090.