Pak. J. Bot., 39(1): 211-221, 2007.

SCREENING OF SOME MEDICINAL PLANTS FOR ISOLATION OF ANTIFUNGAL PROTEINS AND PEPTIDES

[*]AMER JAMIL, MUHAMMAD SHAHID, [1]M. MASUD-UL-HAQ KHAN AND [2]MUHAMMAD ASHRAF

Molecular Biochemistry Lab., Department of Chemistry,
University of Agriculture, Faisalabad-38040, Pakistan
[1]Department of Chemistry, University of Education, Lahore, Pakistan.
[2]Department of Botany, University of Agriculture, Faisalabad-38040, Pakistan
[*]Corresponding author: amerjamil@yahoo.com

Abstract

About three quarters of the world population rely mainly on plants and plant extracts for health care. The global annual sale of botanicals is over $ 62 billion. This data clearly depict the importance of medicinal plants in health and economy. The extracts of some potential medicinal plants such as *Hygrophila auriculata*, *Abrus precatorius*, *Moringa oleifera*, *Withania somnifera*, *Croton tiglium*, *Solanum nigrum* and *Psoralea corylifolia* were investigated against pathogenic fungal strains of *Aspergillus tamarii, Rhizopus solani, Mucor mucedo* and *Aspergillus niger*. After extraction the extracts were purified by ammonium sulphate precipitation followed by gel filtration chromatography (Sephadex G-100) by using Tris HCl as an extraction buffer. Antifungal activity of the extracts was determined by disc diffusion assay. Antifungal activity was found lost in many extracts after treatment with trypsin, which shows that the activity was due to proteins or peptides, but not due to some other compounds.

Introduction

Extracts of many plants are highly efficient against parasitic as well as microbial infections. It is estimated that around 70,000 plant species, from lichens to tall trees, have been used at one time to other for medicinal purposes (Purohit & Vyas, 2004). The use of different parts of several medicinal plants to cure specific ailments has been in vogue from ancient times. The indigenous system of medicine namely Ayurvedic, Siddha and Unani have been in existence for several centuries. This system of medicine caters to the needs of nearly 70% of the population residing in villages. Besides the demands made by these systems as their raw material, the demands for medicinal plants made by the modern pharmaceutical industries has also increased manifold (Gupta *et al.*, 1999; Ashraf & Orooj, 2006; Ashraf *et al*., 2006).

The medicinal plants are the plants whose parts (leaves, seeds, stem, roots, fruits, foliage etc.), extracts, infusions, decoctions, powders are used in the treatment of different diseases of humans, plants and animals (Nostro *et al.,* 2000). The medicinal plants occupy a significant place in modern medicine as a raw material for some important drugs, although synthetic drugs and antibiotics brought about a revolution in controlling different diseases. But these synthetic drugs are out of reach to millions of people. Those who live in remote places depend on traditional healers, whom they know and trust. The judicious use of medicinal herbs can even cure deadly diseases that have long defied synthetic drugs (Bhattacharjee, 2001).

The fungi are eukaryotic, achlorophyllous and heterotrophic thallophytes comprising about 1.5 million species, of which only 74,000 species are described (Hawksworth, 2001) and more than 300 species are potentially pathogenic or cause allergy symptoms in man (Gupta *et al.,* 2002). Many species of fungi develop their association with plants and animals as parasites, saprophytes, symbionts, lichens and micorrhiza. The parasitic fungi cause serious diseases of useful and non-useful plants. The useful plants attacked by fungi include crop plants like wheat, rice, maize, barley, oat, cruciferous plants, potatoes, tomatoes and other fruit plants. So, fungi are regarded as the chief causative agents of plant pathology (Campbell *et al*., 2000). Similarly, man and other mammalians, fishes, amphibians and reptiles are also susceptible to fungal infections (Alexopoulos & Mims, 1979; Dube, 1990). New antimycotics are badly needed and the world market for antifungals is expanding dramatically (Pocsi *et al.,* 2001).

Many low molecular mass proteins or peptides with antibacterial or antifungal activity have been isolated in recent years from various plants (Osborn *et al.*, 1995; Huynh *et al*., 2001; Ye & Ng, 2002), and are believed to be involved in a defense mechanism against fungi. Most of these gene-encoded peptides are mobilized shortly after microbial infection to neutralize a broad range of microbes (Reddy *et al.,* 2004).
Many of the antibiotics and other synthetic drugs show sensitization reactions and other undesirable side effects, and there is a feeling that the herbal drugs are relatively more safe than others of multifarious nature. Microorganisms develop resistance to the synthetic drugs, antifungal and antibiotics. Another discrepancy of the synthetic drugs and antibiotics is that they may also make interactions with the body system to disturb the metabolic processes. Scientists therefore, are working on the extraction of anti-infectional compounds including antifungal peptides/proteins from natural sources like plants and animals. The anti-infectional compounds show broad spectrum bioactivity against infection-causing agents such as bacteria, fungi, protocists, protozoans, viruses, yeasts etc, (Lehrer *et al.,* 1991; Hancock, 1997; Conlon *et al*., 2003).

During the past 10-15 years, interest in their antimicrobial nature has expanded due to their increased resistance of fungal pathogens to antifungal drugs being currently used, and the toxicity or adverse host reactions of other anti-infectives. The antimicrobial peptides are very effective against microbes. They show their activity by lysis, by binding to, and disruption of the outer membrane of microbes. Others penetrate the membrane and interact with specific internal targets or cause pore formation resulting in leakage of important intracellular contents (Haung *et al*., 2000).

Keeping in view the importance of the subject, proteins and peptides from some local potential medicinal plants were isolated and examined for their antifungal activity.

Materials and Methods

The research work was conducted in the Molecular Biochemistry Lab., University of Agriculture, Faisalabad.

1. Medicinal plants: Leaves and seeds of the following medicinal plants were screened for the study: *Hygrophila auriculata* (Talmakhana), *Abrus precatorius* (Kaincha), *Moringa oleifera* (Sohanjna), *Croton tiglium* (Jamal Gota), *Withania somnifera* (Ashwaganda), *Solanum nigrum (*Mako), *Psoralea corylifolia* (Babchi).

2. Preparation of crude extract: Leaves of the medicinal plants were collected from the Botanical Garden, University of Agriculture, Faisalabad and the seeds were purchased from the local market. The leaves and seeds were freed from dirt by washing with distilled water. The moisture content was removed by air drying. After excising, the leaves were mixed with 10 m*M* sodium acetate buffer (Terras *et al*., 1993). The seed (after milling) extracts were prepared in a solution mixture of 10 m*M* Na_2HPO_4, 15 m*M* NaH_2PO_4, 100 m*M* KCl, 2 m*M* EDTA, 1.5% polyvinylpoly pyrrolidone (PVPP), 1m*M* phenyl methyl sulfonylfloride (PMSF) and 2 m*M* thiourea. The leaf and seed mixtures were centrifuged at 12,000 x*g* at 4^oC for 20 min. The supernatant was filtered to remove debris present in the supernatant. The filtrates were stored at -20^oC in 100 mL sterilized bottles (Terras *et al*., 1992).

3. Treatment of extract with trypsin: The crude extracts (2 mL) were treated with trypsin (Sigma Aldrich) to final concentration of 0.5 mg /mL. Samples were incubated at 37^oC for 2 hours followed by incubation at 100^oC for 5 minutes. The samples were subjected to antifungal assay.

4. Partial purification: Partial purification of the extracts was performed at the 80% saturation level of ammonium sulphate. After precipitation, the extracts were centrifuged at 12,000 x*g* and 4^oC, and the supernatants were dialyzed (Huynh *et al.*, 2001).

5. Purification by gel filtration: The extracts were further purified by gel filtration. For this purpose Sephadex G-100 gel column with10 m*M* Tris HCl buffer was used (Deutscher, 1990).

6. Protein contents determination: The protein contents of crude, supernatants and dialyzed samples of leaves and seeds were determined spectrophotometrically by Bradford method (Bradford, 1976) using bovine serum albumin (BSA) as standard.

7. Fungal strains: Following fungal strains were used during the study: *Aspergillus niger*, *Mucor mucedo*, *Aspergillus tamarii* and *Rhizopus solani*. The fungal strains were characterized by the Department of Veterinary Microbiology, University of Agriculture, Faisalabad.

The fungal strains were grown on Sabouraud's glucose agar medium (glucose 40 g/L, agar 20 g/L, peptone 10 g/L). The pH of medium was adjusted to 5.4. It was autoclaved at 121^oC for 15 minutes at 15 psi pressure and transferred to autoclaved test tubes and set to slanting positions and allowed the medium to be solidified (Cruickshank *et al*., 1975). The solidified slants were kept in the incubator at 32^oC for 48 h as a check for contamination. The fungal strains were cultured to the slants in the sterilized laminar air flow from the pure culture. These cultured slants were incubated at 32^oC for 4-5 days for multiplication of fungal strains. The inocula of fungal strains were prepared by pressurized addition of 1% glucose solution to the slants with the help of a sterilized syringe. The spores were mixed with glucose solution with the help of a sterilized glass rod to form fine spore suspensions that were used for further growth.

Table 1. Expression of results for antifungal assay.

S. No.	Mathematical sign	Zone size (mm)	Interpretation
1.	–	0	No or poor antifungal activity
2.	+	6	Moderate activity
3.	++	6–9	Strong activity
4.	+++	9–12	Very strong activity

8. Antifungal assay: The sterilized growth medium was transferred to autoclaved Petri plates (about 20-25 mL medium per Petri plate). Before the transfer of fungal culture to the Petri plates, chloramphenicol solution (40 mg/L; 100 uL per 30 mL) was added to the growth medium to make it selective for fungal growth (Emmons *et al*., 1970). The inoculum, 200 uL per 30 mL of growth medium, was administered. The Petri plates were incubated at 32^oC for 48 h, for fungal growth. After this, filter paper discs were laid flat on the growth medium having fungal growth. The extract (100 uL) was put on each disc. The Petri plates were then again incubated at 32^oC for 48 hours, for the growth of fungus. The extracts having antifungal activity inhibited the fungal growth, and exhibited clear zones around the discs. The zones of inhibition were measured in millimeters using a zone reader (Haung *et al*., 2000; Huynh *et al*., 2001; Rehman *et al.,* 2001).

Results and Discussion

Selected medicinal plants were screened for antifungal activity. The plants with a high antifungal activity were subjected to partial purification of the compounds. The antifungal activity was determined in terms of zones of inhibition (Table 1).

1. Antimicrobial assay of crude extracts: The results indicate that some plant extracts had a broad spectrum of activity by forming clear zones of inhibition, while others had negligible zones of inhibition and had very poor activity against the strains. Negative results of some plants indicate that the plants had no active compound or if present it had either very low concentration or it might have lost its activity. From the literature it can be deciphered that there are certain cases wherein the isolation of the peptides was not effective, and the activity shown was due to the presence of certain other active compounds (Harborne, 1992).

2. Antifungal activity: Most of the crude extracts and dialysed samples were found exhibiting antifungal activity (Tables 2 and 3). Supernatants of some plants also showed fungal inhibition. Antifungal activity of crude extracts and supernatants may have been due to the presence of lipophilic compounds that may bind within or internal to the cytoplasmic membrane (Boyd & Beveridge, 1979; 1981), quinines (Mahoney *et al*., 2000) or thionine, which affect growth of filamentous fungi mainly by causing membrane permeablization (Huang *et al.,* 2001). The selected plants showed antifungal activiy against the *Mucor mucedo and Aspergillus niger* more strongly than for *Aspergillus tamarii* and *Rhizopus solani*.

Nwosu & Okafor (1995) tested the antifungal activities of the extracts of 10 medicinal plants including *Moringa oleifera* against pathogenic fungi using the broth dilution and agar diffusion methods. All the extracts inhibited the growth of *Basidiobolus haptosporus* and *B. ranarum*, but did not inhibit that of *Asperigillus fumigatus* and *Candida albicans*. Das *et al* (1997) evaluated the antifungal activity of phyto-extracts of eight plants including *Moringa oleifera.* Plant extracts at 10% concentration showed a maximum antifungal activity.

Table 2. Average antifungal activity of leaf extracts of medicinal plants against selected fungal strains.

Medicinal plants	*Aspergillus tamarii*	*Rhizopus solani*	*Mucor mucedo*	*Aspergillus niger*
Hygrophila auriculata	–	–	++	++
Abrus precatorius	+	–	–	+++
Moringa oleifera	+	+	++	++
Withania somnifera	+	–	+++	++
Croton tiglium	+	–	+++	++
Solanum nigrum	++	+	+++	++
Psoralea corylifolia	–	+	++	++

Table 3. Average antifungal activity of seed extracts of medicinal plants against selected fungal strains.

Medicinal plants	*Aspergillus tamarii*	*Rhizopus solani*	*Mucor mucedo*	*Aspergillus niger*
Hygrophila auriculata	+	++	+	++
Abrus precatorius	+	+	+	+
Moringa oleifera	+	+	+	+
Withania somnifera	+	+	++	++
Croton tiglium	+	++	++	++
Solanum nigrum	++	++	++	++
Psoralea corylifolia	++	++	++	++

During the present study, the extracts from the young parts of plants exhibited antimicrobial activity greater than those from old parts. The reason behind this point is that newly developed plant organs, at the start, are more susceptible to microbial attack. And at a later stage, these parts naturally get enriched with bioactive compounds to overcome this kind of problem. Because of their stronger innate immunity, young plant organs are protected against microbial attack. Some previous reports confirm this statement (Alwadi & Baka, 2001). This is because of the specificity of microbial compounds to various parts of plants i.e., bioactive compounds may be specific to only roots, stem, flowers, fruits, leaves, seeds or bark. Huynh *et al.*, (2001) have reported the specificity of antifungal compounds to different parts of plant, *Engelmannia pinnatifida.*

Some previous studies revealed a strong antimicrobial activity of tested medicinal plant species, but no report relating to their antimicrobial activity against tested fungal strains came to sight. Antimicrobial activity (Reda *et al.,* 1990; Dimetry *et al.,* 1992; Agina *et al.,* 2000; Molgaard *et al.,* 2001), uterotonic and antidiarrhoel activity (Nwodo, 1991; Nwodo & Alumanah, 1991), teratogenic activity (Sethi, 1991), gastroentrital activity (Brito *et al.,* 1996) and antiplatelet, anti-inflammatory and antiallergic activity (Kuo *et al.,* 1995; Anam, 2001) of *Abrus precatorius* have been reported. Similarly, antimicrobial activity (Nwosu & Okafor, 1995; Das *et al.,* 1997), anti-inflammatory activity (Ezeamuzie *et al.,* 1996), hypotensive activity (Faizi *et al.,* 1998) and antihepatotoxic properties (Ruckmani *et al.,* 1998) of *Moringa oleifera* have also been reported. Previous reports also reveal antimicrobial activity (Dahot *et al.,* 1997) and anticancerous properties (Negi *et al.,* 2000) of *Withania somnifera;* HIV inhibitory activities (Mekkawy *et al.,* 1999) of *Croton tiglium;* anticarcinogenic activity (Aruna & Sivaramakrishnan, 1992) and insecticidal properties (Niber, 1994) of *Solanum nigrum* and antimicrobial activity (Katsura *et al.,* 2001) of *Psoralea corylifolia.*

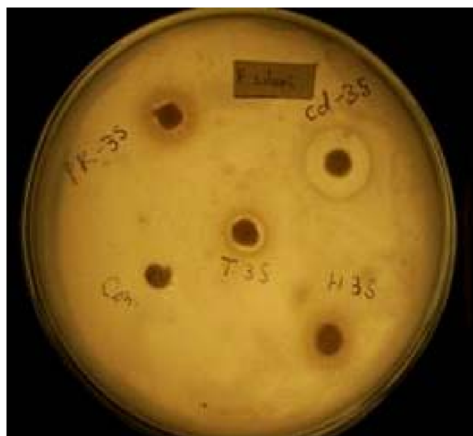

Fig. 1A. Antifungal activity of crude extracts (cd) and trypsin treated extract from seeds of *Moringa oleifera* (3S) against *Fusarium solani*.

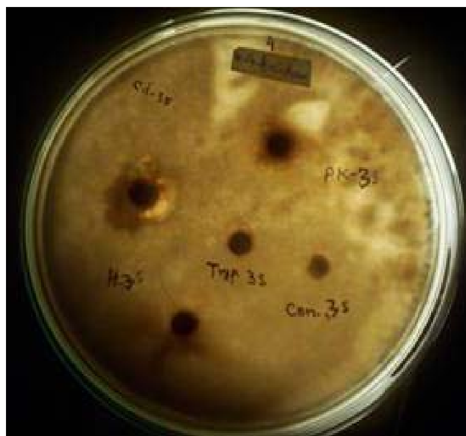

Fig. 1B. Antifungal activity of crude extracts (cd) and trypsin treated extract (Tryp) from seeds of *Moringa oleifera* (3S) against *Ganoderma lucidum*.

Fig. 2A. Antifungal activity of crude extracts (cd), and trypsin treated extract (Tryp) from seeds of *Psoralea corylifolia* (7S) against *Fusarium solani*.

Fig. 2B. Antifungal activity of crude extracts (cd) and trypsin treated extract (Tryp) from seeds of *Psoralea corylifolia* (7S) against *Metarhizium aniscoplea*.

Tang *et al.*, (1993) purified croton I and II from *Croton tiglium,* having Mr 40 kDa and 19 kDa, respectively. Terras *et al.*, (1992) isolated an antifungal protein from the seeds of 4 Brassica species. Dahot *et al*., (1997) separated 7 and 14 peptides on Sephadex G-25 from acetone and ethanol prepared samples of *Moringa oleifera.* Ye & Ng (2002) isolated a variety of antifungal proteins from some leguminous plants.

3. Effect of trypsin treatment on antifungal activity: Trypsin treatment reduced the antifungal activity in many cases. Significant results were achieved for *Moringa oleifera* against *Fusarium solani* and *Ganoderma lucidum* (Fig. 1A and 1B). Big zones of inhibition against crude extract clearly demonstrate high antifungal activity. The activity was reduced to a significant level in the treated extracts. This shows that the antifungal activity was due to the proteins present in the extract, and not due to any other compounds.

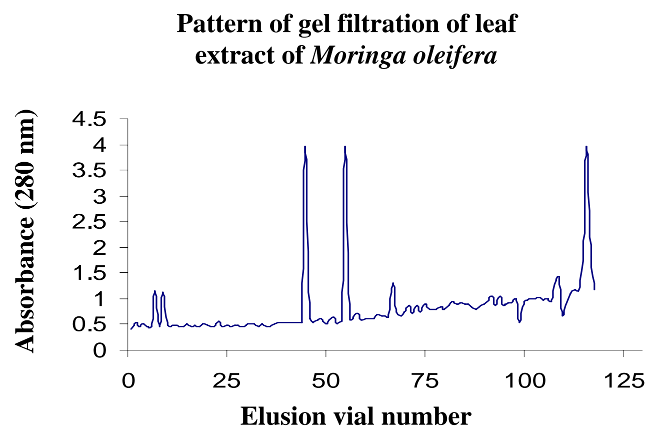

Fig. 3. Representative chromatogram obtained after gel filtration of plant extracts.

Similarly, *Psoralea corylifolia* also gave significant results against *Fusarium solani* and *Metarhizium aniscoplea* (Fig. 2A and 2B). This also demonstrates that the antifungal activity was mainly due to proteins or peptides, and not because of some other compounds. Similarly, crude and treated extracts from different plants exhibited different levels of antifungal activity.

4. Antifungal assay of gel filtration fractions: Fig. 3 shows a representative chromatogram of the sample applied to Sephadex-G100. Samples within individual peaks were pooled together and subjected to antifungal assay. As shown in Tables 4 and 5, a significant activity was observed in many fractions of seed extracts of the plants. However, the activity was low in case of leaf extracts.

Antibacterial activity of the gel filtration extracts also showed positive results (data not shown). High antibacterial activity was obtained before the gel filtration. The reason may also be that the antibacterial activity of plant extracts before gel filtration was due to the presence of bioactive compounds.

5. Antifungal assay of gel filtration fractions after ammonium sulphate precipitation: All medicinal plants leaf and seed dialyzed samples after ammonium sulphate precipitation were run on gel filtration and the selected fractions were tested for antifungal activity. The antifungal activity of fractions from ammonium sulphate dialyzed was greater than those from crude extracts. Their greater bioactivity might have been as a result of purification and higher concentration of bioactive protein /peptide. Fractions from *Psoralea corylifolia* and *Solanum nigrum* had maximum antifungal activity while those from *Withania somnifera* exhibited no activity against *A. niger, A. tamarii* and *R. solani*. Fractions from extracts of all the remaining plants demonstrated a pronounced antifungal activity against all the fungal strains examined. Average antifungal activity of ammonium sulphate dialysed and gel filtrate fractions of leaf and seed extracts are presented in Tables 6 and 7. On the other hand, there were only a few fractions in the leaves of selected plants exhibiting the antifungal activity. The literature also supports this finding that many of antimicrobial proteins/peptides were reported from plant seeds (Chrisrtenes *et al*., 2002; Thevissen *et al*., 2003; Peter *et al*., 2004; Rengente *et al*., 2005).

Table 4. Average antifungal activity of gel filtration fractions of leaf extracts of medicinal plants against selected fungal strains.

Medicinal plants	*Aspergillus tamarii*	*Rhizopus solani*	*Mucor mucedo*	*Aspergillus niger*
Hygrophila auriculata	–	–	++	+++
Abrus precatorius	++	++	–	–
Moringa oleifera	–	+	–	+
Withania somnifera	–	–	+	–
Croton tiglium	+	–	–	+
Solanum nigrum	–	+	+	+
Psoralea corylifolia	+	–	+	+

Table 5. Average antifungal activity of gel filtration fractions of seed extracts of medicinal plants against selected fungal strains.

Medicinal plants	*Aspergillus tamarii*	*Rhizopus solani*	*Mucor mucedo*	*Aspergillus niger*
Hygrophila auriculata	++	++	++	+++
Abrus precatorius	++	++	+++	+++
Moringa oleifera	+++	++	++	+++
Withania somnifera	–	–	++	–
Croton tiglium	++	+++	+++	++
Solanum nigrum	+++	+++	+++	+++
Psoralea corylifolia	+++	+++	+++	+++

Table 6. Average antifungal activity of leaf extracts of medicinal plants after ammonium sulphate precipitation, dialysed and gel filtration.

Medicinal plants	*Aspergillus tamarii*	*Rhizopus solani*	*Mucor mucedo*	*Aspergillus niger*
Hygrophila auriculata	–	–	+	+++
Abrus precatorius	–	–	–	+
Moringa oleifera	–	–	–	+
Withania somnifera	+	–	+	+
Croton tiglium	–	–	+	+
Solanum nigrum	–	–	+	++
Psoralea corylifolia	–	–	+	+++

Table 7. Average antifungal activity of seed extracts of medicinal plants after ammonium sulphate precipitation, dialysis and gel filtration.

Medicinal plants	*Aspergillus tamarii*	*Rhizopus solani*	*Mucor mucedo*	*Aspergillus niger*
Hygrophila auriculata	++	++	++	+++
Abrus precatorius	++	++	++	++
Moringa oleifera	+++	++	++	+++
Withania somnifera	++	+	+++	++
Croton tiglium	++	+++	++	+++
Solanum nigrum	+++	+++	+++	+++
Psoralea corylifolia	+++	+++	+++	+++

Conclusion

The present findings show that various plant extracts had a high antifungal activity due to proteins or peptides. Partial purification of the antifungal proteins and peptides also demonstrated the presence of such peptides and proteins in the leaf and seed extracts of the plants tested in the project. Further research work is underway to purify and characterize the antifungal proteins/peptides from the plants.

Acknowledgement

The research work reported in the paper was conducted under a research grant from Higher Education Commission, Government of Pakistan.

References

Agina, S. E., A.O. Olaolu and H. Husaini. 2000. Antibacterial effect of the ethanolic testa extracts of seeds of some leguminous plants. *Legume-Research*, 23: 97-101.

Alexopoulos, C.J. and C.W. Mims. 1979. Introductory Mycology. 3rd ed. John Wiley and Sons, New York, pp. 390-409.

Alwadi, H.N. and Z.A.M. Baka. 2001. Microorganisms associated with *Withania somnifera* leaves. *Microbiological Research*, 156: 303-309.

Anam, L. 2001. Anti inflammatory activity of compounds isolated from the aerial parts of *Abrus precatorius* (*Fabaceae*). *Phytomedicine*, 8: 24-27.

Aruna, K. and V.M. Sivaramakrishnan. 1992. Anticarcinogenic effects of some Indian plant products. *Food and Chemical Toxicology*, 30: 953-956.

Ashraf, M. and A. Orooj. 2006. Salt stress effects on growth, ion accumulation and seed oil content in an arid zone medicinal plant ajwain (*Trachyspermum ammi* (L.) Sprague). *Journal of Arid Environments*, 64: 209-220.

Ashraf, M., Q. Ali and Z. Iqbal. 2006. Changes in chemical composition of fixed and essential oil of black cumin (*Nigella sativa* L.) seeds collected from plants grown at different soil nitrogen habitats. *Journal of the Sciences of Food and Agriculture*, 86: 871-876.

Bhattacharjee, S.K. 2001. *Handbook of Medicinal Plants*. 3rd Ed. Pointer Pub. Jaipur (India).

Boyd, I. and E.G. Beveridge. 1979. Relationship between the antibacterial activity towards *E. coli* NCTC 5933 and the physicochemical properties of some esters of 3,4,5-trihydrobenzoic acid (gallic acid). *Microbios*., 24: 173-184.

Boyd, I. and E.G. Beveridge. 1981. Antimicrobial activity of some alkyl esters of gallic acid (of 3,4,5-trihydrobenzoic acid) against *E. coli* NCTC 5933 with particular reference to n-propil gallate. *Microbios*., 30: 73-85.

Bradford, M.M. 1976. A rapid and sensitive for the quantitation of microgram quantitites of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72: 248-254.

Brito, M. de-F., A.G. Armien, C.H. Tokarnia, B.M. De. Farias and De-Farias-Brito-M. 1996. Experimental poisoning of sheep with seeds of the leguminous plant *Abrus precatorius*. *Pesquisa-Veterinaria-Brasileira*, 16: 59-66.

Campbell, N.A., L.G. Mitchell and J.B. Reece. 2000. *Biology Concepts and Connections*. 3rd ed. Addison Wesley Longman, Inc. New York. Page, pp. 672-674.

Chrisrtenes, A.B., B.H. Cho, M. Naesby, P.L. Gregersen, J. Brandt, K.M. Ordana, D. Collinge and H.T. Christensen. 2002. The molecular characterization of two barley proteins establishes the novel PR-17family of pathogenesis-related proteins. *Molecular Plant Pathology*, 3: 135-144.

Conlon, J.M., A. Sonnevend, M. Patel, C. Daviuudson, P.F. Npelsen, T. Pasl and L.A.R. Smith. 2003. Isolation of peptides of the brevinin-1 family with potent candidacidal activity from the skin secreations of the frog Rana boylii. *Journal of peptide Research*, 62: 207.

Cruickshank, R., J.P. Duguid, B.P. Marmion and R.H.A. Swan. 1975. *Medical Microbiology*, 12th ed. 2: 136.

Dahot, M.U., Z.H. Soomro and M. Ashiq. 1997. Antimicrobial peptides isolated from *Moringa oleifere* seeds. *Pak. Journal of Pharmacology*, 14: 15-21.

Das, S.R., B.K. Pani and S. Kar. 1997. Evaluation of some plant extracts against *Sclerotium rolfsii* causing stem rot in tuberose. *Environment and Ecology*, 15: 975-976.

Deutscher, M.P. 1990. *Methods in Enzymology, Guide to Protein Purification*. Academic press, U.S.A. 182: 317.

Dimetry, N.Z., S.E. Gengaihi, A.S. Reda and S.A.A. Amer. 1992. Biological effects of some isolated *Abrus precatorius L.* alkaloids towards *Tetranychus urticae* Koch. *Anz.-Schaedlingskd-Pflanzenschutz-Umweltschutz*, 65: 99-101.

Dube, H.C. 1990. *An introduction to fungi*. 2nd Rev. Ed. Vikas Pub. House Pvt. Ltd. pp. 141-176.

Emmons, C.W., C.H. Binford and J.P. Utz. 1970. *Medical Mycology*. 2nd ed. London, Henry Kimpton, p. 464.

Ezeamuzie, I.C., A.W. Ambakederemo, F.O. Shode and S.C. Ekwebelem. 1996. Antiinflammatory effects of *Moringa oleifera* root extract. *International Journal* of *Pharmacognosy*, 34: 207-212.

Faizi, S., B.S. Siddiqui, R. Saleem, K. Aftab, F. Shaheen and A.U.H. Gilani. 1998. Hypotensive constituents from the pods of *Moringa oleifera*. *Planta Medica*., 64: 225-228.

Gupta, M., U.K. Mazumder, S. Chakrabarti, M. Gupta and S. Chakrabarti. 1999. CNS activities of methanolic extract of *Moringa oleifera* root in mice. *Fitoterapia*, 70: 244-250.

Gupta, S.K., J. Prakash, S. Srivastava. 2002. Validation of tradition claim of Tulsi, *Ocimum sanctum* L. as a medicinal plant. *Indian* J. *Exp. Biol*., 40: 765-73.

Hancock, R.E. 1997. Peptide antibiotics. *Lancet*, 349: 418-422.

Harborne, J.B. 1992. *Chemicals as defense agents. In: Introduction to ecological Biochemistry*. (Ed.): J.B. Harborne. Academic Press. Harcourt Brace and Co Publishers, New York, pp. 131-158.

Haung, X., W.J. Xie and Z.Z. Gong. 2000. Characterization and antifungal activity of a chitin binding protein from *Ginkgo biloba. FEBS*., 478: 123-126.

Hawksworth, D.L. 2001. The magnitude of fungal diversity: the 1.5 million species estimate revisited. *Mycol. Res*., 12: 1422-1432.

Huynh, Q.K., J.R. Borgmeyer, C.E. Smith, L.D. Bell and D.M. Shah. 2001. Isolation and Characterization of a 30 kDa protein with antifungal activity from leaves of *Engelmannia pinnatifida*. *J. Biol. Chem*., 316: 723-727.

Katsura, H., R. Tsukiyama, A. Suzuki and M. Kobayashi. 2001. In vitro antimicrobial activities of bakuchiol against oral microorganisms. *Antimicrobial-Agents-and-Chemotherapy*, [Antimicrob.-Agents-Chemother.], 45: 3009-3013.

Kuo, S.C., S.C. Chen, L.C. Chen, J.B. Wu, J.P. Wang, C.M. Teng, S.C. Kuo, S.C. Chen, L.C. Chen, J.B. Wu, J.P. Wang and C.M. Teng. 1995. Potent antiplatelet, anti-inflammatory and antiallergic isoflavanquinones from the roots of *Abrus precatorius*. *Planta Medica*, 61: 307-312.

Lehrer, R.I., M. Rosnman, S.S. Harwig, R. Jackson and P. Eisenhouer. 1991. Ultra sensitive assay for endogenous antimicrobial polypeptide. *Immunol. Methods*., 137: 167-173.

Mahoney, N., R.J. Molyneux and B.C. Campbell**.** 2000. Regulation of aflatoxin production by napthoquinones of walnut (*Juglans regia*). *J. Agric. Food Chem*., 48: 4418-21.

Mekkawy, E.S., M.R. Meselhy, N. Nakamura, M. Hattori, T. Kawahata and T. Otake. 1999. 12-O-Acetylphorbol-13-decanoate potently inhibits cytopathic effects of human immuno-deficiency virus type 1 (HIV-1), without activation of protein kinase C.*Chemical* and *Pharmaceutical Bulletin*, 47: 1346-1347.

Molgaard, P., S.B. Nielsen, D.E. Rasmussen, R.B. Drummond, N. Makaza and J. Andreassen. 2001. Anthelmintic screening of Zimbabwean plants traditionally used against schistosomiasis. *Journal of Ethnopharmacology*, 74: 257-264.

Negi, M.S., A. Singh and M. Lakshmikumaran. 2000. Genetic variation and relationship among and within *Withania* species as revealed by AFLP markers. *Genome*, 43: 975-980.

Niber, B.T. 1994. The ability of powders and slurries from ten plants including *Solanum nigrum,* to protect stored grains from attack by *Prostephanus truncates* Horn (Coleoptera: Bostrichidae) and *Sitophilus oryzae* L. (Coleoptera: Curculionidae). *J. Stored Prod. Res*., 30: 297-301.

Nostro, A., M.P. Germano, V. Angelo, A. Marino and M.A. Cannatelli. 2000. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. *Letters* in *Applied Microbiology*, 30: 379-348.

Nwodo, O.F. 1991. Studies on *Abrus precatorius* seeds. I: Uterotonic activity of seed oil. *J. Ethnopharmacol*, 31: 391-394.

Nwodo, O.F. and E.O. Alumanah. 1991. Studies on *Abrus precatorius* seeds. *II:* Antidiarrhoeal activity. *J. Ethnopharmacol*, 31: 395-398.

Nwosu, M.O. and J.I. Okafor. 1995. Preliminary studies of the antifungal activities of some medicinal plants against *Basidiobolus* and some other pathogenic fungi. *Mycoses*, 38: 194-195.

Osborn, R.W., G.W. De-Sanbianx, K. Thevissen, I. Goderis, S. Torrekens, F.V. Leuven, S-Attenborough, S.B. Rees and W.F. Broekaert. 1995. Isolation and characterization of plant defensins from seeds of *Asteraceae, Fabaceae, Hippocastanaceae and Saxifragaceae. FEBS Lett*., 368: 257-262.

Peter, W.H., P.J. V-D, Meijs, A. S. Ponstein, B. H. Simons, L. S. Melchers and M. H. Stuiver. 2004. Isolation and characterization of a carbohydrate oxidases from higher plants with a role in active defense. *The Plant*, 39: 147-160.

Pocsi, I., L. Sami, E. Leiter, L. Majoros, B. Szabo, T. Emri and T. Pusztahelyi. 2001. Searching for new type antifungal drugs. *Acta Microbiol. Immunol. Hung*., 48: 533-543

Purohit, S.S. and S.P. Vyas. 2004. *Medicinal plants cultivation a scientific approach including processing and financial guidelines*. 1st edition. Publishers Agrobios, Jodhpur, India, pp. 1-3.

Reda, A.S., N.Z. Dimetry, S.A.A. Amer and S.E. Gengaihi. 1990. The role of *Abrus precatorius* alkaloid on settling, reproduction and development in *Tetranychus urticae* Koch. *Annals of Agricultural Science, Moshtohor*, 2894: 2643-2653.

Reddy, K.V.R., R.D. Yedery and C. Aranha. 2004. Antimicrobial peptides: premises and promises. *Antimicrobial agents*, 24: 536-547.

Regente, M.C., A.M. Giudic, J. Villalain and L. De la Canal. 2005. The cytotoxic properties of a plant lipid transfer protein involve membrane permeabilization of target cells. *Letters* in *Applied Microbiology*, 40: 183-189.

Rehman, A., M.I. Choudhary and W.J. Thomson. 2001. *Bioassay techniques for drug development*. 1st ed. Harwood Academic Publishers, Netherlands, pp. 16-20.

Ruckmani, K., S. Kavimani, R. Anandan and B. Jaykar. 1998. Effect of *Moringa oleifera* Lam on paracetamol-induced hepatotoxicity. *Indian Journal of Pharmaceutical Sciences*, 60: 33-35.

Sethi, N. 1991. Teratogenic activity of some indigenous medicinal plants in rats. *International Journal of Toxicology, Occupational and Environmental Health*, 1: 131.

Tang, G., C. Minghuang, W. HongJing, G.Y. Tang, M.H. Chen and H.J. Wu. 1994. Isolation and determination of croton toxin by high performance gel filtration chromatography (GFC) with photodiode array detector. *Chinese Journal of Chromatography*, 12: 244-246.

Terras, F.R.G., H.M.E. Schoofs, M.F.C. De Bolle, F.V. Leuven, S.B. Rees, J. Vanderleyden, A.B.P. Cammue and W.F. Broekaert. 1992. Analysis of two novel classes of plant antifungal proteins from Radish (*Raphanus sativus*) seeds. *J. Biol. Chem*., 267: 15301-15309.

Terras, F.R. G., S. Torreken, F.V. Leuven, R.W. Osborn, J. Vanderleyden, B.P.A. Cammue and W.F. Broekaert. 1993. New family of basic cysteine-rich plant antifungal proteins from *Brassicacea* species. *FEBS*, 316: 233-240.

Thevissen, K., D.C. Warnecke, I.E.J.A. Francois, M. Leipelt, E. Heinz, C. Ott, U. Zahringer, B. P.H.J. Thomma, K.K.A. Ferket and B.P.A. Cammue. 2003. Defensins from insects and plants interact with fungal Gllucosylceramides. *J. Biol. Chem*., 297: 3900-3905.

Ye, X.Y. and T.B. Ng. 2002. A new antifungal peptide from rice beans. *J. Peptide Res*., 60: 81-87.

(Received for publication 28 December 2006)