Alkaloid extracts of *Prosopis juliflora* (Sw.) DC. (Mimosaceae) against *Alternaria alternata*

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ABSTRACT

The antifungal activity of aqueous, petroleum ether, benzene, chloroform, methanol and ethanol extracts and alkaloid extract of *Prosopis juliflora* (Sw.) DC. leaves (Mimosaceae) was evaluated for antifungal activity by poisoned food technique against *Alternaria alternata* a causal organism of brown spot of tobacco. Aqueous extract recorded highly significant antifungal activity at 24% concentration. Among different solvent extracts tested, methanol and ethanol extract recorded highly significant antifungal activity. Methanol extract was further subjected to fractionation guided by antifungal activity leading to the isolation of alkaloid extract, which was also recorded highly significant antifungal activity against the test fungus and the minimum inhibitory activity was recorded at 1000 ppm. The antifungal activity of alkaloid extract was compared with synthetic fungicides viz., blitox, captan, dithane M-45 and thiram at their recommended dosage of 2000 ppm indicating that the alkaloid extract was highly effective even at the dosage lesser than the synthetic fungicides.

Key words: *Prosopis juliflora*, *Alternaria alternata*, antifungal activity

INTRODUCTION

Prosopis juliflora (Sw.) (Mimosaceae) commonly known as mesquite (Azhar, 1998) is a shrub or small tree native to Mexico, South America and the Caribbean. It has become established as a weed in Asia, Australia and elsewhere. *Prosopis* species form a major component in dryland ecosystems in the Americas, Africa and Asia as it is fast growing, hardy and drought-resistant tree with remarkable coppicing power (Nadkarni and Nadkarni, 1976). Its uses include forage, wood and environmental management. Pharmacological properties under *in vitro* conditions shows that it has antibacterial (Shankarmurthy and Siddiqui, 1948; Ahmad *et al.*, 1986; Aqeel *et al.*, 1989; Kanthasamy *et al.*, 1989a), antifungal (Ahmad *et al.*, 1989a; Kanthasamy *et al.*, 1989a), hemolytic (Kanthasamy *et al.*, 1989b) and anti-inflammatory (Ahmad *et al.*, 1989a) activities were attributed to piperidine alkaloids present in the extracts of *P. juliflora* leaves (Ahmad *et al.*, 1978; Ott-Longoni *et al.*, 1980; Daetwyler *et al.*, 1981; Batatinha, 1997). Additionally, cytotoxic and antitumoral activity against human epithelial tumour cells (HeLa), human hepatic tumour (HepG2), and two fibroblast lineage F26 and F57 was also recoded (Batatinha, 1997).

Many alkaloids such as juliflorine, julifloricine and julifloridine (Ahmad *et al.*, 1978), juliprosine (Daetwyler *et al*., 1981), juliprosinine and juliflorinine (Ahmad *et al*., 1989b), benzene insoluble alkaloidal fraction (containing 2 major and 3 minor alkaloids) (Ahmad *et al*., 1989b) have been isolated and their *in vitro* biological activities demonstrated. Analogous alkaloids such as 3'-oxo-juliprosopine, sceojuliprosopinol, 3-oxojuliprosine and 3'-oxo-juliprosine were isolated and their growth inhibitory activity reported (Nakano *et al*., 2004).

Recently, julifloravizole a novel alkaloid from leaves of *P. juliflora* was reported by Raghavendra (2007). The alkaloid recorded broad spectrum antifungal activity against species of *Fusarium*, *Drechslera* and *Alternaria*, which prompted us to screen further pathogens for susceptibility test to alkaloids of *P. juliflora*.

Considering the availability of many biologically important alkaloids in the leaves, the pooled alkaloid extract was obtained from the leaves and subjected to antifungal activity against important phytopathogen *Alternaria alternata* associated with brown spot of tobacco. Aqueous extract of the plant helped in the initial screening of the antifungal activity. Minimum Inhibitory Concentration (MIC) studies was also conducted to study the comparative efficacy of the alkaloid extract with that of synthetic pesticides which are under debate due to their toxic effects on human health (Bajaj and Ghosh, 1975; Javaraska, 1978; Toteja *et al.*, 2003), hence identifying pesticides of biological origin will be a fruitful alternative

to overcome the pesticide pollution problems and in view of this the present study was conducted.

MATERIALS AND METHODS

Sample collection and extraction

Healthy disease free, mature leaves of *Prosopis juliflora* collected from Mysore, Karnataka, India was used for the preparation of aqueous and different solvent extracts. A voucher specimen of the plant has been deposited in the herbarium of DOS in Botany, University of Mysore, Mysore. Leaf samples (100g) of the plant were thoroughly washed, blot dried and macerated with 100ml sterile distilled water in a waring blender (Waring international, New Hartford, CT, USA) for 10 min. The macerate was first filtered through double layered muslin cloth and then centrifuged at 4000 *g* for 30 min. The supernatant was filtered through Whatmann No. 1 filter paper and sterilized at 120 °C for 30 min. This served as an aqueous extract stock. 25g of dried leaf material was sequentially extracted for 48 h with petroleum ether, benzene, chloroform, ethanol and methanol solvents. Each solvent was evaporated to dryness using rotary flash evaporator for further usage.

Extraction of alkaloids

The extract containing alkaloids (AE) from *Prosopis juliflora* leaves was obtained by acid/basic extraction method previously described by Harborne (1998). In brief, the air dried plant material was extracted in Soxhlet extractor with methanol (100 mL/25g) for 48 h. The methanolic extract was concentrated using rotary flash evaporator. Evaporated methanol extract was acidified with dilute HCl and extracted with ether to remove the resins, fats, oils and colouring matters. The combined aqueous-acid solution was neutralized with ammonia until it reached pH 11 and was extracted with chloroform. Aqueous ammonical solution was discarded. The resulting solution, an extract containing alkaloids, was washed with water, evaporated to dryness and confirmed for their positivity using the Dragendorff's reagent identification test (Wagner *et al.*, 1983). Chloroform solution of alkaloid was subjected to antifungal activity assay and determination of MIC. 24 per cent concentration for aqueous extract and 500 ppm concentration for solvent extracts were used in the antifungal bioassay.

Test fungi and antifungal activity bioassay

Alternaria alternata was isolated from infected tobacco leaves collected from Hunsur, Mysore district, Karnataka state and identified using standard manuals (Barnet and Hunter, 1998). The pathogen was selected based on the previous reports available from our laboratory (Raghavendra, 2007). For aqueous extract, Czapek Dox agar medium with 4%, 8%, 12%, 16%, 20% and 24% of the aqueous extract were prepared. About 15 ml of the medium was poured into each petriplate, allowed to cool and solidify. Five mm disc of 7-day-old culture of the test fungus was placed at the center of the petriplates and incubated at 25±2 °C for seven days. After incubation the colony diameter was measured in millimeter. For each treatment three replicates were maintained. Czapek Dox agar medium without the aqueous extract served as control. Thus the percent inhibition of mycelial growth (PIMG) (Pinto *et al.*, 1998) if any was determined by the following formula:

$$\text{PIMG} = \frac{dc - dt}{dc} \times 100$$

where dc = Average increase in mycelial growth in control plates, dt = Average increase in mycelial growth in treated plates.

The commonly used chemical fungicides *viz*., Blitox, Captan, Dithane M-45 and Thiram at the recommended dosage of 2 g/L (2000 ppm - field recommended dose) were also subjected to antifungal activity assay by poisoned food technique (PFT). The results were compared with that of the active fraction of the methanol fraction at different concentration.

Determination of MIC of the alkaloid fraction

Known weight of the active fraction was dissolved in known amount of methanol, diluted with Czapek Dox Broth (CDB) to obtain required concentrations (100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 ppm); later agar was added and sterilized (Rai and Linganna, 2000). Sterilized CDA media with active fraction was transferred to sterilized petriplate. The test fungus was subjected to antifungal activity assay as described earlier and the percentage of inhibition was recorded. The concentration in which complete inhibition of the mycelial growth observed was recorded as the Minimal Inhibitory Concentration (MIC). The mean data was subjected to ANOVA using SPSS for Windows software. The significances were expressed at 5 % level.

RESULTS

The results revealed that aqueous extract of *Prosopis juliflora* significantly ($P < 0.05$) inhibited the mycelial growth of the test fungi. Significant increase in the inhibition of mycelial growth was observed with increase in concentration of aqueous extract from 4 to 24% (57.06 ± 0.32, 58.99 ± 0.54, 63.11 ± 0.28, 63.60 ± 0.62, 67.22 ± 0.37 and 71.59 ± 0.26 for 4, 8, 12, 16, 16, 20 and 24 per cent respectively). Among different solvent extracts tested at

500 ppm concentration, methanol and ethanol extracts recorded highly significant antifungal activity, whereas no activity was observed in other solvent extracts. The MIC study of the isolated alkaloid fraction indicated that the complete inhibition of the pathogen was observed at 1000 ppm concentration (75.68 ± 0.62, 77.23 ± 0.54, 77.43 ± 0.37, 78.91 ± 0.32, 79.03 ± 0.28, 79.03 ± 0.26, 81.00 ± 0.22, 84.07 ± 0.12 and 100 ± 0.00 for 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 ppm respectively).

Among different synthetic fungicides tested, the fungus showed varied degree of susceptibility to each fungicide, thiram was found highly effective followed by dithane M-45, blitox and captan. Comparative evaluation of alkaloid extract with that of synthetic fungicides indicated that the alkaloid extract completely inhibited the mycelial growth of *A. alternata* at 1000 ppm, where as the fungicides used in the present study did not show complete inhibition of the pathogen even at the recommended dosage of 2000 ppm concentration (Fig. 1). Comparative evaluation of aqueous extract with that of synthetic fungicides was also conducted in view of developing management strategies for *A. alternata* infection in tobacco. The result is highly promising as the activity of aqueous extract was also highly significant ($P < 0.05$) compared to synthetic fungicides tested except thiram (Fig. 1).

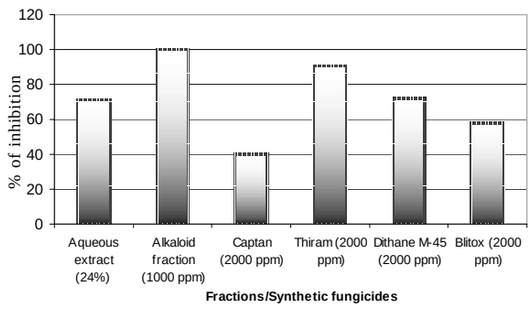

Figure 1. Comparative evaluation of different fractions of leaves of *P. juliflora* and synthetic Fungicides

DISCUSSION

A special feature of higher plants is their capacity to produce a large number of organic chemicals of high structural diversity. Collectively plants produce remarkably diverse array of over 1,00,000 low molecular mass natural products, also known as secondary metabolites. Most of these are derived from the isoprenoid, phenylpropanoid and alkaloid or fatty acid/polyketide pathways (Dixon, 2001). It is quite interesting to know that the *P. juliflora* seeds and leaves are known to contain several alkaloids of pharmaceutical importance as already mentioned. Research into plant metabolites for human disease management are many as against phytopathogens management, it is hence the present study is conducted to demonstrate the possible application of herbal remedy for the plant disease management.

Prosopis juliflora is used for feed of livestock, shade, windbreak, charcoal, live fence, and firewood as well as house construction (Mohamed, 1997 and Hailu, 2002). However, it is jeopardizing the daily activities of the nomadic pastoralist and agriculturalists, on the other hand. It invades the farmlands, rangelands, irrigation canals, narrowing roads (Zewudie, 1999 and Hailu, 2002), hence it is now a days considered as weed. The meaningful use of this weed depends on exploitation of its biological activity in agriculture; in view of this the present study is highly successful in indicating its antifungal potential against phytopathogen.

Species of *A. alternata* is known to cause brown spot of tobacco which is a new threat to FCV tobacco crop cultivation in Karnataka (Murthy *et al.*, 2003) and also involved in grain discoloration of paddy seeds (Agarwal *et al.*, 1989) world wide. The present study has demonstrated, for the first time, the antifungal potential of *P. juliflora* against *Alternaria alternata*, phytopathogenic fungi of tobacco plant. Aqueous extract of the leaves was also tested in keeping view of developing management strategies in combination with synthetic fungicides or the aqueous extract alone. The further toxicological studies at the dosage tested is warranted as the pooled alkaloids recorded several neurological disorders in test animals (Silva *et al.*, 2007). Hence the use of this alkaloid fraction in combination with already available fungicides which will reduce the concentration of both will be clever choice, which could be arrived at by further studies.

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REFERENCES

Agarwal, P. C., Mortensen, C. N. and Mathur, S. B. 1989. *Seed-borne diseases and seed health testing of rice*. Technical Bulletin No. 3. Phytopathological papers No. 30. Danish Government Institute of seed pathology for developing countries and CAB International Mycological Institute.

Ahmad, A., Ali K. K., Ahmad V. U. and Qazi, S. 1986. Antibacterial activity of juliflorine isolated from *Prosopis juliflora*. *Planta Medica*, **1:** 285-288,

Ahmad, A., Khursheed, A.K., Sabiha, Q. and Viqaruddin, A. 1989a. Antifungal activity of some hydrosoluble *Prosopis juliflora* alkaloids. *Fitoterapia*, **60:** 86-89.

Ahmad, V. U., Basha, A. and Haque, W. 1978. New alkaloids from *Prosopis juliflora*. *Z. Naturforsch*, **33**: 347-348.

Ahmad, V.U., Sultana, A. and Qazi, S. 1989b. Alkaloids from the leaves of *Prosopis juliflora*. *Journal of Natural Products*, **52:** 497-501.

Aqeel, A., Khursheed, A. K., Viqaruddin, A. and Sabiha, Q. 1989. Antimicrobial activity of julifloricine isolated from *Prosopis juliflora*. *Arzneim. - Forsch. Drug Research*, **39:** 652-655.

Azhar, I. 1998. Chemical and biological studies on some medicinal Leguminaces. Ph.D. thesis submitted to Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi, Karachi.

Bajaj, B.S. and Ghosh, A.K. 1975. Antifungal antibiotics in perspective. In: *Advances in Mycol. Plant Pathol*. Sagar printers. New Delhi. 279-302 **PP**.

Barnett, H.L. and Hunter, B.B. 1998. Illustrated Genera of Imperfect Fungi, 4th Edition. Minnesota, USA: The American Phytopathological Society Press.

Batatinha, M.J.M. 1997. Investigations on toxic influences of *Prosopis juliflora* D.C. (Algarobeira) on cell cultures as well as on ruminal fermentation in cattle (*in vitro*). Dissertação (mestrado)- Veterinary Medicine University, Hannover. 189 **P**.

Daetwyler, P., Ott-Longoni, R., Schöpp, E. and Hesse, M. 1981. Over juliprosine, a further alkaloid from *Prosopis juliflora* A. DC. *Helvetica Chimica Acta*, **64:** 1959-63.

Dixon, R.A. 2001. Phytochemistry in the genomics and post genomics era. *Phytochemistry*, **57:** 145-148.

Hailu, S. 2002. *Some Biological Characteristics that Foster the Invasion of Prosopis juliflora (SW.) DC. at Middle Awash Rift Valley Area, Northeastern Ethiopia*. M.Sc. Thesis. Addis Ababa University, Addis Ababa, Ethiopia.

Harborne, J.B. 1998. *Phytochemical methods*. Chapman and Hall publications, London. 7-8 **PP.**.

Javaraska, T. 1978. Effect of combined herbicides on the occurrence of morphoses in spikes of spring barley. *Agrochemica*, **18**: 37-42.

Kanthasamy, A., Subramanian, S. and Govindasamy, S. 1989a. Bactericidal and fungicidal effects of *Prosopis juliflora* alkaloidal fraction. *Indian Drugs*, **26:** 390-394.

Kanthasamy, A., William, S. and Govindasamy, S. 1989b. Hemolytic effects of *Prosopis juliflora* alkaloids. *Current Science*, **58:** 142-144.

Mohamed, A.E.F. 1997. *Tropical Forestry Report: Management of Prosopis juliflora for Use in Sudan* (Luukkanen, O. ed.). Ph.D. Thesis. Helsinki, Finland.

Murthy, K., Shenoi, M. M. and Sreenivas, S. S. 2003. Perpetuation and host range of *Alternaria alternata* causing brown spot disease of tobacco. *Indian Phytopathology*, **56:** 138-141.

Nadkarni, K. M. and Nadkarni, A.K. 1976. Indian Materia Medica, Bombay Popular Prakashan, **1**: 26.

Nakano, H., Nakajima, E., Hiradate, S., Fujii, Y., Yamada, K., Shigemori, H. and Hasegawa, K. 2004. Growth inhibitory alkaloids from mesquite *Prosopis juliflora* (Sw.) DC. leaves. *Phytochemistry*, **65:** 587-591.

Ott-Longoni, R., Viswanathan, N. and Hesse, M. 1980. The structure of the alkaloid juliprosopin from *Prosopis juliflora* A. DC. *Helvetica Chimica Acta*, **63:** 2119-2129.

Pinto, C. M. F., Maffia, L. A., Casali, V. W. D. and Cardoso, A. A. 1998. *In vitro* effect of plant leaf extracts on mycelial growth and sclerotial germination of *Sclerotium cepivorum*. *Journal of Phytopathology*, **146**: 421-425.

Raghavendra, M.P. 2007. Isolation and characterization of antimicrobials of plant origin. Ph.D. thesis submitted to University of Mysore, Mysore, Karnataka, India.

Rai, K.M.L. and Linganna, N. 2000. Synthesis and evaluation of alkylated 2-amino-1, 3, 4-oxadiazole derivatives. *IL Farmaco*, **55:** 389-392.

Shankarmurthy, P. and Siddiqui, S. 1948. Antibiotic activity of leaf extracts of *Prosopis juliflora*. *Journal of Scientific and Industrial Research*, **7**: 188.

Silva, A. M. M., Silva, A. R., Pinheiro, A. M., Freitas, S. R. V. B., Silva, V. D. A., Souza, C. S., Hughes, J. B., El-Bachá, R. S., Costa, M. F. D., Velozo, E. S., Tardy, M. and Costa, S.L. 2007. Alkaloids from *Prosopis juliflora* leaves induce glial activation, cytotoxicity and stimulate NO production. *Toxicon*, **49:** 601-614.

Toteja, G. S., Mukherjee, A., Diwarkar, S., Singh, P. and Saxena, B. N. 2003. Residues of DDT and HCH pesticides in rice samples from different geographical regions of India; a multicentre study. *Food Additives and Contaminants*, **20:** 933-939.

Wagner, H., Bladt, S., Zgainski, E.M. and Analyse, D. 1983. In: Verlag Springer, (ed.) Dünnschichtchromatographie Anlyse von Arzneidrogen. Berlin: Heidelberg New York Publishers. 249-250 **P**.

Zewudie, K. 1999. Prosopis *(Prosopis juliflora)* in Ethiopia (Fassil Reda, Tanner, D.G., eds.) *Arem*. **5**: 96-102. Ethiopian Weed Science Society, Addis Ababa, Ethiopia.

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