Antifungal Activity of Leaf Extracts of *Alseodaphne andersonii* Against Different Pathogenic Fungal Cultures

Atul Kaushik[1], Versha Parcha[2], Jeevan Jyoti Kaushik[1] and Mohan Singh Rawat, [3] Debesai Gaim[1]

Department of Pharmaceutical Chemistry, School of Pharmacy, Asmara College of Health Sciences, Eritrea, North East Africa
[2]Department of Pharmaceutical Sciences, S.B.S (P.G) Institute of Biomedical Sciences & Research, Balawala, Dehradun (U.A).248 161 INDIA
[3]Department of Chemistry, H.N. B. Garhwal University, Srinagar, (U.A.) INDIA

Summary

To evaluate the antifungal activity of leaf extracts of *Alseodaphne andersonii* (King ex Hook.f.) Kosterm (Lauraceae), various pathogenic fungal cultures *viz Phialophora verrucosa, Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum*, *Cladosporium sp*., *Trichoderma viride* were used. Inhibition of fungal growth was investigated using disk diffusion methods. Methanol leaf extract of *Alseodaphne andersonii* was active against assayed fungal cultures. The minimal inhibitory concentration (MIC) values and minimal fungicidal concentration (MFC) values were also determined. Results indicated that all the extracts of leaf showed good antifungal activity against fungal cultures as reflected by the zone of inhibition but acetone and methanol extracts showed a significant control of growth of *Aspergillus niger*, *Fusarium oxysporum* and *Phialophora verucosa*. The MIC and MFC of methanol extract of leaves were found (6.25 and 12.5 mg/mL) for *Fusarium oxysporum,* (1.25 and 6.25 mg/mL) for *Phialophora verucosa* and (3.12 and 6.25 mg/mL) for *Aspergillus niger* respectively. *Alseodaphne andersonii* might provide promising therapeutic agents against infections with fungal cultures.

Key words: *Alseodaphne andersonii*; Anti fungal; Methanol extract.

Introduction

Higher plants have severed humankind as sources of medicinal agents since its earliest beginnings. Infact, natural products once served as the source of all drugs. Today, natural products still represent over 50% of all drug in clinical use, with higher plant-derived natural products representing 25% of the total. On numerous occasions, the folklore records of many different cultures have provided leads to plants with useful medicinal properties (1). *Alseodaphne andersonii* (King ex Hook. f.) Kosterm. (Lauraceae), a plant growing in the Himalayan region, has been selected for the current study. There is a very little report about this plant. The plant is reported to be used as timber in Yuan-Nan (2). Only five gamma lactones were isolated from the bark and roots of *A. andersonii* (3). However, there were no reports about the antifungal activity of this plant. Therefore, an attempt has been made to investigate antifungal activity of *A. andersonii* leaf extracts. It is hoped that this study might lead to the discovery of new compounds that could be used to formulate new and more potent antimycotic drugs that might over come the problem of resistance to the currently available antimycotic agents.

Materials and Methods

Collection of Plant material

Fresh plant leaves were collected from the botanical garden of Forest Research Institute (F.R.I.) Dehradun and authenticated by Dr. H.C. Pandey, taxonomist, Botanical survey of India, FRI, Dehradun, India. Voucher specimens A-29 were prepared and stored in the, department of pharmacognosy, SBSPGI, Balawala, Dehradun (U.A.), India.

Preparation of extracts

Fresh 500g leaves were shade dried at room temperature, ground into a fine powder and extracted in soxhlet assembly with solvents *viz* petroleum ether, chloroform, acetone and methanol. The extracts were concentrated under reduced pressure in rotary evaporator. The yields of dry extracts were (6.25, 3.0, 3.5 and 4.0 % w/w respectively) recorded and stored in a clean glass bottles for antifungal activity.

Organisms

The fungal cultures were collected from the Department of microbiology, SBSPGI, Balawala, Dehradun, India. The fungal cultures were *Phialophora verrucosa, Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum*, *Cladosporium sp*., *Trichoderma viride*. All the organisms were maintained on specified media slants at 4^{O} C and revived prior to use.

Antifungal activity

The disc diffusion method was used to determine *in vitro* antifungal activity of the extracts (4). The cultures were subculture in agar medium and incubated at 27^{0}C for 72-120 h and from this, the spore suspension was prepared containing 10^{5} cell/ml. Turbidity of the organism suspension was adjusted to the Mc-Farland standard (0.5) and 100µl of suspension was plated on agar medium. Sterile empty discs (Hi-media) were allowed to soak and absorb the extract for 24 hrs before draining off the excess and drying in the oven at 60 ^{O}C (5). These discs were placed on the agar plates against the control (solvents) and standard (nystatin). Plates were incubated at 25-27^{0}C for 72-120 h and observed for the zone of inhibition. Disc diameter (6 mm) was deducted while recording the zone size. The tests were conducted in triplicate (Table- 1).

Minimum inhibitory concentration

Minimum inhibitory concentration (MIC) was evaluated by tube dilution method against *Aspergillus flavus* and *Fusarium oxysporum* only. Methanol extract MIC was determined by dilution of the extract to various concentrations (0.097-50 mg/ml) and compared with a standard (Nystatin). All the tubes were incubated at suitable temperature for 72-120 h. The tubes were observed for the appearance of any growth. The MIC was interpreted as the lowest concentration of the extract that did not permit any visible growth when compared with control tubes.

Minimum fungicidal concentration

Minimum Fungicidal Concentration (MFC) was determined by sub culturing methods (6). Subcultures made from samples obtained from those test tubes which showed no visible turbidity or growth in MIC assays, were made on freshly prepared agar plates. After 72 h incubation, the MFC was regarded as the lowest concentration of the extract that did not permit any growth on the agar plate surface used.

Results and discussion

The leaf extracts (petroleum ether (60-80), chloroform, acetone and methanol) were subjected to qualitative analysis for the presence of various phytoconstituents. Acetone and methanol extracts have shown the presence of alkaloids, glycosides, proteins, amino acids and sugars while

petroleum ether and chloroform extracts showed the presence of steroids. Among all the extract, methanol extract revealed remarkable effect on fungi like, *Phialophora verrucosa* (12 mm), *Aspergillus niger*(14 mm), *Aspergillus flavus* (15 mm), *Fusarium oxysporum* (15 mm), *Cladosporium sp*.(17 mm), *Trichoderma viride* (12 mm) respectively in terms of zone of inhibition while other extracts showed negligible effects (Table). The MIC and MFC of methanol extract of leaf for *Aspergillus flavus* was found to be 6.25 and 12.5 mg/ml and for *Fusarium oxysporum* 1.56 and 6.25 mg/ml respectively (Figure).

In the initial stages of development of modern medicine, plants and plant products formed an important part of pharmacopoeia; however, because of significant development in synthetic drug chemistry and antibiotics, there was certain amount of decline in the use of plants in modern medicine. But despite the dramatic advances and advantages of conventional medicine, or biomedicine as it is also known, it is clear that herbal medicine has much to offer.

Results revealed that *Alseodaphne andersonii* leaf extracts exerted inhibitory effects against certain pathogenic fungi associated with severe infections justifies the reasoning behind the use of these leaves as herbal remedy against mycotic diseases. Further research is expected to boost the use of this plant in the nearby future against fungal infections.

Table. Anti-fungal activity of different extracts of *Alseodaphne andersonii* leaf.

S.No	ORGANISMS	Extracts (100 mg/ml)			
		Pet. Ether	Chloroform	Acetone	Methanol
1	*Fusarium oxysporum*	12	15	17	23
2	*Aspergillus flavus*	13	15	18	21
3	*Aspergillus niger*	12	14	17	20
4	*Cladosporium sp.*	15	17	20	23
5	*Phialophora verucosa*	NZ	NZ	NZ	12
6	*Trichoderma viride*	NZ	NZ	NZ	12

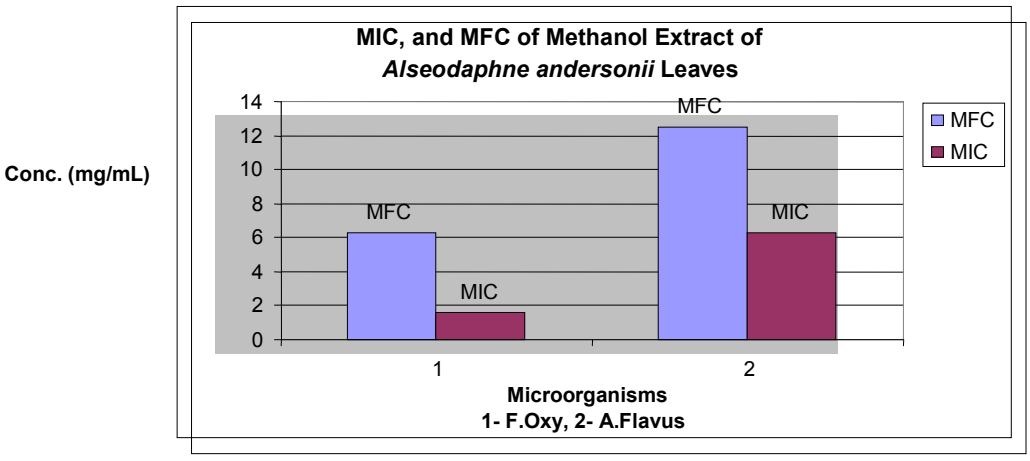

Figure 1. MIC & MFC of methanol extract of *Alseodaphne andersonii* leaves

Acknowledgement

The authors are highly thankful to Dr. Ram Lal thakur, Head, Department of Microbiology, S.B.S (P.G) Institute of biomedical Sciences & Research, Balawala, Dehradun (U.A). INDIA, for their valuable guidance and special thanks to Dr H.C. Pandey, for authentication of the plant.

References

1. Balandrin, MF, Klocke, JA, Wurtele, ES et al. Science. 228: 1154-1160, 1985
2. Anon. Flora of Yuan-Nan. Shanghai. Kunming Institute of Botany, Chinese Academy of Sciences, Scientific Publication Comp. Chinese Academy of Sciences; 1983 pp. 189–190
3. Lee SS, Chang SM, Chen CH. Chemical constituents from *Alseodaphne andersonii*. J. Nat. Prod. 64: 1548–1551, 2001.
4. Bauer AW, Kirby WMM, Sherris JC et al. Antibiotic susceptibility testing by standardized single disc method. Am. J. Clinc. Pathol. 45: 493-496, 1996
5. Olukoya DK, Odugbemi TO, Bangbose SOA. Some aspects of traditional therapy of gonorrhoea in Lagos. Nigeria J. Res. Ethnomed. 1: 26–29, 1986
6. Alade, PI, and Irobi, ON. Antimicrobial activities of crude leaf extracts of *Acalypha wilkesiana*. J. Ethanopharmacol. 39: 171-174, 1993.

Corresponding author:
Dr. Atul Kaushik
Department of Pharmaceutical Chemistry
School of Pharmacy
Asmara College of Health Sciences,
Eritrea, North East Africa
E- mail: atul_kaushik29@rediffmail.com