Sindh Univ. Res. Jour. (Sci.Ser.), Vol 33 (2): 35 – 40 (2001)

ANTIFUNGAL ACTIVITY OF OCIMUM BASILICUM AGAINST SOME HUMAN PATHOGENIC FUNGI

Nasreen Khalid Thebo, Hidayatullah Abro,Wazir Shaikh, Abdul Qayoom Soomro and Javed Anwar*

Department of Botany, University of Sindh, Jamshoro, Pakistan

Abstract

Methanolic extract of *Ocimum basilicum* leaves was investigated for its antifungal activities on four species of Human pathogenic fungi.

In vitro the extract exhibited high activity on species of *Microsporum gypsum* and *Trichophyton mentagrophyte.*

Methanolic extract of *Ocimum basilicum* showed a strong antifungal activity against the human pathogenic fungi tested. The inhibitory effect is strong against. *Trichophyton rubrum* and *Microsporum gypsum*. However *Trichophyton mentagrophyte and Microsporum canis* showed weak inhibition at low concentration.

Key Words: Antifungal activity, Ocimum Basilicum, Pathogenic fungi

Introduction

Ocimum basilicum belaongs to the family *Labiatae*, it is common weed in the cultivated fields of Pakistan. Kritikar, K, Basu, B.D (1987). Its local names: Babuitulsi (Bangla); Babri (Punjabi): Nazbo (Kashmiri) and English name sweet basil. According to distribution, it is commonly planted in the gardens of Pakistan. *O. basilicum* has been identified as jangli-Tulsi by Physicians of Greoc-Arab medicine. A medium sized, strongly scented, erect herb; leaves opposite, exstipulate, simple, ovate, entire 3-7.5 cm. Long; flowers 1-1.2 cm. long, white, pink or purplish; pedicels shorter than the calyx in front; the 2 lower calyx-teeth longer than the upper; bracts petioled; nutlets 4, about 4 mm. each, black. RIPPON, J.W (1998).

It has slender leaves which are closer to ruefragrant and bearing violets streaks as in blam mint. Its buds last throughout the winter and the summer, it is also potent against heat irritation and headache. It is sporofic and its seeds are antidiorhoeal Kirtikar, K.R. and Bush (1988). Several structures have been carried out on the biochemical analysis and biological structures of *O. basilicum.*

Proximate analysis and chemical composition have been carried out by Gule-Rana et al. (1990). Many medicinal properties are attributed to the *O.basilicum* in the indigenous system of medicine .

The plant considered to be warm and dry, and demulcent. Expectorant and antiperiodic, diuretic emmenergogue. Seeds are mucilaginous and given in gonorrhoa, dysentery and chronic diarrhoea. The leaves are useful for croup for which the warm juice with honey is given. Externally, the juice is used in skin disease and as ear drops and deafness.

Decoction of leaves is given in gastric and hepatic disorders and is useful in catarrh and bronchitis.

Jain and Jan (1921) reported that phenolic and non phenolic fractions of essential oil of *O. basilicum* showed maximum

* Department of Dermatology, LUMHS, Jamshoro

treatment of diverse skin infections. Mhasker investigated the correlation between the chemical composition of anthetmintus and the therapeutic values. Oxides had week vormicidal but no antifungal properties. Jain, S.R Jain, M.L (1921). A clinical study was carried out to determine to the efficacy of the methanolic extract from leaves of *O. basillicum*. Finally, in order to evaluate the possible fungicidal properties of this extract. The present paper reports the results obtained in these studies which confirm the antifungal properties of this popular plant.

Material and Methods

Identification: - Plant material.

The *Ocimum basillicum* (STIMSON 1962) leaves were collected from department of Botany and other area of the Sindh University. All the plant material were identified by assistant professor Syed Abdul Ghaffar Shah. Department of Botany University of Sindh Jamshoro.

Preparation of the extract

The collected leaves were washed with distilled water and then dried in shade at room temperature and finely powdered. One kilogram, of powdered leaves were macerated for one month with the solvent methanol at room temperature for 2 weeks. The dried leaves were ground powered in a blender and dipped in 2 liter of methanol for one month. The solvent of each extracted material was removed. The methanol solvent of extracted material was separated with the rotary evaporator and by drying at water bath at 40 °C. with the help of separate blades/slides scratched and the dried powder from the walls and bottom and kept in a desicator. The methods are desired and followed by Mc Clary 1989, Darah 1991, Anie riched 1992.

Microorganism

The microorganisms used in this consisted of four species of dermatophytic fungi namely*Trichophyton rubrum,Trichophyton mentagrophyte*, *Microsporum* canis and *Microsporum gypsum*. All pathogenic micro organisms were clinically isolated. The affected areas were cleaned with 70% ethanol. Hairs, scales or crusts, and nail particles were taken from lesion especially from the active margin using sterile scalpel. Pieces of infected nails were taken using sterile scissor 20% of KoH. These all cultures were identified from Microbiology Department University of Karachi. All the fungal spores were grown and maintained on Sabouraud glucose agar. The inoculated agar slants were incubated at 25 °C.

Preparation of culture for fungi

Sabouraud glucose agar media with following composition was used for this purpose (Merck).

Peptone = 10g, Glucose 40 g; Agar = 20g
Distilled water = 1000 ml with pH = 5.4

All the contents were mixed and dissolved in distilled water. The solution was autoclaved at 121^0c and 15 ib/sq inch pressure for 15 minutes.

Antimicrobial activity test

The screening method was based on the commonly decribed procedures reported for the study of crude natural products with antimicrobial activity. (Lannatte, *et al.*, 1985; Rios *et al.*,). Tests were performed by the diffusion method, (Darah, I. And Annie-C Lara, A. 1992) using 1 ml of inoculum containing 10^5 fungal spores perplate. The inoculum was applied as a spot with a loop.

Preparation of drug dilutions

10 drug concentration or dilution of stock drug extract were prepared by dispensing the desired amount of sterilized distilled water under aseptic conditions. Concentration of drug used interms of percentage were 100%, 90%, 80%,70%, 60%, 50%, 40%, 30%, 20%, 10% (Palanichamy, S. and Nagarjan 1990).

The effect of *Ocimum bailicum* leaf extract on four species of human pathogen fungi

On milliliter of fungal suspension 4 x 10^5 spores/ml was prepared 4 wells were bored in the medium. The four peripheral wells were used for dispensing the dilutions of crude drug extracts marked as 100%, 90%, 80%,70%, 60%, 50%, 40%, 30%, 20%, 10%. The petriplates were the left at 35^0c for incubation and after 5 days the inhibition zones were measured and data were noted for each fungus.

Determination of the minimum inhibitory concentration (MIC).

MIC was determined by the liquid dilution method. Dilution series setup with 100%, 90%, 80%,70%, 60%, 50%, 40%, 30%, 20%, 10%of (SGA) broth medium. The lowest concentration which did not show any growth of the tested microorganism. On the other hand the highest concentration showed strong activity against human pathogen fungi.

Result and Discussion

The ethnobotonical bibliography on *Ocimum basilicum* used by traditional medicine of other cultures can be summarized as follows Leaves extracted in water are used in India for external treatment of skin disease (Bhakuri, 1969).

Although research work on the antimicrobiol activity of *O.basilicum* has been done in the past Akhtar, K.A, (1986). The *Ocimum basilicum* showed maximum antiwormal response under the test conditions. The flowers are reported carminativediuretic and demulcent. The juice of leaves are remedy for the skin infection specially for the local apply on infected region, W.R, (1971); Rios, J.L. MC, (1988).

The present phytochemical investigation of antimicrobiol agents from this local plant was carried out. Results obtained during the investigation have been presented in table No (1). Table (2) shows the isolated members of *Trichophyton* and *Microsporum* on clinical observation.

Total four species of dermatomycosis were tested in this investigation the zones of inhibition produced by the crude drug plant against the micro-organizm used. Results show that maximal antifungal activity as performed by the liquid dilution method is exhibited by methanolic extract of leaves of *Ocimum basilicum*. Fungal strains showed susceptibility towards the extracts.

The degree of susceptibility varied depending on the species. Results have revealed that *Ocimum basilicum* is most effective at highest concentration against dermatomycosis of superficial skin infection namely disease are, .Tinea corporis, Tinea pedis, Tinea cruris, Tinea barbae, Tinea ungium, Tinea capitis. EMMONS, C.W., *et al.* (1977).

Maximum inhibition was recorded against *Trichophyton rubrum* at 100%, 90% & 80% concentration.

The weakest activity was observed by drug plant against *Microsporum canis.*

Leaf extract at 100% 90%, 80% & 70% found to be effective on *Microsporum gypsum.* Inhibition of *Trichophyton mentogrophyte* tookplace at 100%, 90%, 80% and 70% but not strong effect on the zones of fungal growth.

The results indicates that the antifungal activity of the *Ocimum basilicum* gave a well defined response. It could be concluded that *O. basilicum* have antifungal properties.

Table No.1 Antifungal activity of *Ocimum basilicum* against test organisms

Name of Drug plant	Concentration	Zones of inhibition (m.m)		*M. Canis*	*M. gypsum*
		T. rubrum	*T.mentagrophyte*		
O. basilicum	100%	+++	++	+	++
	90%	++	+	+	++
	80%	++	-	+	+
	70%	++	-	-	+
	60%	+	-	-	+
	50%	+	-	-	-
	40%	-	-	-	-
	30%	-	-	-	-
	20%	-	-	-	-
	10%	-	-	-	-

Key: - no inhibition Zone; + inhibition zones of < 10mm diameter: ++, inhibition of > 10mm diameter.

Table N0.2 Characteristics of the genera of the dermatophytes on clinical observtion

(The common species are reported in Pakistan).

S.No	Pathogen	Diagnosis	Clinical
1.	*Trichophyton mentgrophyta*	Tinea corporius, T. pedis T, cruris. T. barbae	Members may attack Skin, hair nail
2.	*Trichophyton rubrum*	T, barbae. T. corporis T. ungium. T. pedis	Members may attack Skin, hair, nail
3.	*Microsporum canis*	T.Capitis	Members attack hair, nail
4.	*Microsporum gypsum*	T.Capitis. T.corporis	Members attack hair, nail

References:

1. Akhtar et al; (1992). Phyto chemistry 31 (8): 821-824.
2. Darah I. And Annie-clara A. (1992). Antimicrobial activity of *rhinancanthus nasutus leaf extract.* Cassia atata Jourof Ethnopharmaclogy 21(1), 71-75.
3. General Emmons, C.W. Binford, C.H., UTZ, J.P. and kwon-chung, K. J. (1977). Medical mycology, 3rd edition.
4. Jain, S.R., Jan, M.L. plants medica research 8, 737-40 (1921).
5. Kirtikar K. Basu BD Indian medicinal plants 2nd Ed. Vol 1, (PEPR 1987). Pp. 153-4.
6. Planichamy. S. and Nagarajan S. (1990). Antifungal activity of cassia alatza leaf extract Journal of Ethnopharmacology 29, 337-340.
7. Rios. J.L. Recio, M.C. and Villar, A. (1988) Screening methods for natural products with antimicrobial activity a review of the litrature Journal of ethnopharmacolaogy 23; 127-150.
8. Rippon, J., 1988. Medicalmycolog: The pathogenic fungi and the pathogenic actinomycetes, 2nd Ed. The W.B. Saunders Co. Philadelphia.
9. Stimson, W.R. (1971). Ethnobotanical notes from Puerto Rico Llaydia 34; 165-170.
10. Kiritkar, K.R. and Basu BD (1988). Indian medicinal plants,vol.2.870-872. Jayyed Press Delhi.