Mycopath (2013) 11(2): 91-94 **Research Article**

Antifungal activity of *Clitoris ternatea* L. extracts against different fungal species

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Abstract

Crude, methanol and ethanol extracts of seeds, leaves and callus of Butterfly pea (*Clitoria ternatea* L.) were studied for antifungal activity against three fungal strains i.e. *Aspergillus niger*, *Alternaria solani* and *Rhizopus oryzae* using agar well diffusion method. It was observed that extracts of seeds, callus, leaves of tissue-cultured and pot-grown plants showed antifungal effect against *A. niger* and *A. solani* and none of these extracts exhibited pronounced effect against *R. oryzae.* The zone of inhibition acquired by the leaves extracts from pot-grown plants was more than that of tissue-cultured plants against fungal strains. The MIC values for different extracts ranged from 0.1–1.4 cm in diameter. Hence, it was observed that the extracts obtained from *C. ternatea* had good antifungal properties.

Keywords: *Alternaria solani,* antifungal activity, *Aspergillus niger, Clitoria ternatea*, *Rhizopus oryzae.*

Introduction

Clitoria ternatea L. is a perennial herb that belongs to the family Fabaceae and subfamily Papilionaceae. The various parts of *C. ternatea* have been reported for anti-inflammatory, antipyretic, and antimicrobial activities (Mukherjee *et al*., 2008). The member of this plant family exhibited strong anti-fungal activity against number of important fungi including *Aspergillus niger* and *Candida albican* (Bishnupada *et al*., 2011). The *C. ternatea* leaves extract illustrated the strong antifungal activity against *A. niger* with a minimum inhibitory concentration (MIC) 0.8 mg mL^{-1} (Kamilla *et al*., 2009). In an other study, the crude extract from *C. ternatea* seeds showed strong good antifungal activity against *A. niger* and *A. ochraceous* (Mhaskar *et al*., 2010).

Few reports are available regarding *in vitro* antifungal activity of *C. ternatea* (Malabadi and Nataraja, 2001), however antimicrobial activity of seeds and callus extracts of *C. ternatea* has been reported against fish pathogen (Ponnusamy *et al*., 2010). It has further been investigated that the antifungal properties of *C. ternatea* L. is attributed due to the presence of cystein rich protein and finotin (Shahid *et al*., 2009).

Keeping in view the importance of the plant the present study was conducted to compare the antifungal activity of methanolic and ethanolic extracts obtained from seed, callus, leaves of tissue-cultured and pot grown plants of *C. ternatea*.

Materials and Methods

Extraction of plant material

Seeds, callus cultures, tissue-cultured and pot-grown plants of *C. ternatea* were ground and 30 g of plant material was placed in extraction chamber, hanging above the flask having the solvent (250 mL) and below a condenser. As the flask was heated, methanol evaporated and moved into the condenser where it converted into a liquid that trickled into the extraction chamber containing the plant material. When the solvent surrounding the sample rose at a certain level, it ran over and dropped back into the boiling flask. This cycle was allowed to repeat at least 3-4 times. The flask containing the methanol extract was removed and methanol or ethanol was evaporated with the help of rotary evaporator.

To acquired extract by steady state extraction, the material was poured in the flask containing 250 mL of solvent. The flask was left for 15 days on shaker after which the material was filtered. To obtain the extracts by circulatory extraction, the plant material was added into a flask containing 250 mL of the solvent and placed on orbital shaker. The material was filtered and dried with the help of rotary evaporator.

Assay for Antimicrobial Activity

Methanolic and ethanolic extracts of seeds, callus, *in vitro*-grown and pot-grown leaves were tested for their antifungal activity against three different fungal strains i.e. *Aspergillus niger* (IIB 21), *Alternaria solani* (GCU 32) and *Rhizopus oryzae* (IIB57)The test microorganisms used were procured from Government College University,

Lahore, Pakistan and maintained by sub-culturing periodically on nutrient agar and preserved at 4 °C. The MIC was determined by agar dilution technique.

Different concentrations of *C. ternatea* were prepared in two organic solvents methanol and ethanol. The antifungal activity was experimented by agar well diffusion method (Mukherjee *et al*., 1995). Wells of 7 mm diameter were made with sterile borer after the solidification of Petri plates prefilled with potato dextrose agar and fresh inoculam of each fungus. The wells were then filled with 0.1 mL of different concentrations (from 0.9 to 0.1 mg mL^{-1}) of the extracts. For antifungal assay plates were incubated at 37 ºC for 48 h. After incubation, the zones of inhibition diameter around each of the well were measured.

Table 1: Antifungal action of leaves of pot-grown plants (LPP) and seeds extracts of *Clitoria ternatea*.

Concentration of extracts		Extraction procedure	Zone of Inhibition (cm)					
			Aspergillus niger		*Alternaria solani*		*Rhizopus oryzae*	
			Methanol	Ethanol	Methanol	Ethanol	Methanol	Ethanol
0.3 mg mL^{-1}	Seeds	Soxhlet	0.3±0.02	-	-	-	-	-
		Circulatory	0.5±0.01	0.5±0.02	0.2±0.01	0.1±0.01	-	-
		Steady state	0.1±0.02	-	-	-	-	-
	LPP	Soxhlet	-	-	-	-	-	-
		Circulatory	0.3±0.01	0.2±0.01	0.2±0.01	0.1±0.01	-	-
		Steady state	-	-	-	-	-	-
0.4 mg mL^{-1}	Seeds	Soxhlet	0.3±0.01	0.3±0.01	0.1±0.01	-	-	-
		Circulatory	0.8±0.02	0.7±0.02	0.3±0.04	0.2±0.02	-	-
		Steady state	0.1±0.03	0.1±0.02	-	-	-	-
	LPP	Soxhlet	0.2±0.01	-	-	-	-	-
		Circulatory	0.3±0.02	0.3±0.01	0.2±0.01	0.2±0.01	-	-
		Steady state	-	-	-	-	-	-
0.5 mg mL^{-1}	Seeds	Soxhlet	0.5±0.03	0.4±0.02	0.2±0.02	0.1±0.02	-	-
		Circulatory	0.9±0.01	0.7±0.02	0.5±0.02	0.5±0.04	-	-
		Steady state	0.2±0.03	0.1±0.01	0.1±0.03	0.1±0.03	-	-
	LPP	Soxhlet	0.3±0.01	0.3±0.01	0.1±0.01	0.1±0.01	-	-
		Circulatory	0.5±0.02	0.4	0.4±0.02	0.3±0.01	-	-
		Steady state	0.2±0.01	0.1±0.01	-	-	-	-
0.9 mg mL^{-1}	Seeds	Soxhlet	1.3±0.02	1.1±0.02	0.8±0.02	0.7±0.01	-	-
		Circulatory	1.4±0.01	1.3±0.01	1.0±0.02	0.9±0.05	-	-
		Steady state	0.5±0.01	0.4±0.03	0.3±0.01	0.2±0.01	-	-
	LPP	Soxhlet	0.9±0.01	0.9±0.09	0.8±0.03	0.7±0.02	-	-
		Circulatory	1.2±0.01	1.1±0.01	0.9±0.01	0.9±0.01	-	-
		Steady state	0.8±0.01	0.7±0.04	0.7±0.02	0.6±0.01	-	-

Table 2: Antifungal action of leaf callus extracts of *Clitoria ternatea.*

Concentration of extracts		Zone of Inhibition (cm)					
		Aspergillus niger		*Alternaria solani*		*Rhizopus oryzae*	
		Methanol	Ethanol	Methanol	Ethanol	Methanol	Ethanol
0.5 mg mL^{-1}	TCL	-	-	-	-	-	-
	Callus	-	-	-	-	-	-
0.6 mg mL^{-1}	TCL	0.7	0.6	-	-	-	-
	Callus	-	-	-	-	-	-
0.7 mg mL^{-1}	TCL	0.3	0.2	0.1	0.1	-	-
	Callus	0.1	0.1	0.1	0.1	-	-
0.9 mg mL^{-1}	TCL	0.6	0.5	0.5	0.5	-	-
	Callus	0.4	0.4	0.4	0.3	-	-

TCL: Tissue cultured leaves

Table 3: Comparison between zone of inhibition of different fungal strains of *in vivo* leaves and *in vitro* leaves extracts of *Clitoria ternatea*.

Fungal species	Concentrations of extracts (mg mL^{-1})	Zone of inhibition (cm)			
		In vivo leaves		*In vitro* leaves	
		Methanol	Ethanol	Methanol	Ethanol
Aspergillus niger	0.4	-	-	-	-
	0.5	0.2±0.05	0.1±0.04	0.1±0.025	-
	0.6	0.4±1.0	0.3±0.05	0.2±0.02	0.1±0.025
	0.7	0.6±0.03	0.4±0.02	0.3±0.02	0.2±0.01
	0.8	0.7±0.01	0.6±0.25	0.4±0.15	0.4±0.01
	0.9	0.8±0.03	0.7±0.05	0.6±0.051	0.5±0.01
Alternaria solani	0.5	-	-	-	-
	0.6	0.1±0.01	0.1±0.01	-	-
	0.7	0.4±0.002	0.3±0.01	0.2±0.00	0.1±0.02
	0.8	0.5±0.01	0.4±0.02	0.4±0.01	0.3±0.00
	0.9	0.7±0.02	0.6±0.01	0.5±0.01	0.5±0.01
	0.9	-	-	-	-

Minimum inhibitory concentration (MIC) was determined as the lowest concentration of ex-plant extracts inhibiting the growth of the organism. All the experiments were performed in triplicate.

Statistical analysis

For the experiment, a completely randomized design with 3 replicates was used. The SAS program was used for analysis of variance of antifungal activity of *C. ternatea* extracts at $P \leq 0.05$.

Results and Discussion

Methanolic and ethanolic seeds, callus, pot-grown and tissue-cultured leaf extracts

Table 1-3 indicated the zone of inhibitions by extracts of seeds, leaves and callus against fungal strains. The methanolic and ethanolic seeds extract through circulatory extraction showed inhibition zone of 1.4 cm to 1.3 cm against *A. niger*, respectively. Whereas, leaves methanolic extracts from pot-grown plant showed 1.2 cm inhibition zone against *A. niger*. The callus extracts of *C. ternatea* showed MIC against *A. niger* and *A.solani* at high concentration of 0.7 mg mL^{-1} and the inhibition zone was 0.1 cm. The methanolic and ethanolic extract of *C. ternatea* seeds by circulatory extraction process showed inhibition zone at 0.2 mg mL^{-1} against fungal strains than callus, pot-grown and *in vitro* leaves extract. At the highest concentration of 0.9 mg mL^{-1} callus extracts showed 0.4 cm zone of inhibition against *A. niger*. The concentrations range of 0.1 mg mL^{-1} to 0.5 mg mL^{-1} of methanolic and ethanolic callus extracts didn't show inhibition zone against fungi. Present results were in uniformity with findings of Kamilla *et al*. (2009) and Mhaskar *et al*. (2010).

Comparison between antifungal activity of leaf extracts of pot-grown and tissue-cultured plants

It is evident from Table 3 that both the pot-grown and tissue-cultured leaf extracts didn't show any response against *Rhizopus* sp. The extracts from leaves of plants grown in pots showed high inhibition zone than tissue-cultured leaves extracts against *A. niger* and *A. solani* with the values of 0.8 cm and 0.6 cm, respectively. The minimum inhibition concentration of methanolic and ethanolic *in vivo* leaves extracts was 0.5 mg mL^{-1} against *A. niger* but ethanolic *in vitro* leaves extracts against *A. niger* showed 0.6 mg mL^{-1}.

The results of the present study conclude that the extracts of *C. ternatea* were effective against *A. niger* and *A. solani*, but not against *R. arrhizus.* The methanolic extracts obtained through circulatory extraction from seeds and pot-grown leaves of *C. ternatea* showed maximum inhibition zone against *A. niger*. The *C ternatea* seeds extract was more resistant against fungal strains than the leaves extracts.

References

Bishnupada B, Saha D, Koley A, Sur D, Jana SB, Jena A, Sarkar A, 2011. Anti-fungal activity of leaf extract of *Derris indica*. *Int. J. Appl. Biol. Pharm. Technol*., **2**: 30-32.

Kamilla L, Mansor SM, Ramanathan S, Sasidharan S, 2009. Effects of *Clitoria ternatea* leaf extract on growth and morphogenesis of *Aspergillus niger*. *Microsci. Microanal*., **15**: 366-372.

Malabadi RB and Nataraja K, 2001. Shoot regeneration in leaf explants of *Clitoria ternatea* L. cultured *in vitro*. *Phytomorphology,* **51**: 169-171.

Mhaskar AV, Prakesh K, Vishwakarma KS, Maheshwari VL, 2010. Callus Induction and antimicrobial activity of seed and callus extracts of *Clitoria ternatea* L., *Curr. Trends Biotechnol. Pharm*, **3**: 561-567.

Mukherjee PK, Balasubramanian P, Saha K, Saha PB, Pal M, 1995. Antibacterial efficiency of *Nelumbo nucifera* (Nymphaeaceae) rhizomes extract. *Indian Drugs*, **32**: 274-276.

Mukherjee PK, Kumar V, Kumar NS, Heinrich M, 2008. The Ayurvedic medicine *Clitoria ternatea* from traditional use to scientific assessment. *J. Ethnopharmcol*., **120**: 291-301.

Ponnusamy S, Gnanaraj WE, Antonisamy JM, Selvakumar V, Nelson J, 2010. The effect of leaves extracts of *Clitoria ternatea* Linn. against the fish pathogens. *Asian Pac. J. Trop. Med.*, **3**: 412-420.

Shahid M, Shahid A, Anis M, 2009. Antibacterial potential of the extracts derived from leaves of medicinal plants Pterocarpus marsupium Roxb., *Clitoria ternatea*. *Orient. Pharm. Exp. Med.*, **9**: 174-181.