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ANTIFUNGAL AND PHYTOTOXIC PROPERTIES OF CRUDE METHANOLIC EXTRACT AND VARIOUS FRACTIONS FROM *STROBILANTHES URTICIFOLIA WALL. EX KUNTZE*

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Abstract

In the present study crude methanolic extract and various fractions (n-Hexane, chloroform, ethyl acetate and aqueous) of Strobilanthes urticifolia Wall.ex Kuntze were used to investigate its antifungal and phytotoxic properties. Antifungal assay was carried out against seven pathogenic fungal strains (Aspergillus niger, Fusarium oxysporium, Triticum harzianum, Rhizopus stolonifera, Alternaria solani, Candida albicans, Microsporum canis) using tube dilution method. The crude methanolic extract and various fractions showed low to good activities against the fungal strains while only aqueous fraction showed significant activity against Candida albicans. Phytotoxic assay was used to investigate the phytotoxic property of the plant extracts against the Lemna minor at different concentrations (10,100 and 1000 µg/ ml) and showed highest phytotoxic activity at the concentration of 1000 µg/ml. The results obtained from in vitro studies of antifungal and phytotoxic activities clearly inferred that the crude methanolic extract and various fractions of Strobilanthes urticifolia Wall.ex Kuntze have significant properties against fungi and phytotoxic substances. For future perspective, extensive work will be needed, for the isolation and characterization of active ingredients from S. urticifolia to facilitate its pharmacological assessment.

Key words: *Strobilanthes urticifolia Wall.ex Kuntze, crude methanolic extract, fractions, antifungal, phytotoxic*

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INTRODUCTION:

Infectious diseases are one of the major problems of the world and almost 57 million lives were affected because of these diseases worldwide every year [1]. In last three decades, many new drugs have been produced by pharmaceutical industries but due to the expansion of toxic properties and microbial confrontation to the chemically produced drugs, men turned to ethnopharmacognosy [2]. Medicinal plants not only possess a history of regular use as medicine throughout the subcontinent [3] but the use of herbal medicine for therapeutic purpose is attaining a worldwide popularity [4,5]. A large portion of the world population; especially developing countries depends upon the traditional system of medicine for a variety of infectious diseases. In general, the varieties of plant groups are used in traditional medicine and it is an essential source of potent and powerful drugs in tropical countries [6]. The world health organization's statistics has revealed that more than 85% of the world population uses traditional herbal medicine for treatment of various health concerns [7].

The purpose of the present study is to evaluate antifungal and phytotoxic activities of the crude methanolic extracts and various fractions of *Strobilanthes urticifolia Wall.ex Kuntze*. Antifungal assay was tested against standard fungal strains of Aspergillus niger, Fusarium oxysporium, Triticum harzianum, Rhizopus stolonifera, Alternaria solani, Candida albicans, Microsporum canis while phytotoxic assay was screened against lemna minor plant.

MATERIALS AND METHODS:

Plant Material and Extraction

The plant sample was collected from Swat, the Northern region of Pakistan in 2016 and identified by Mr. Ghulam Jelani, a taxonomist at the Department of Botany, University of Peshawar, Khyber Pakhtunkhwa, Pakistan. The plant materials (12 kg) were air dried in the shade at room temperature and then crushed to powder. Dry powder was subjected to soaking using methanol for 15 days, twice, and then filtered using Whatman No. 1 filter paper. The extract was concentrated using rotary evaporator under reduced pressure at 40°C (Farid et al., 2017). Methanolic extract (880 g) was mixed with 500 ml distilled water and soaked for 24 hours, then partitioned with n-hexane, CHCl3, EtOAc to obtain their respective soluble fractions. The remaining was considered as water soluble fraction i.e. aqueous fraction.

Antifungal activity

Antifungal assay was performed by using agar tube dilution method [8] against *Aspergillus niger, Fusarium oxysporium, Triticum harzianum, Rhizopus stolonifera, Alternaria solani, Candida albicans, Microsporum canis.* The stock solution of crude plant extract and various fractions were prepared in DMSO (<1%) at concentration of 24 mg/ml. For the growth of fungal species, Sabouraud dextrose agar (SDA) was prepared and autoclaved. Upon cooling of the media to about 40-45°C, 4 ml sterile medium was transferred to each test tube and plant extracts (66.66 µl) were added to it. Test tubes were placed in slanting position to solidity and incubated overnight at 28±1°C for sterility check. Next, 5-7 days old cultured fungal strains were inoculated into test tubes using flame sterilized inoculating loop. DMSO was used as negative control and Miconazole as positive control. The % inhibition was calculated as:

$$\text{Percent Inhibition} = 100 - \frac{\text{Linear growth in test (mm)}}{\text{Linear growth in control (mm)}} \times 100$$

Phytotoxic Activity

The phytotoxic activity of the test samples (methanolic crude extract and various fractions) were screened against Lemna minor [9]. Stock solutions (20 mg/ml) of crude methanolic extract and fractions were prepared in methanol and E-medium was prepared for the growth of L. minor. 10, 100 and 1000 µg/ml from the stock solutions were transferred to three petri plates and kept at room temperature until the organic solvent was evaporated. 20 ml of the E-medium and ten healthy mature L. minor plants with a rosette of three fronds were added to each petri plate and incubated at 28 ± 1°C in a growth chamber for one week. Paraquat (0.015 µg/ml) was used as positive control while E-Medium and L.minor only used as negative control. Results were recorded after seven days of incubation. The % growth inhibition was calculated as:

$$\text{Growth inhibition (\%)} = 100 - \frac{\text{No. of fronds in sample}}{\text{No. of fronds in control}} \times 100$$

RESULTS AND DISCUSSION:

Antifungal activity

The antifungal activity was determined using the agar tube dilution method by measuring the linear growth of fungus on the SDA slant and by calculating the

percentage inhibition. The (Fig.1). Our analysis indicated that the aqueous fraction of *S. urticifolia* showed highest activity against C. albicans (82.33%), A.solani (74.22%), R.stolonifer (67.18%) and less growth inhibition against M.canis (10.27%), T.harzianum (12%) and A.niger (16.55%) as compared to other extracts while exhibited moderate activity against F.oxysporum (33.21%). The antifungal activity was moderate for the methanolic (A.niger, T.harzianum, R.stolonifer, C. albicans and M.canis with percentage inhibition of 20.35%, 30%, 25.5%, 32.15% and 30.21% respectively), n-hexane (A.niger, F.oxysporum, T.harzianum, R.stolonifer, A.solani, C. albicans and M.canis with percentage inhibition of 25%, 22%, 33.27%, 20%, 28.15%, 22.35% and 28.10% respectively), chloroform (F.oxysporum, T.harzianum, R.stolonifer, A.solani, C. albicans and M.canis with percentage inhibition of 20.43%, 23.32%, 36.11%, 20%, 22% and 25.31% respectively) and ethyl-acetate (F.oxysporum, T.harzianum, A.solani, C. albicans and M.canis with percentage inhibition of 25%, 20%, 22.25%, 36.50% and 34% respectively) extracts. Further, the methanolic (F.oxysporum and A.solani with percentage inhibition of 55.5% and 47.26% respectively) chloroform (A.niger with percentage inhibition of 45.30%) and ethyl-acetate (A.niger and R.stolonifer with percentage inhibition of 40.16% and 44.50% respectively) extracts showed good inhibitory activity against various fungal strains. The presented result may have indicated that the tested extracts showed different activity for each fungal strain studied.

Table 1: Antifungal activity of *Strobilanthes urticifolia Wall.ex Kuntze*

Fungal Species	Percent inhibition (400µg/ml)						
	Negative control	Positive control	Cr. Met. Ext.	*n*-hexane Fraction	Chloroform Fraction	Ethyl acetate Fraction	Aqueous Fraction
A.niger	0	100	20.35	25	45.30	40.16	16.55
F.oxysporum	0	100	55.5	22	20.43	25	33.21
T.harzianum	0	100	30	33.27	23.32	20	12
R.stolonifer	0	100	25.5	20	36.11	44.50	67.18
A.solani	0	100	47.26	28.15	20	22.25	74.22
C. albicans	0	100	32.15	22.35	22	36.50	82.33
M.canis	0	100	30.21	28.10	25.31	34	10.27

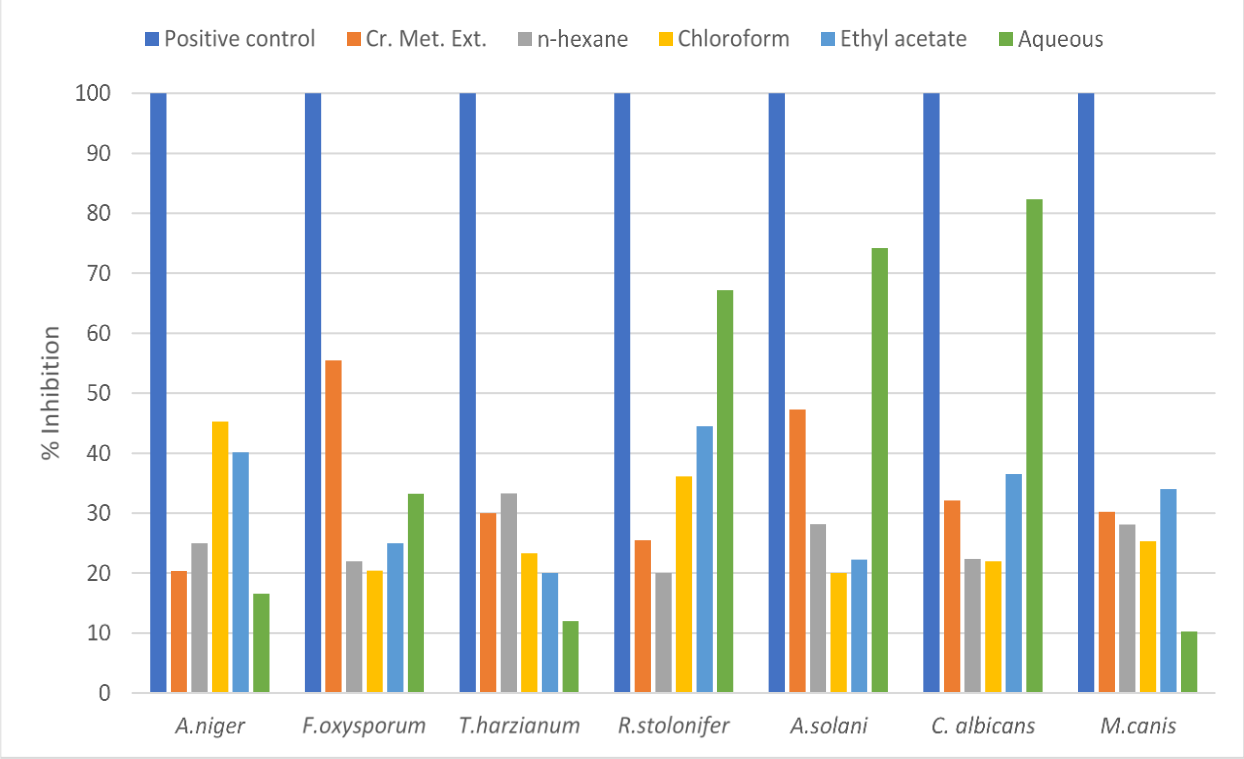

Fig. 1: Antifungal assay of *Strobilanthes urticifolia Wall.ex Kuntze*

Phytotoxic Activity

The result of crude methanolic extract and various fractions against *L. minor* are summarized in Figure 2. The phytotoxic activity of crude methanolic extract showed (40 and 30%), n-hexane (50 and 30%), EtOAc (40 and 20%) while CHCl3 and aqueous fractions showed (50 and 20%) growth inhibition at concentrations of 1000 and 100 µg/ml, respectively. The extracts were not toxic or slightly toxic at 10 µg/ml.

Table 2: Phytotoxic activity of *Strobilanthes urticifolia Wall.ex Kuntze*

Name of plant	Concentration of sample (µg/ml)	Percent Growth Inhibition					
		Cr. Met. Ext.	*n*-hexane	Chloroform	Ethyl acetate	Aqueous	Control
Lemna minor	1000	40	50	50	40	50	100
	100	30	30	20	20	20	100
	10	10	0	0	0	10	100

*Paraquat was used as a standard drug (µg/ml)

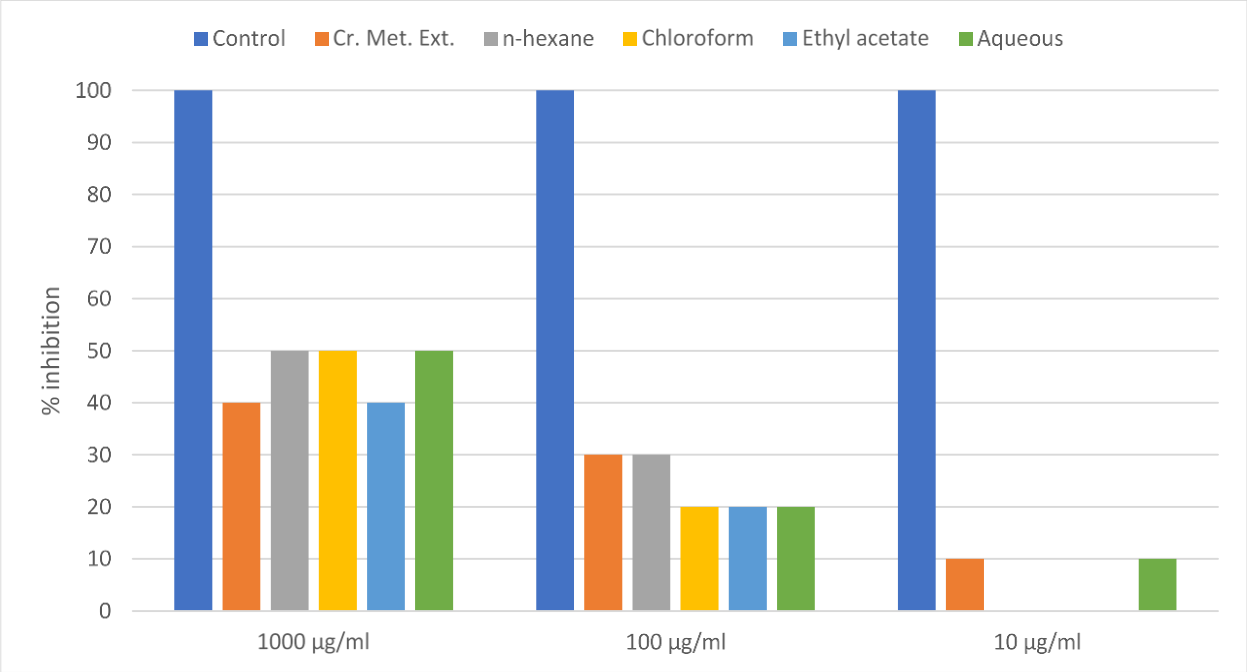

Fig. 2: Phytotoxic assay of *Strobilanthes urticifolia Wall.ex Kuntze*

CONCLUSION:

From the results of the present study, it can be concluded that the methanolic, n-hexane, chloroform and aqueous extracts possess antifungal and phytotoxic potential. However, further studies should be done to isolate the active constituents from the extracts in order to investigate their mechanism of action and potential to be developed as antifungal and herbicide agents.

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