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Antimicrobial Activity of *Solanum torvum* Swart. Against Important Seed Borne Pathogens of Paddy

[1]V. Lalitha, [2]K.A. Raveesha and [3]B. Kiran

[1]Department of Studies in Botany and Microbiology,
Maharanis Science College for Women, Palace Road, Bangalore Karnataka State, India
[2]Department of Studies in Botany, Manasagangotri,
University of Mysore, Mysore- 570 006, Karnataka State, India
[3]Department of Microbiology and Biotechnology,
CMR Institute of Management Studies, Kalyan nagar, Bangalore

Abstract: Aqueous and Solvent extracts of leaves of *S. torvum viz.*, Petroleum ether, Benzene, Chloroform, Methanol and Ethanol were tested *in vitro* for antimicrobial activity following the procedures of poisoned food technique and cup diffusion method against some important seed borne pathogens of paddy viz, *Pyricularia oryzae, Alternaria alternata, Bipolaris oryzae, Tricoconis padwickii, Dreschlera tetramera, D. halodes, Curvularia lunata, F. oxysporum, F. moniliformae, F. solani and Xanthomonas oryzae.* Aqueous extracts of leaves (at 25% concentration) showed 100, 47.44, 60.47, 71.50, 56.11, 63.33, 78.62, 66.66, 47.44 and 46.27% inhibition of the test pathogens respectively. Highly significant antifungal activity was observed in Methanolic and ethanolic extract. The percentage inhibition of the test pathogenic fungi in Methanolic extract was 100% (*P.oryzae*), 74.42 (*A. alternata*), 65.68 (*B oryzae*), 87.62 (*C. lunata*), 100% (*T.* padwickii), 63.33 (*D.halodes*), 60.31 *D. (tetramera*), 76.01 (*F.moniliformae*), 59.21 (*F. oxysporum*), 43.91% (*F.solani*) and zone of inhibition of *Xanthomonas campestris pv oryzae* was 18 and 30mm in Methanol and ethanol extract.

Key words: Seed borne pathogens % Antimicrobial activity % *Solanum torvum* % Paddy

INTRODUCTION

The search for naturally occurring materials with biological activity and the use of naturally occurring antifungal substances in plant chemotherapy is gaining more importance [1]. Plants contain hundred or thousands of metabolites. Medicinal and aromatic plants a gift of nature are being used against various infections and diseases in the world since past history [2]. Among the estimated 2, 50, 000 to 5, 00, 000 plants species, only a small percentage has been investigated phytochemically and the fraction submitted to biological screening is even smaller. Plant kingdom represents an extraordinary reservoir of novel molecules. Plant derived products have been used for medicinal purposes for centuries [3] and plants have been an important source of medicine for thousands of years [4]. About three quarter of the world's population relies on plants and plant extracts for healthcare [5]. The World Health Organisation estimated that 80% of the population of developing countries still relies on traditional medicines [6]. Higher plants are a treasure house of phytochemicals which serve as valuable drugs that helped combat several fatal diseases world over. Increasing resistance of many pathogens to currently available synthetic pesticides has become a serious problem around the globe. Because of the strict requirements of their efficacy, selectivity, toxicology and general impact on the environment [7, 8]. The presence of phytochemicals in medicinal plants have attracted a great deal of attention, concentrated on their role in preventing diseases [9]. The antimicrobial activity of plants extracts has formed the basis of many applications in food preservetions, pharmaceuticals, alternative medicines and natural therapies[10]. Researchers are increasingly turning

Corresponding Author: V. Lalitha, Department of Studies in Botany and Microbiology, Maharanis Science College for Women, Palace road, Bangalore, Karnataka State, India. E-mail: Lali76v@rediffmail.com, Tel: +91-9844142520

their attention to natural products looking for new leads to develop better drugs against microbial infections [11]. In Philippines an estimated 1.2 million hectares of crop land which is roughly 1/4th of the total area under cultivation has been severely degraded by the application of synthetic pesticides and chemical fertilizers [12]. The latest WHO figures suggests that at least 3 million and perhaps 25 million agricultural workers are prone to poison each year by pesticides and some 20,000 deaths. Thus it is necessary to search for natural pesticides for sustainable agriculture and healthy environment [13]. Consequently there is an increasing interest in evaluating other mechanisms of control including the effect of plant metabolites. Many angiosperm plants are store houses of effective chemotherapeutants and results of biological screening of these plants for a wide range of activities proved that these can be used for treating diseases [14-17]. One such plant which has shown significant antimicrobial activity during preliminary screening was *Solanum torvum*. Hence a detailed systematic investigation was conducted to test *in vitro* the antimicrobial activity against important seed borne pathogens of paddy.

MATERIALS AND METHODS

Cultures: Important seed borne pathogens of Paddy viz., *Pyricularia oryzae, Bipolaris oryzae, Alternaria alternata, Tricoconis padwickii, Drechslera tetramera, D.halodes, Curvularia lunata, Fusarium moniliformae, F.oxysporum, F.solani* and *Xanthomonas oryzae* were isolated from infected paddy seeds by standard procedures [18] and pure cultures were maintained for further studies.

Aqueous Extract: One hundred grams of fresh leaves of *Solanum torvum* free from diseases were collected, washed and macerated with 100ml sterile distilled water in a waring blender (waring international, new hart-ford, CT, USA) for 5mins. The macerate was filtered through double layered muslin cloth and then centrifuged at 4000g for 30 minutes. The supernatant was filtered through Whatman No.1 filter paper and sterilized at 120°C for 10minutes, which served as mother aqueous extract (100%). The extract was preserved aseptically in a brown bottle at 4°C until further use [9].

Solvent Extract: Fresh disease free leaves were collected, washed, shade dried and pulverized to obtain fine dry powder. The dry powder of the plant material was extracted successively with different solvents viz., Petroleum ether, Benzene, Chloroform, Methanol and Ethanol depending on its polarity using soxhlet apparatus and it is condensed under reduced pressure in rotary flash evaporator to serve as mother extract. The extracts were preserved in airtight brown bottle until further use for antifungal activity and antibacterial activity assay [20].

Antifungal Activity Assay: The antifungal activity of aqueous and solvent extracts was tested against ten important seed borne fungal pathogens of paddy. The antifungal activity was tested by poisoned food technique [10,21,22].

Antibacterial Activity Assay: The antibacterial activity of aqueous and solvent extracts was tested against *Xanthomonas campestris* pv *oryzae* by cup diffusion method [16]. Streptomycin disc (25mcg), Gentamycin and Co-trimaxazole antibiotics were also tested for comparative efficacy.

Statistical Analysis: The data were subjected to Tukey's HSD analysis. Data on percentages were transformed to arcsine and analysis of variance (Anova) was carried out with transformed values. The means were compared for significance using Tukey's HSD (P=0.05) .

RESULT AND DISCUSSION

Antifungal Activity Assay

Aqueous Extract: The results revealed a significant inhibitory activity. The percentage inhibition of test pathogenic fungi in aqueous extract at 25% concentration was 100% for (*P.oryzae*), 47.44 for (*B.oryzae*), 60.47 for (*A.alternata*), 71.50 for (*T padwickii*), 56.11 for (*D. tetramera*), 63.33 for (*D.halodes*), 66.66 for (*F.moniliformae*), 47.44 for (*F.oxysporum*) and 46.26 for (*F.solani*) (Table 1).

Solvent Extract: Chloroform, Methanol and Ethanol extracts showed antifungal activity. The activity was highly significant in Methanol and Ethanol extracts. The concentration of 1000ppm was effective in mycelial inhibition of test pathogens (Table 1). Methanol extract recorded more than 90% and least 43.91 inhibitions of test pathogens at 1000ppm. Ethanolic extract also revealed significant activity against test pathogens. Cent percent inhibition observed in *P.oryzae* and more than 50% inhibition observed in rest of the pathogen.

Table 1: Effect of Aqueous and solvent extracts of *Solanum torvum* swart. against important seed borne fungal pathogens of paddy

		Percent Inhibition					
		Chloroform extract		Methanol extract		Ethanol extract	
Organisms	Aqueous extract (25%)	500ppm	1000ppm	500ppm	1000ppm	500ppm	1000ppm
P. oryzae	100.00 ± 0.00^{h}	69.10±0.81^{i}	68.97±0.68^{efg}	80.95±0.95^{i}	100.00±0.00^{h}	79.79±0.86^{g}	100.00±0.00^{h}
B. oryzae	47.44±0.39^{b}	22.08±0.40^{b}	24.49±0.40^{ab}	49.80±0.39^{ab}	65.68±0.39^{a}	16.96±0.60^{a}	67.87±0.60^{b}
A. alternata	60.47±0.47^{d}	44.05±0.69^{de}	44.66±0.66^{c}	68.94±0.45^{f}	74.42±0.45^{e}	38.19±0.69^{c}	50.69±0.69^{c}
T. padwickii	71.50±0.60^{f}	29.52±0.95^{c}	50.47±0.47^{cd}	71.85±0.74^{h}	100.00±0.00^{h}	17.77±0.11^{a}	44.44±0.11^{a}
C. lunata	56.11±0.42^{c}	40.44±0.44^{d}	52.44±0.44^{cd}	44.08±0.54^{de}	60.31±0.49^{d}	27.77±0.69^{b}	81.66±0.83^{g}
D. tetramera	63.33±0.83^{d}	77.43±0.51^{j}	77.43±0.51^{g}	50.83±0.83^{e}	63.33±0.83^{d}	57.77±0.11^{f}	82.22±0.11^{g}
D. halodes	78.62±0.42^{g}	53.45±0.62^{f}	65.07±0.79^{ef}	78.97±0.51^{fg}	87.62±0.53^{g}	35.65±0.77^{c}	77.51±0.77^{f}
F. oxysporum	66.66±0.71^{e}	54.66±0.66^{f}	62.66±0.66^{ef}	75.55±0.55^{fg}	76.01±0.58^{ef}	48.60±0.69^{de}	71.52±.0.69^{de}
F. moniliformae	47.44±0.39^{b}	63.80±0.95^{h}	92.37±0.95^{h}	35.68±0.39^{c}	59.21±0.39^{c}	48.44±0.44^{de}	67.10±0.44^{d}
F. solani	46.27±0.39^{bc}	26.66±0.41^{c}	31.66±0.41^{b}	42.03±0.80^{d}	43.91±0.39^{ab}	30.41±0.41^{bc}	44.16±0.41^{a}

C Values are the means of three replicates ± standard error.

C P<0.001

C In column 'a-h' means with different letters are significantly different each other.

Table 2: Effect of solvent extracts of *Solanum torvum* against *Xanthomonas campestris* pv *oryzae*

	Concentration (µ lt) >< zone of inhibition (mm)									
Solvent extracts	10	20	30	40	50	60	70	80	90	100
Petroleum ether	-	-	-	-	-	-	-	-	-	-
Benzene	-	-	-	-	-	-	-	-	-	-
Chloroform	-	-	-	-	-	-	-	-	-	-
Methanol	10mm	12	12	15	15	15	18	18	18	18mm
Ethanol	12mm	19	19	20	20	22	26	26	28	30mm

Standard: Streptomycin -29mm

Gentamycin -35mm

Co-trimaxazole -35mm

C Values are the means of three replicate.

Antibacterial Activity Assay: Only Methanolic and Ethanolic extracts of *S.torvum* showed the activity against *Xanthomonas campestris* pv *oryzae* at different concentrations. The antibacterial activity, measured as the zone of inhibition of Methanolic and Ethanolic extracts of *Solanum torvum* and the antibiotics on the pathovar *X. oryzae*, shown in Table 2. The maximum zone of inhibition in Methanolic extract of *S.torvum* was 19mm at 100µlt concentration and minimum 10mm at 10µlt concentration.

Ethanolic extract of *S.torvum* was highly active compared to methanolic extract against *X.oryzae* where zone of inhibition was 30mm at 100µlt concentration and can be compared with commercial synthetic antibiotic, where Streptomycin, Gentamycin and Co-trimaxazol showed 29mm, 35 and 35mm zone of inhibition against *X. oryzae* (Table 2). *In vitro* screening of *S.torvum* has given encouraging results, indicating this as the potential candidate plant for further work on isolation and characterization of the antimicrobial compound and its subsequent exploitation for the management of seed borne pathogens of paddy.

Seeds serve as important microcosm for saprophytic and pathogenic micro-organisms and paddy seeds are no exception to this. Fungi and bacteria invade and colonize paddy seeds at pre and post harvest stages causing considerable loss in yield and quality [23]. To overcome the loss due to these seed borne phytopathogenic fungi, many synthetic fungicides are used, which are effective and efficient, but often are not ecofriendly due to their toxicity and biological magnification in food chain. Similarly to protect the paddy crop from seed borne phytopathogenic bacteria a few synthetic antibiotics have to be applied which is generally cost prohibitive and also not ecofriendly. Consequently, efforts are underway to evaluate other mechanism of control including the effect of plant metabolites on plant pathogens to develop alternative ecofriendly approaches to crop disease management [9,15,21,24,25,26].

The present investigation not only demonstrates the antimicrobial activity of *S.torvum* but also reveal that the extract can significantly control the seed borne pathogenic fungi and bacteria. The results also indicated the necessity for further investigation to isolated and characterize active principle responsible for the activity and its subsequent exploitation for paddy disease management.

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