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Achyranthes aspera L.: As a Source of Bio-fungicide

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ABSTRACT

Achyranthes aspera is a herb in amaranthaceae family, traditionally used in treatment of several diseases (inflammation, diabetes, hypertension, wounds, pain, pneumonia, diarrhea, dysentery, asthma, cough, dropsy, ulcers, piles, rheumatism, scabies and other skin diseases. and fever etc). The present study was carried out to investigate the antimicrobial activities of the methanolic extract and to investigate phytochemical profile of aqueous extract of vegetative parts of *Achyranthes aspera*. The methanol extracts were screened for antifungal activity against four fungi *Fusarium oxysporum*, *Alternaria solani, Athelia rolfsii and Rhizoctonia solani* by food poisoning method. The extracts of fresh *Achyranthes aspera* showed a significant and remarkable activity against all these four fungal species when compared to standard. The observed IC_{50} values for these funguses are 43.75µg/ml for *Fusarium oxysporum,* 27.5µg/ml for *Alternaria spp.,* 19.37µg/ml for *Sclerotium rolfsii.* And 18.75µg/ml for *Rhizoctonia solani*. The phytochemical screening of the aqueous extract indicates that the fresh plant contain tannin, phlobatannin, terpenoid, flavonoid, cardiac glycoside, phenol and alkaloid. Phytochemical screening indicate the absence of saponin, steroid, free anthraquinone, and carbohydrate.

Keywords: Medicinal plants, *Achyranthes aspera,* Antimicrobial activity, Phytochemical screening, Plant pathogen.

INTRODUCTION

Generally plant resources constitute an important natural wealth of a country. They play a significant role in providing primary health care services to rural people in addition to large number of economic products. It is in fact secondary metabolites, like alkaloids, glycosides, tannins, phlobatannin, terpenoid, flavonoid, phenol, volatiles oils and many more compounds which serve as important therapeutic agents. According to the World Health Organization, more than 80 % of the world's

population relies on traditional herbal medicine for their primary health care[1]. These medicines are relatively safer and cheaper than synthetic or modern medicine.

Herbal remedies used in folk medicine provide an interesting and still largely unexplored source for the creation and development of potentially new drugs for therapy which might help overcome the growing problem of resistance and also the toxicity of the currently available commercial antibiotics/pesticides and discovery of new bioactive compounds. Therefore, it is of great interest to carry out a screening of these plants in order to validate their use in folk medicine and to reveal the active principle by isolation and characterization of their constituents. In this background *Achyranthes aspera* used as herbal drugs has been identified and studied for phytochemical screening and antimicrobial studies[2].

Achyranthes aspera L. (Amaranthaceae) is an important medicinal herb found as a weed throughout India. Though almost all of its parts are used in traditional systems of medicines, seeds, roots and shoots are the most important parts which are used for their medicinal properties[3].

Achyranthes aspera is a species of plant in the Amaranthaceae family. The plant is a perennial stiff erect herb, 0.2-2.0 m high, is growing up to 1000 m height. Stems are square, leaves elliptic ovate or broadly rhombate, 5-22 cm long, 2-5 cm broad, and adpressed pubescent. The inflorescences are 8-30 cm long, with many single, white or red flowers, 3-7 mm wide. Flowering time is in summer. Wide numbers of phytochemical constituents have been isolated from the plant which possesses activities like antiperiodic, diuretic, purgative, laxative, antiasthmatic, hepatoprotective, anti-allergic and various other important medicinal properties. Traditionally, the plant is used in pneumonia, diarrhea, dysentery, asthma, cough, dropsy, ulcers, piles, rheumatism, scabies, snake bite and other skin diseases[4]. A fresh piece of root is used as tooth brush. It is one of the 21 leaves used in the Ganesh Patra Pooja done regularly on Ganesh Chaturthi day.

Pathogenic fungi are the main infectious agents in plants, causing alterations during developmental stages including post-harvest

Fusarium oxysporum is a large genus of filamentous fungi widely distributed in soil and in associated with plants. It causes Fusarium wilt disease in various plant. The host include Tomato, tobacco, cucurbits, sweet potatoes and banana. *Fusarium* generally produces symptoms such as wilting, chlorosis, necrosis, premature leaf drop, browning of the vascular system, stunting and damping off. The most important of these are vascular wilt[5].

Alternaria solani is fungal pathogen that produces a disease in tomato and potato plants called early blight. Distinguishing symptoms of *A. solani* include leaf spot and defoliation, which are most pronounced in the lower canopy[6].

Sclerotium rolfsii, an omnivorous, soilborne fungal pathogen, causes disease on a wide range of agricultural and horticultural crops. Susceptible agricultural hosts include sweet potato, pumpkin, corn, wheat and peanut. Mature plants are attacked just below the soil surface and are completely girdled. The mycelium often grows over the diseased tissue and surrounding soil forming a white mat of mycelial threads with the typical tan-to-brown, mustard-seed-sized sclerotia. The tops wilt and die rapidly, often the entire root system is destroyed. Slightly sunken, yellow spots develop on invaded fruit, which rapidly decay, collapse, and become covered by a white fungal mass with numerous sclerotia[7].

Rhizoctonia solani is a soil-borne plant pathogenic fungus causes rot disease with a wide host range and worldwide distribution. *Rhizoctonia solani* is best known to cause various plant diseases such as collar rot, root rot, damping off and wire stem. *Rhizoctonia solani* attacks its host (s) when they are in their juvenile stages of development such as seeds and seedlings, which are typically found in the soil. The most common symptom of *Rhizoctonia* is "damping off", or the failure of infected seeds to germinate. *Rhizoctonia solani* can also cause hypocotyl and stem cankers on mature plants of tomatoes, potatoes and cabbage. Roots will turn brown and die after a period of time[8].

Name of the fungus and disease caused by them with their host

See table 4.

MATERIALS AND METHODS

Collection of the plant

The plant *Achyranthes aspera* were collected from the Lalpur locality of the city Ranchi (Jharkhand). The taxonomic identity of this plant was confirmed at Department of Botany, Ranchi University.

Drying

For extraction, the freshly collected plant were thoroughly washed with tap water followed by sterile distilled water. Then the collected plant samples were sad dried inside the hot air oven at 50°C, and then samples were powdered using mechanical grinder.

Preparation of methanolic plant extract

The dried plant powder was taken on a conical flask and was soaked with methanol (the volume of methanol was 10 times than the amount of plant powder. The flask was covered with aluminum foil to avoid evaporation and then kept for 48 hours. After 48 hours the solution was filtered by using whatman filter paper no.1 and the filtrate was collected in a beaker. Then the filtrate was kept under room temperature to evaporate the solvent (methanol) until a gummy substance was obtained. 128 mg of *A. aspera* extract was obtained from 5 g of dried leaf powder. The prepared extract was then stored for further use.

Antimicrobial assay

Methanolic plant extracts of *A. aspera* thus obtained were immediately evaluated for antifungal activities using poisoned food technique.

Food poisoned technique

The antifungal activity of plant extracts was evaluated against food-associated fungi by using poisoned food technique. In poisoned food technique, all the food-associated fungi were inoculated on Potato dextrose agar (PDA) plates and incubated for 25^0 C for 3 to 7 days, to obtain young, actively growing colonies of fungus.

Different concentration (12.5, 25, 37.5, 50, 62.5 µg/ml) of the plant extract were mixed with 16 ml of cooled (45^0 C) molten PDA medium and allowed to solidify at room temperature. One more plate containing only media without any plant extract was taken as positive control. A mycelium from periphery of 3 to 7 day old cultures, was aseptically inoculated with the help of inoculation loop onto the agar plates containing the plant extract. The plates were then sealed. The inoculated plates were then incubated at 25^0 C. After 48 hours the colony diameter were regularly measured and recorded at an interval of 24 hours.

CALCULATION

The percentage zone of inhibition was calculated using the formula-

$\%\ \text{Zone of inhibition} = G_C - G_T / G_C \times 100.$

Where, GC = Growth of mycelia colony in control set,

GT=Growth of mycelia colony in treatment set.

Phytochemical screening

Chemical tests were carried out on the aqueous extract and on the powdered specimens using standard procedures to identify the constituents[9,10].

Test for tannins

About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

Test for phlobatannins

Deposition of a red precipitate when an aqueous extract of each plant sample was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins.

Test for terpenoids (Salkowski test)

Five ml of each extract was mixed in 2 ml of chloroform, and concentrated H_2S0_4 (3 ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

Test for saponin

About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

Teat for flavonoids

Three methods were used to determine the presence of flavonoids in the plant sample. 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H_2S0_4. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing.

Few drops of 1% aluminium solution were added to a portion of each filtrate. A yellow colouration was observed indicating the presence of flavonoids.

A portion of the powdered plant sample was in each case heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration was observed indicating a positive test for flavonoids.

Test for Cardiac glycosides (Keller-Kiliani test)

Five ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Test for phenols

Crude extract was mixed with 2ml of 2% solution of $FeCl_3$. A blue green or black coloration indicated the presence of phenols.

Test for steroids

Two ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml H_2S0_4. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Test for alkaloids

Crude extract was mixed with 2ml of 1% HCl and heated gently. Mayer's reagent was then added to the mixture. Turbidity of

the resulting precipitate was taken as an evidence for the presence of alkaloids.

Test for free anthraquinone

Take 5 ml of plant extract and add 5 ml of chloroform and shake for 5 minute. Filter the solution. The filtrate solution were again shaken with equal volume of 10% ammonia solution, these experiment show the colour of bright pink colour in the aqueous layer it determined the presence of free anthraquinones.

Test for carbohydrates

Crude extract was mixed with 2ml of iodine solution. A dark blue or purple colouration indicated the presence of the carbohydrate.

RESULTS

Screening of the plant extract for antimicrobial activity

Six different concentration (6.25, 12.5, 25, 37.5, 50, 62.5 µg/ml) of methanolic extract of *Achyranthes aspera* used to test against four fungi, namely *Fusarium oxysporum, Alternaria spp., Sclerotium rolfsii*, *and Rhizoctonia solani,* The fungal (mycelia) growth (cm) on different concentration of plant extract are presented in table no. 1; the selected fungi showed growth rate in the order *Sclerotium rolfsii* > *Rhizoctonia solani* > *Alternaria spp* > *Fusarium oxysporum.* Growth inhibition percent and IC_{50} value are calculated to compare with control (table 2, graph 1). The fungal growth decreased with the increasing concentration of plant extract in media. *Rhizoctonia solani* show the highest sensitivity to plant extract, the IC_{50} value was observed at 18.75 µg/ml concentration of plant extract; at the concentration of 62.5 µg/ml complete growth was inhibited. The methanolic extract of *A. aspera* show IC_{50} value against *Sclerotium rolfsii* and *Alternaria spp.* at the concentration of 19.37 µg/ml and 27.5 µg/ml respectively whereas *Fusarium oxysporum* are less sensitive as compare to other fungal stain, 50 percent inhibition was obtained at 43.75 µg/ml concentration. Thus the sensitivity of these fungus against the plant *Achyranthes aspera* methanolic extract in the decreasing order is *Rhizoctonia solani* > *Sclerotium rolfsii* > *Alternaria spp* > *Fusarium oxysporum.* Thus among the four fungus strain the most sensitive is *Rhizoctonia solani* and the less sensitive is *Fusarium oxysporum.*

Phytochemical screening of the plant extract

In the present study phytochemical screening was also carried out to investigate phytochemical profile of aqueous extract of vegetative parts of *Achyranthes aspera*. The phytochemical screening of the aqueous extract indicates that the fresh plant contain tannin, phlobatannin, terpenoid, flavonoid, cardiac glycoside, phenol, and alkaloid. However saponin, steroid, anthraquinone and carbohydrate were not reported in aqueous extract. Result were shown in Table 3 and Fig.2.

DISCUSSION

Folk medicine is first hand source of information about the therapeutic efficacy of phytochemicals against different kinds of diseases. The present study plant under investigation show presence of various secondary metabolites, namely tannins, phlobatannins, terpenoids, flavonoids, cardiac glycosides, phenols and alkaloids etc. Moreover, antimicrobial studies have been carried of different types of fungi. Similar antimicrobial studies have been done by several workers[11-15]. Antimicrobial activities have been attributed to different phytochemicals.

Different concentrations of methanolic extract of A. *aspera* shows the inhibition efficacy against almost all the fungi undertaken. At conc. of 50 ug/ml the highest

efficacy has been recorded for *Alternaria* followed by R. *solani*, S. *rolfsii*, and F. *oxysporum* but at conc. of 62.5 ug/ml 100% inhibition was recorded for both in R. *solani* and *Alternaria* sp. This implies that graph is not in a straight line. Moreover, the IC 50 values are minimum for R. *solani* followed by S. *rofsii*, *Alternaria* sp., and F. *oxyporum* with 18.75, 19.37, 27.5 and 43.75 µg/ml, respectively.

CONCLUSION

The antimicrobial activities of plant extract of A. *aspera* on different fungal species suggest that extract can be used as biopesticides or bioinsecticides. As the plant grows widely so it could be a cheaper and indigenous source for the frames. Moreover, it is also reported that it is a good source of medicines for the treatment in human. Therefore, an intensive study is required in terms of bioactive compounds.

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Table 1. Effect of different concentration of plant extract of *A. aspera* against fungus

Fungus name	Conc.	Growth of the fungal colony in diameter (in centimeter)				
		2nd day	3rd day	4th day	5th day	6th day
Fusarium oxysporum	Control	2.4	3.7	4.6	5.7	6.4
	12.5 µg/ml	1.9	3.1	3.8	4.8	5.7
	25.0 µg/ml	1.8	3.0	3.7	4.5	5.3
	37.5 µg/ml	1.5	2.6	3.2	3.9	4.5
	50.0 µg/ml	0.7	1.2	1.4	1.7	2.1
	62.5 µg/ml	0.4	0.7	0.8	1.0	1.2
Alternaria sps.	Control	2.35	4.2	5.7	7.2	8.1
	12.5 µg/ml	2.2	4.1	5.5	6.7	7.6
	25.0 µg/ml	1.4	2.6	3.6	4.5	5.2
	37.5 µg/ml	-	0.4	0.6	0.9	1.1
	50.0 µg/ml	-	-	-	-	0.3
	62.5 µg/ml	-	-	-	-	-
Sclerotium rolfsii	Control	3.6	7.8	8.8	+	+
	12.5 µg/ml	1.0	3.9	8.1	+	+
	25.0 µg/ml	0.9	3.0	6.2	8.8	+
	37.5 µg/ml	0.8	2.0	3.8	5.5	7.25
	50.0 µg/ml	-	0.9	1.6	3.3	4.9
	62.5 µg/ml	-	0.7	1.4	2.35	3.1
Rhizoctonia solani	Control	4.6	5.7	8.5	+	+
	12.5 µg/ml	3.7	4.7	7.5	+	+
	25.0 µg/ml	0.95	1.05	1.65	2.0	2.5
	37.5 µg/ml	0.5	0.6	0.8	0.9	1.3
	50.0 µg/ml	-	0.3	0.35	0.6	0.7
	62.5 µg/ml	-	-	-	-	-

Note: + (Plus) sign indicating the overgrowth of the fungus,
- (Minus) sign indicating the complete inhibition of growth of fungus.

Table 2. Percentage of zone of inhibition

Fungus name	Concentration	% of zone of Inhibition by different concentration of plant extract at different days					Mean ± SD	IC_{50} µg/ml)
		2nd day	3rd day	4th day	5th day	6th day		
Fusarium oxysporum	Control	0	0	0	0	0	0	43.75
	12.5µg/ml	20.83	16.21	17.39	15.78	10.93	16.22±3.563	
	25.0µg/ml	25.00	18.91	19.56	21.05	17.18	20.34±2.952	
	37.5µg/ml	37.50	29.72	30.43	31.57	29.68	31.78±3.287	
	50.0µg/ml	70.83	67.56	69.56	70.17	67.18	69.06±1.612	
	62.5µg/ml	83.33	81.08	82.60	82.45	81.25	82.14±0.953	
Alternaria sps.	Control	0	0	0	0	0	0	27.50
	12.5µg/ml	6.38	2.38	3.50	6.94	6.17	5.07±2.007	
	25.0µg/ml	40.25	38.09	36.84	37.50	35.80	37.69±1.661	
	37.5µg/ml	100	90.47	89.47	87.50	86.41	90.77±5.400	
	50.0µg/ml	100	100	100	100	96.29	99.25±1.659	
	62.5µg/ml	100	100	100	100	100	100±0.000	
Sclerotium rolfsii	Control	0	0	0	Data not measurable due to overgrowth of fungus in control		0	19.37
	12.5µg/ml	75.00	50.00	7.95			44.31±33.884	
	25.0µg/ml	72.22	61.53	29.54			54.43±22.208	
	37.5µg/ml	77.77	74.35	56.81			69.64±11.244	
	50.0µg/ml	100	88.46	81.81			90.09±9.203	
	62.5µg/ml	100	91.02	84.09			91.70±7.976	
Rhizoctonia solani	Control	0	0	0			0	18.75
	12.5µg/ml	19.56	17.54	11.76			16.28±4.048	
	25.0µg/ml	79.34	81.57	80.58			80.49±1.117	
	37.5µg/ml	89.13	89.47	90.58			89.72±0.758	
	50.0µg/ml	100	94.73	95.88			96.87±2.770	
	62.5µg/ml	100	100	100			100±0.000	

Table 3. Phytochemical screening of the plant

Serial No.	Phytochemical	Observation	Present/Absent
1.	Tannin	Brownish black ppt	Present
2.	Phlobatannin	Red precipitate formed	Present
3.	Terpenoid	A reddish brown colour formed	Present
4.	Saponin	Frothing not observed	Absent
5.	Flavonoid	Yellow colour	Present
6.	Cardiac glycoside	Ring formed	Present
7.	Phenol	Reddish black	Present
8.	Steroid	Red colour observed	Absent
9.	Alkaloid	Turbidity obtained	Present
10.	Anthraquinone	Pink colour not observed	Absent
11.	Carbohydrate	Black colour not observed	Absent

Table 4. Name of the fungus and disease caused by them with their host

Fungus	Disease	Host
Fusarium oxysporum	Fusarium wilt	Tomato, tobacco, cucurbits, sweet potatoes and banana
Alternaria solani	Early blight	Tomato, potato, eggplant, and other members of the *Solanum* family.
Sclerotium rolfsii	Southern blight	Sweet potato pumpkin, corn, wheat and peanut.
Rhizoctonia solani	Rot disease	Common beet, soyabean

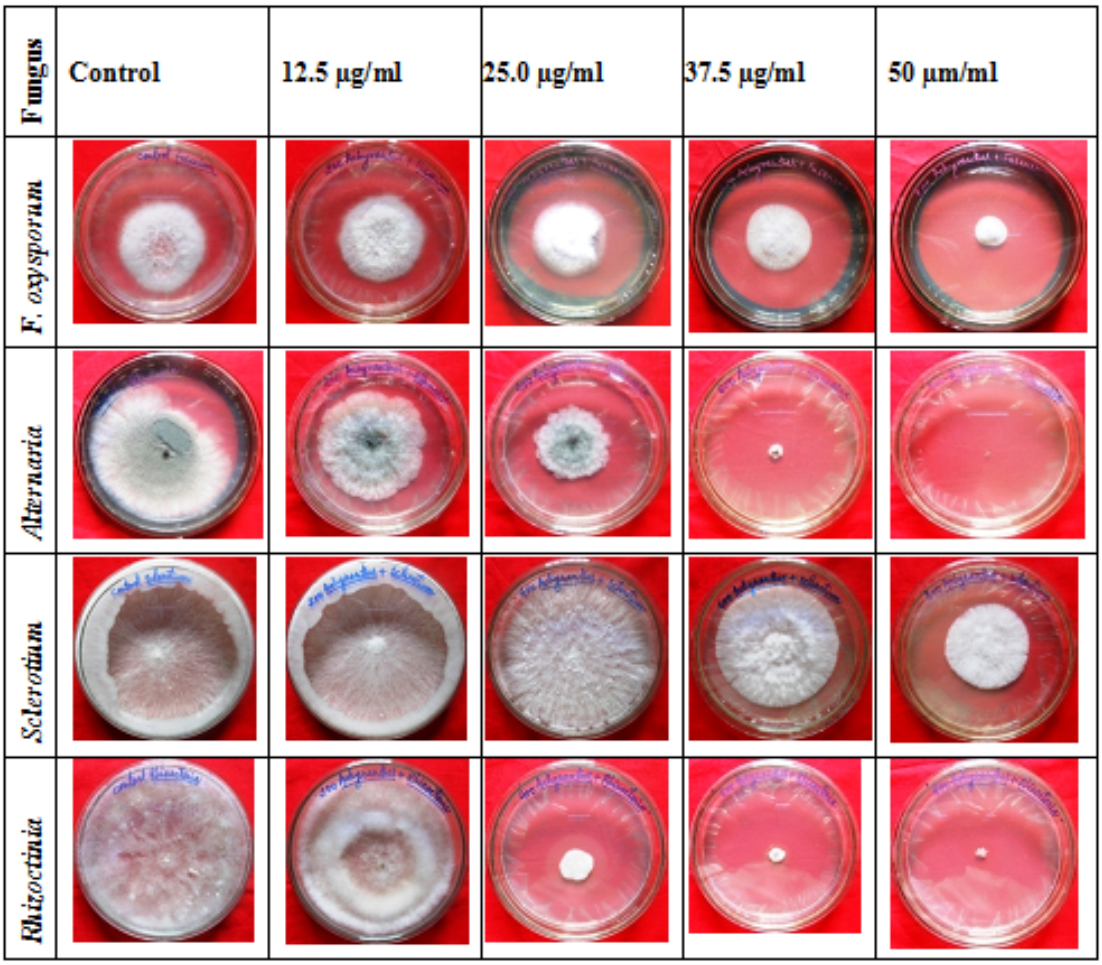
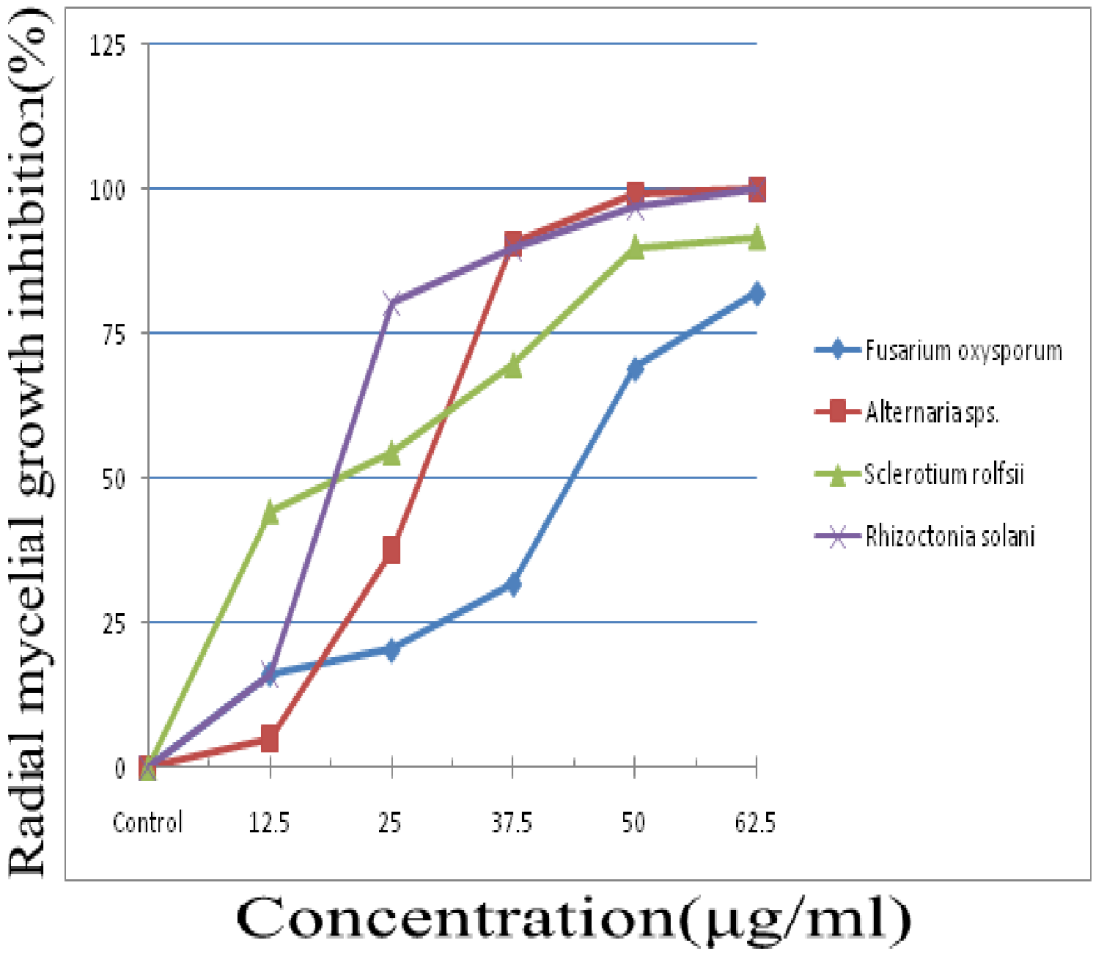


Figure 1. Comparative growth of the four different fungi on 5th day at different concentration of the plant extracts



Graph 1. Percentage of mycelial growth inhibition at different con. of plant extract