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PHYTOCHEMICAL SCREENING AND ANTIFUNGAL ACTIVITY OF *SEMECARPUS ANACARDIUM* L. (AN ANTI-CANCER PLANT)

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Semecarpus anacardium, antifungal activity, Bioactive compounds, Phytochemical screening.

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ABSTRACT: The spread of antibiotic resistant pathogens is one of the most serious menaces to successful treatment of microbial diseases. Medicinal and aromatic plants are widely used as traditional medicines and constitute a major source of natural organic compounds. *Semecarpus anacardium* Linn (Family: Anacardiaceae), is a plant well known for its medicinal value in Ayurvedic and Siddha system of medicine. The plant was selected on the basis of its reported ethnobotanical uses. The present investigation was undertaken to evaluate the phytochemicals and antifungal activity of methanolic extract of *Semecarpus anacardium* L. nuts oils. The preliminary phytochemical studies showed the presence of alkaloids, saponins, tannins, flavonoids, steroids, glycosides. Different Concentration of methanolic extract (6.25, 12.5, 25, 37.5, 50, 62.5 µg/ml) of *S. anacardium* were tested against four fungal strains namely *Fusarium oxysporum*, *Rhizoctonia solanii*, *Alternaria spp.*, and *Sclerotium rolfsii*. The excellent inhibitory activity was observed against *Rhizoctonia solanii* (100%) followed by *Sclerotium rolfsii* (92.59 %), *Alternaria spp.* (72.34 %) and *Fusarium oxysporum* (47.19 %) at 62.5 µg/ml. Among different fungi tested *R. solanii* and *Sclerotium rolfsii* were found to be more sensitive to crude extract when compared to others. The results of the study provide scientific basis for the use of the plant extract in the future development as antifungal agent.

INTRODUCTION: Nature has provided a complete store house of remedies to cure all ailments of mankind. The natural or herbal remedies are still the backbone of medicines. In fact, plants produce a diverse range of bioactive molecules making them a rich source of different types of medicines. Over the past few decades there has been much interest in natural materials as sources of new antimicrobial agents. Different extracts from traditional medicinal plants have been tested.

Many reports show the effectiveness of traditional herbs against microorganisms; as a result, plants have become one of the bases of modern medicine¹. The exploitation of plants by man for the treatment of diseases has been in practice for a very long time. Herbal drugs, mostly secondary metabolites constitute a major part in all the traditional system of medicines². These secondary metabolites showed various biological activities and act in plant defense mechanisms. Higher plants, as sources of medicinal compounds continue to play a dominant role in maintenance of human health since antiquities.

Screening of medicinal plants for antimicrobial agents has gained much importance because lately World Health Organization (WHO) is keenly interested in the development and utilization of

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medicinal plant resources in the traditional system of medicine in the developing countries so as to extend the health care to maximum number of population in these countries³.

Pathogenic fungi are the main infectious agents in plants, causing alterations during developmental stages including post-harvest. In fruit and vegetables, there is a wide variety of fungal genera causing quality problems related to; nutritional value, organoleptic characteristics, and limited shelf life. In addition, in some cases fungi are indirectly responsible for allergic or toxic disorders among consumers because of the production of mycotoxins or allergens.

Generally, phytopathogenic fungi are controlled by synthetic fungicides; however, the use of these is increasingly restricted due to the harmful effects of pesticides on human health and the environment. The increasing demand of production and regulations on the use of agrochemicals and the emergence of pathogens resistant to the products employed, justifies the search for novel active molecules and new control strategies.

Fusarium species are important plant pathogens causing various diseases such as crown rot, head blight, and scab on cereal grains and they may occasionally cause infection in animals. *F. oxysporum* strains are ubiquitous soil inhabitants that have the ability to exist as saprophytes, and degrade lignin^{4, 5} and complex carbohydrates^{6, 7, 8} associated with soil debris.

F. oxysporum is also pathogenic to humans. The diseases associated with *Fusarium* include:

- **Fungal keratitis** - The fungal infection of the cornea that can infect the eyeball and causes abscesses to form on it.
- **Onychomycosis** - The fungal infections of the nail that can cause fingernails or toenails to thicken, discolor, disfigure, and split.
- **Hyalohyphomycosis** - A fungal infection of the skin that can result in an extreme rash or penetrate the dermis and cause infection or internal bleeding. It can cause severe losses in many vegetables and flowers, field crops, such as cotton, and plantation crops, such as banana, date palm and oil palm.

Rhizoctonia solani is a soil-borne fungal pathogen that causes disease in a wide range of plants worldwide. Strains of the fungus are traditionally grouped into genetically isolated anastomosis groups (AGs) based on hyphal anastomosis reactions.

Although these different AGs show differences in their host ranges, generally *R. solanii* is a phytopathogenic species with a wide spectrum of hosts. It has the ability to grow as a saprotroph, which further complicates its behaviour as a parasite. The losses caused by *R. solanii* are very important and need a sustainable management strategy. The patchy appearance of the disease caused by this pathogen is well-known. The severity of infection can vary and for highly infected patches, severity of the infection can be very devastating to the farmer. Some of these consequences are: major yield losses (ranging from 25%-100%), increased soil tare (because the soil sticks to the fungus' mycelium), and poor industrial quality of the crops based on increased levels of sodium, potassium and nitrogen. Due to the vast number of hosts that the pathogen attacks, these consequences are numerous and detrimental to a variety of crops.

Alternaria is a genus of ascomycete fungi. *Alternaria* species are known as major plant pathogens. They are also common allergens in humans, growing indoors and causing hay fever or hypersensitivity reactions that sometimes lead to asthma. They readily cause opportunistic infections in immunocompromised people such as AIDS patients.

It is ubiquitous and includes large number of saprobes and pathogens. *Alternaria* spp. are one of the major contaminants of small grains causing "black point" disease. They are normal agents of decay and decomposition. The spores are airborne and found in the soil and water, as well as indoors and on objects.

The club-shaped spores are single or form long chains. They can grow thick colonies which are usually green, black, or gray⁹. At least 20% of agricultural spoilage is caused by *Alternaria*

species; most severe losses may reach up to 80% of yield, though⁹.

Due to their growth even at low temperature, *Alternaria* spp. are well known post harvest pathogens, responsible for spoilage of food during refrigerated transport and storage¹⁰. Economical losses are mainly related to quality reduction due to decreased nutritive value, discoloration and insipidness¹¹.

In addition to losses in food and feed production, many *Alternaria* species are mycotoxin producers with different toxicological properties. The most important *Alternaria* toxins are alternariol (AOH), alternariol monomethyl ether (AME), altenuen (ALT), tenuazoic acid (TEA) and altertoxins¹².

S. rolfsii is a ubiquitous soil-borne pathogen characterized by presence of whitish mat of mycelia and formation of brownish sclerotia. The sclerotia constitute the primary inoculum and overwintering structure of the pathogen. It causes disease on a wide range of agricultural and horticultural crops. Susceptible agricultural hosts include sweet potato (*Ipomea batatas*), pumpkin (*Cucurbita pepo* L.), corn (*Zea mays*), wheat (*Triticum vulgare*) and peanut (*Arachis hypogea*). Horticultural crops affected by the fungus are included in the genera *Narcissus*, *Iris*, *Lilium*, *Zinnia*, and *Chrysanthemum*¹³.

***Semecarpus anacardium* Linn.** (Family: Anacardiaceae) is a plant well-known for its medicinal value in Ayurvedic and Siddha system of medicine. Chemical and phytochemical analyses of its nut reveal the presence of biflavonoids, phenolic compounds, bharalwanols, minerals, vitamins and amino acids. A variety of nut extract preparations from this source are effective against many diseases, viz., arthritis, tumors, infections and so on.

Semecarpus anacardium, also known as oriental cashew or Indian cashew, is indigenous to India. It is usually found growing in the eastern portion of the country. The nut is commonly known as "marking nut" and in the vernacular as "Ballataka" or "Bhilwa". The genus *Semecarpus* contains some

40 species distributed throughout the tropical regions of the world. It is also widely distributed in the forests of Similpal Biosphere Reserve, Orissa. The tribals of Similpal Biosphere Reserve use it for curing headache, hydrocoel and as antiseptic etc. In this study we have evaluated the antimicrobial activity and phytochemical properties of nuts and oils of *Semecarpus anacardium*.

Semecarpus anacardium is one of most popular medicinal valuable plant in world of Ayurveda. Charak, Sushrut and Vagbhata, the main three treatises of Ayurveda have described the medicinal properties of *Semecarpus anacardium* and its formulation. *Semecarpus anacardium* is classified in Ayurveda under the category of toxic plants. Since Bhallataka is extremely hot and sharp in its attributes, it should be used with caution. Individuals showing allergic reactions to it should stop and avoid the usage of Bhallataka.

It should not be used in small children, very old persons, pregnant women and individuals of predominant pitta constitution. The use of the same should be restricted in summer season.

It is also use as a brain tonic, blood purifier and haematinic tonic. *Semecarpus anacardium* is used for various medicinal properties. The fruit and nut extract shows various activities like antiatherogenic, antiinflammatory, antioxidant, antimicrobial, anti-reproductive, CNS stimulant, hypoglycemic, anticarcinogenic and hair growth promoter. More efforts are needed to study the traditional uses of the plant and the subsequent validation of activity and the mechanism of action. Reports have shown noticeable impact on illnesses related to the heart, blood pressure, respiration, cancer and neurological disorders¹⁴.

Due to the toxic activities, large size, allergic effect are loss of traditional knowledge generation by generation, most of the peoples don't know the importance and proper use of *Semecarpus anacardium*, that's why now a day's peoples are avoiding to grow it in surrounding area. Now *Semecarpus anacardium* plant has become a wild plant, it found only in forest area.

MATERIALS AND METHOD:

Collection of plant material: The fresh and healthy nuts of *Semecarpus anacardium* were collected between June and August, 2013 from forest of Parasnath, Giridih district of Ranchi, Jharkhand, India.

Extraction of plant extract: Crude plant extract was prepared by Soxhlet extraction method. 15gm of the shade dried nuts of this plant was crushed with the help of mortar-pestle and plant material was uniformly packed into a thimble paper and extracted with 250ml of methanol. The process of extraction continues for till the solvent in siphon tube of an extractor become colorless (about 48 hours). In order to evaporate excessive solvent, the sample was placed in incubator at 30-40 °C till solvent evaporate. The extract left over appear to be thick, oily and sticky in nature but the colour was black. The extracts were preserved in sterile bottles at 4°C.

Phytochemical screening: Preliminary phytochemical screening was performed as per standardized procedure^{15,16}.

1. **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 browrish green or a blue-black colouration.
2. **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.
3. **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.
4. **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 H₂SO₄. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow coloration 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.

5. **Test for steriods:** Two ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml H₂SO₄. The colour changed from violet to blue or green in some samples indicating the presence of steriods.
6. **Test for terpenoids (Salkowski test):** Five ml of each extract was mixed in 2 ml of chloroform, and concentrated H₂SO₄ (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.
7. **Test for cardiac glycosides (Keller-Killani 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.
8. **Test for alkaloids (Mayer's test):** 1.36gm of Mercuric chloride and 5gm of KI were dissolved in 60ml and 10 ml of distilled water respectively. These two solvents were mixed and diluted to 100ml using distilled water. To 1ml of acidic aqueous solution of

samples few drops of reagent was added. Formation of white or pale precipitate showed the presence of alkaloids.

9. Test for phenols (Ferric Chloride Test):

To 1ml of alcoholic solution of sample, 2ml of distilled water followed by a few drops of 10% aqueous ferric chloride solution were added. Formation of blue or green colour indicated the presence of phenols.

10. Test for anthraquinones: Borntrager's test was used for detecting the presence of anthraquinones. In this case 0.5 g of the plant extract was shaken with benzene layer separated and half of its own volume of 10% ammonia solution added. A pink, red or violet colouration in the ammoniacal phase indicated the presence of anthraquinone.

11. Test for carbohydrates: Plant extract was mixed with 2ml of iodine solution. A dark blue or purple coloration indicated the presences of carbohydrate.

Fungal strains: Four pathogenic fungal strains were selected for investigation namely *Fusarium oxysporum*, *Rhizoctonia solani*, *Sclerotium rolfsii* and *Alternaria spp.* All the fungal strains are maintained in Lab of Plant Physiology and Biotechnology, University Dept. of Botany, Ranchi University, Ranchi.

Preparation of the Potato Dextrose Agar Medium: The weight amount of potato slice was boiled with a little amount of distilled water for 30 minutes and applied for coarse filtration with the help of filter paper. The required amount of dextrose and agar were dissolved in a conical flask containing potato filtrate final volume was made by adding water and pH was adjusted.

Antifungal assay: *In vitro* antifungal screening was performed by **food poisoning technique** against four fungal strains at different concentrations of plant extracts (6.25, 12.5, 25, 37.5, 50 and 62.5 µg/ml) were mixed with Potato Dextrose Agar media and the results were compared with control (not containing plant extract). Zone of mycelium (fungal) spreading was measured with the help of scale after 72 hours of incubation at 27°C. All experiment was performed in aseptic sterilized condition.

Calculation: Inhibition percentage was calculated by using the following formula (Naz et al., 2006):

Percentage of inhibition =

$$\frac{\text{Diameter of colony in control} - \text{Diameter of colony in treatment}}{\text{Diameter of colony in control}} \times 100$$

RESULTS:

Phytochemical Screening

The results of the phytochemical screening of methanolic extract of *S. anacardium* are shown in **Table 1**. The results of the phytochemical screening of *Semecarpus anacardium* have reveals the presence of various metabolites like alkaloids, glycosides, phenolic compound, Saponins, steroids, carbohydrates, tannins, flavonoids and absence of Anthraquinones, phlobatanin. Thus the preliminary tests may be useful in detection of the bioactive principles and subsequently lead to identification and quantification of bioactive compounds.

TABLE 1: PHYTOCHEMICAL COMPONENTS OF NUT EXTRACTS OF SEMECARPUS ANACARDIUM

Sl. No.	Phytoconstituents (compounds tested)	Result (Aqueous Extract)
1	Acidic Compounds	+
2	Phlobatanin	-
3	Flavonoids	+
4	Phenolic compound	+
5	Glycosides	+
6	Alkaloids	+
7	Saponin	+
8	Tannins	+
9	Terpenoids	+
10	Anthraquinones	-
11	Steroids	+

(+) = Present, (-) = Absent

Antifungal assay: Six different concentration (6.25, 12.5, 25, 37.5, 50, 62.5 µg/ml) of methanolic extract of *S. anacardium* were used to test against four fungi, namely *Fusarium oxysporum*, *Rhizctonia solanii*, *Alternaria spp.*, and *Sclerotium rolfsii* (**fig. 1 and 2**). The fungal (mycelia) growth (mm) on different concentration of plant extract is presented in **Table 2**.

The selected fungi showed growth rate in the order *Sclerotium rolfsii* > *Rhizoctonia solanii* > *Alternaria spp* > *Fusarium oxysporum*. Growth

inhibition percent and LC₅₀ value are calculated to compare with control (Table 3). The fungal growth decreased with the increasing concentration of plant extract in media.

Rhizctonia solani show the highest sensitivity to plant extract, the LC₅₀ value was observed at 8.125 µg/ml concentration of plant extract; at the

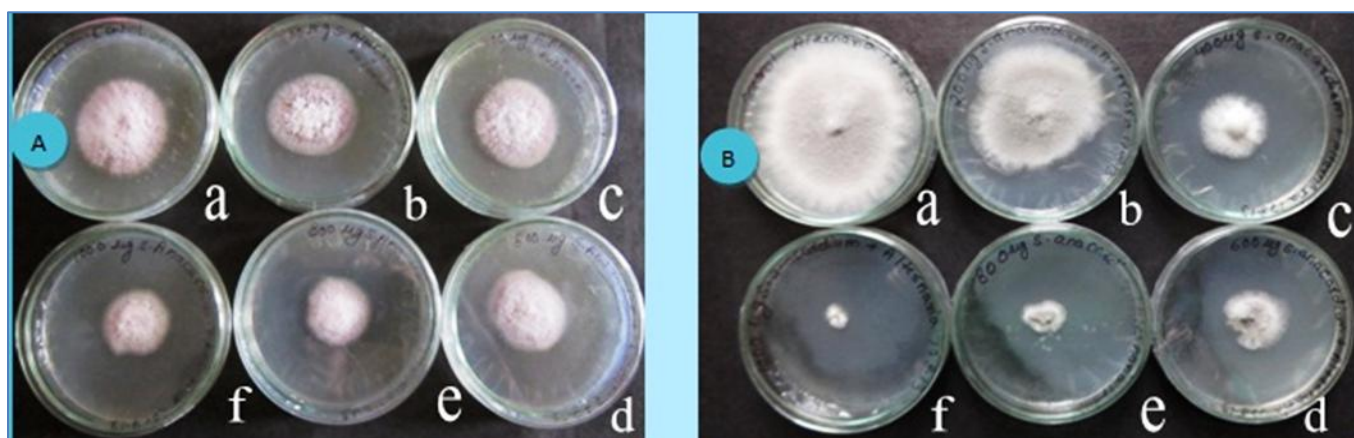
concentration of 62.5 µg/ml complete growth was inhibited. The methanolic extract of *S. anacardium* was show LC₅₀ value against *Alternaria spp.* and *Sclerotium rolfsii* at the concentration of 31.25 and 30µg/ml respectively whereas *Fusarium oxysporum* are less sensitive as compare to other fungal stain, 50 percent inhibition was obtained at 62.5 µg/ml concentration.

TABLE 2: IMPACT OF NUT EXTRACT ON FUNGAL COLONIES GROWTH *IN VITRO*.

Fungus name	Incubation time in days	Diameter of colony (mm)						
		Control	6.25 µg/ml	12.5 µg/ml	25 µg/ml	37.5 µg/ml	50 µg/ml	62.5 µg/ml
<i>Fusarium oxysporum</i>	2 days	12	12	10.5	10	9	6	6
	3 days	19	19	16	15	13	12	10
	4 days	27	27	24	23	20.5	17	13
	5 days	30	31	28	28	24	20	15
<i>Rhizoctonia solani</i>	2 days	30	9	8	7	6	2	0
	3 days	43	22	20	19	11.33	5	0
	4 days	45	24	22	21	12	6	0
	5 days	55	35	30	29	16	8	0
<i>Alternaria spp.</i>	2 days	22	15	12	11	9	7	5
	3 days	32	26	18	16	16	13	9
	4 days	42	39	32	21	19	14	10
	5 days	45	42	35	28	23	20	15
<i>Sclerotium rolfsii</i>	2 days	26	20	7	6	3	2	1
	3 days	45	41	22	21	14	5	3
	4 days	70	62	43	37	22	6	5
	5 days	75	72	58	55	31	9	7

TABLE 3: *IN-VITRO* IMPACT OF NUT EXTRACT ON FUNGAL GROWTH INHIBITION (%)

Sl. No.	Name of fungus	Part used	Zone of Inhibition (%) of fungal growth at different concentrations (µg/ml)							LC ₅₀ (µg/ml)
			Control	6.25µg	12.5µg	25µg	37.5µg	50µg	62.5µg	
1	<i>Rhizctonia solanii</i>	Nuts	0	47.70	53.84	56.15	73.85	87.69	100.00	8.125
2	<i>Alternaria spp.</i>	nuts	0	13.47	31.20	46.09	52.48	61.70	72.34	31.25
3	<i>Sclerotium rolfsii</i>	nuts	0	9.72	39.81	44.90	67.59	89.81	92.59	30
4	<i>Fusarium oxysporum</i>	nuts	0	0.00	11.82	14.60	25.30	38.20	50	62.5



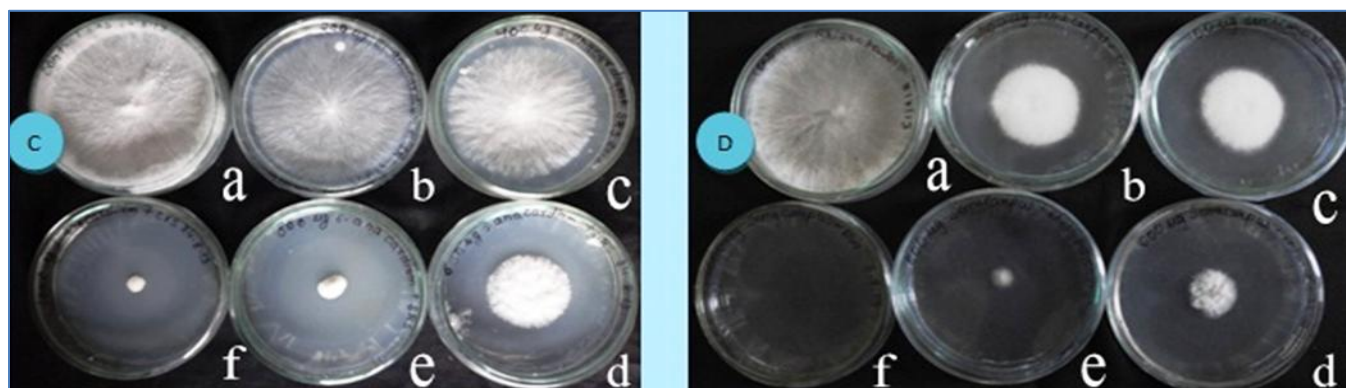


FIGURE 1: ZONE OF INHIBITION OF METHANOLIC EXTRACTS OF PLANT SPECIES ON DIFFERENT FUNGUS. (A) *Fusarium oxysporum* (B) *Alternaria* spp. (C) *Sclerotium rolfsii* (D) *Rhizoctonia solanii*. (a) Control (b) 12.5 μ g/ml extract (c) 25 μ g/ml extract (d) 37 μ g/ml extract (e) 50 μ g/ml extract (f) 62.5 μ g/ml extract

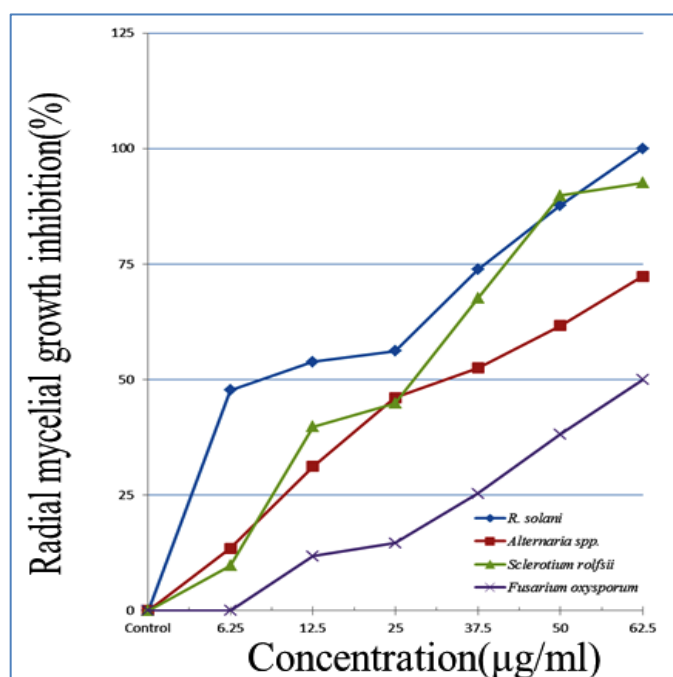


FIG. 2: INHIBITORY EFFECT OF DIFFERENT CONCENTRATION OF *S. ANACARDIUM* EXTRACTS ON SELECTED FUNGAL STRAINS

DISCUSSION: Plants are important source of potentially useful drugs for the development of new chemotherapeutic agent¹⁷. The first step towards this goal is the *in vitro* antibacterial, anti-fungal, antihelmenthic and anti-inflammatory properties¹⁸.

In the present investigation, antifungal activity of *Semecarpus anacardium* is revealed against four fungi, namely *Fusarium oxysporum*, *Rhizctonia solanii*, *Alternaria* spp., and *Sclerotium rolfsii*; the LC₅₀ values, are 62.5, 8.125, 31.25, 30 μ g/ml, respectively.

The present result may be correlated with presence of different types of phytochemicals alkaloids, glycosides, phenolic compound, Saponins, steroids, carbohydrates, tannins, flavonoids etc. Specially nut of *Semecarpus anacardium* is very rich in phenol contents which is involved in multiple mode of action, for example degradation of cell wall, disruption of cytoplasmic membrane, damage of membrane protein and integrated enzymes, leakage of cellular components, Depletion of the proton motive force alteration in nutrient uptake and electron transport^{19,20}.

Similar finding have also been reported for efficacy of plant extracts against fungal growth by different workers^{21,22}.

CONCLUSION: Folk herbal medicines are important source of drug discovery as they are widely used for the control of plant pathogens and human related diseases. *Semecarpus anacardium* hold important position in traditional system of medicines and their anti-fungal activities have been proved in present study under *in vitro* conditions. Their trial needs further to be established in field condition before any final verdict.

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