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Antifungal Activity of Ethanol Extract of Leaves of *Rosmarinus Officinalis* (Rose Mary)

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Abstract
Antifungal activity of ethanol extract of leaves of Rosmarinus officinalis L. on Aspergillus flavus and Fusarium oxysporum was investigated in vitro in Sokoto. A. flavus and F. oxysporum were obtained from stock in mycology laboratory of Usmanu Danfodiyo University Sokoto for the purpose of the study. The plant extract was constituted into five different concentrations (2%, 4%, 8%, 12% and 20%). A Potato Dextrose Agar (PDA) media was prepared according to manufacturer's specifications for the growth of test organisms. The organisms were inoculated in sterilised Petri dishes containing a mixture of potato dextrose agar media and the ethanol extracts. The result indicated that the growth of the organisms was reduced. Growth inhibition of A. flavus was higher compared to that of F. Oxysporum. The result indicated that A. Flavus was more sensitive to the extract than F. Oxysporum. That suggests that the plant has potentiality for antifungal activity and could be recommended as potential source for production of drugs with a broad spectrum activity.
Key words: *Aspergillus flavus, Ethanol extract, Fusarium oxysporum, Leaves, Rosmarinus officinalis L. and PDA.*

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I. Introduction

Rosmarinus officinalis commonly known as rosemary is a woody, perennials herb having a characteristic fragrant, with evergreen needle like leaves and white pink, purple or blue flower, rosemary has a fibrous root system. It is native to the Mediterranean region (Inoue *et al*., 2005). *R. officinalis* is a member of the family Lamiaceae. It is one of the 2-4 species in the genus *Rosmarinus* (Johan *et al.,* 2007). It is easy to grow and it is a pest-resistant plant. Rosemary can grow quite large and retain its attractiveness for many years. It can be pruned into formal shapes and low hedges, and has been used for topiary. It is easily grown in pots. The ground cover cultivars spread widely with a dense and durable texture. Rosemary grows on friable loam with good drainage in an open sunny position. It does not withstand water logging, and some particles are susceptible to frost. It grows best in neutral to alkaline conditions (PH-7.7-8). It can be propagated from an existing plant by clipping a shoot (from a soft new growth 10-15cm) 4-6m long striping a few leaves from the bottom and planting it directly into the sand.

The entire plant (Leaves, flowers, stem, branches) are used for commercial purposes. An essential oil is extracted from the leaves and flower by steam distillation. The leaves contain 1-2.5% essential and falconoid, luteoln, apigenin diosmosing, tannis and saponins additionally, the plant is very high in iron, calcium and vitamin B6. Rosemary has long been known to aid new hair growth. It has been infused into many shampoo products as an aid in fighting dandruff. This herb has been used as an insect repellent; additionally it has been used to get rid of head lice. Some studies claim that the cornodic found in rosemary may shield the brain from radical lowering the risk of strokes and neuro-degrative disease like Alzheimer's disease, dementia and Lou Gehrig's.

Rosemary was believed to be helpful in the digestion of starchy food and vegetables to avoid indigestion and is also believed to relieve flatulence and it commonly used as a circulatory and heart stimulant. In addition it has been shown to have possible antioxidant properties. Rosemary is sometimes used to treat muscle pain and arthritis. Rosemary is used as incense and is also very common in aroma therapy. The herbs have been approved as a medical herb by the German commission. Fresh and dried leaves are often used in traditional Mediterranean cuisine (Martin *et al*., 2008). Rosemary also contain 0.5% to 2.5% of volatile, the major component of the oil include (alpha and beta-pinene), camphene, limonene, camphor (10% to 20%), borneol, cineole, linalool, and verbinol. Rosemary contains a wide variety of volatile and aromatic components. Flavonoids in the plant include diosmetin, diosmin, luteolin, hispidium, and apigenin. One analysis report 3 new flavonoids glucuronoids, also found in rosemary include triterpense oleanolic and ursolic acids and diterpense carnosol (Carrio, 2009).

Micro organisms have a tremendous impact on life, physical and chemical makeup of our planet. It has been estimated that $5x10^3$ microbial cells exist on earth. Excluding cellulose, the cell that constitute about 90% of the cells in our bodies are microbes (Jawatz *et al*., 2010). New spectrum of opportunistic human infections is increasing day after day due to increase in number of AIDs and cancer patients and most microorganisms have developed resistance to conventional antifungal and antibiotics, hence the need for a plant based alternative form of antifungal and antibiotic drug to minimise the growth of those microbes. This research therefore aimed to investigate the anti-fungal efficacy of the leaf extract of *R. officinalis* by determining the efficacy of the leaf extract on *A. flavus and F. oxysporum.*

II. Materials and Methods

Collection of Samples

R. officinalis plant was used for the study. The fresh plant leaves were obtained from National research institute (NARICT) Zaria, Kaduna state. The samples collected were packed in clean sterilized polythene bag and brought to Usmanu Danfodiyo University Sokoto, Department of Biological sciences herbarium for identification. The samples were then transferred to mycology laboratory for the study.

Preparation of plant material:

The samples were washed with tap water and air dried under room temperature of 37±3 ^{0}C, so as to preserve the photochemical constituents in it. The dried material was crushed using mortar and pestle, and then sieved to obtain fine powder. Fifty grams, 50 g of powder was weighed using weighing balance. The powder was transferred into a clean conical flask.

Plant Extraction:

Five hundred (500) militres of ethanol was added in the conical flask containing 50g of the powdered material; the sample was stirred, cupped with aluminum foil and kept for three (3) days. It was then filtered using muslin cloth. The filtrate obtained was collected in a stainless plate and evaporated to dryness using hot plate set at 40 ^{0}C. The resultant extract was scraped off, kept in a clean foil paper and labelled (Sukhdev *et al*., 2008).

Preparation of culture media

Potato Dextrose Agar (P D A) is the nutrient media to be used. It was prepared according to the manufacturer's instructions. Thirty nine grams (39g) of Potato Dextrose Agar was dissolved in 1000ml distilled water. One gram (1g) of streptomycin was added to inhibit the growth of bacteria in the media. The mixture was heated to ensure complete dissolution. It was autoclaved at 121^{0}C for 15min. and allowed to cool at room temperature (BAM, 1998)

Preparation of plant extract:

Five millilitres (5ml) of distilled water was measured using syringed and poured into test tubes and cotton wool was used to plug and aluminum foil to cover the test tubes. It was then autoclaved at 121^{0}c for 15min. 0.01 g, 0.04 g, 0.08 g, 0.12 g and 0. 20 g of the extract were measured separately and added to each of the test tubes and mixed thoroughly to obtain mixtures. The mixtures constitute the concentration of the extract as 2%, 4%, 8%, 12% and 20% for antifungal activity.

Test organisms

The test organisms were collected from stuck in the mycology laboratory of Usmanu Danfodiyo University, Sokoto. The test organisms were *A. flavus* and *F. oxysporum.*

Antifungal assay

Agar incorporation method was used to determine the anti fungal effect of plant extract. Twenty millilitres (20 mls) of the prepare PDA media was dispensed into petri dishes and 2%, 4%, 8%, 12% and 20% of the varying concentrations of plant extract was separately added to the PDA medium in the petri dishes in triplicates, and allowed to solidify at room temperature (37± 3^{0}C). *A. flavus* and *F. oxysporum* obtained from the laboratory stuck culture were separately inoculated in triplicates into each PDA-extract medium. PDA medium without plant extract was used as control and the test organisms were also inoculated in triplicates into it. These were kept in the incubation room for one week and observed daily for growth (Imhof *et al.*, 2003).

Measurement of growth (diameter)

Colony diameter was taken as the means along two directions on two perpendicular lines drawn on the reverse side of the plates. The effectiveness of the extract was recorded in terms of percentage inhibition (Nene and Thapliyal, 1979).

$$\% \text{ Mycellial inhibition} = \frac{\text{Mycellial growth}_{(control)} - \text{Mycellial growth}_{(treatment)}}{\text{Mycellial growth (control)}} \times 100$$

III. Results

The result of the anti fungal assay of ethanol extract of *R. officinalis* on *A. flavus* and *F. oxysporum* indicated that the plant extract inhibited on the growth of those test organisms as shown in **Table 1**. The highest zone of inhibition was $30.49^{\pm}3.18^{a}$ at 20% concentration of the plant extract against *A. flavus* and $26.91^{\pm}37.45^{b}$ was recorded for *F. oxysporum* . There was no significant difference at $p<0.05$ on the growth inhibition of the test organisms at 4%, 8%, and 12% concentrations of the plant extract. The least inhibitory effect was observed for both organisms at 2% concentrations.

Table 1: Antifungal activity of ethanol leaf extract of *R. officinalis* on *A. flavus and F. oxysporum.*

S/N	Concentration(%)	Zone of Growth inhibition(%)	
A.	*flavus*	*F. oysporum*	
1	2	18.39 ± 2.89^{d}	6.67 ± 0.88^{d}
2	4	20.98 ± 3.02^{c}	13.33 ± 0.88^{c}
3	8	25.18 ± 1.01^{bc}	14.88 ± 1.99^{bc}
4	12	26.91 ± 7.45^{b}	18.22 ± 1.56^{b}
5	20	30.49 ± 3.18^{a}	26.22 ± 0.53^{a}
6	Control	$0.00^{\pm}0.00$	$0.00^{\pm}0.00$

Values are mean $\pm$ standard error of 3 replication means in a column with different superscript are significantly different ($P< 0.05$)

IV. Discussion

The research work as observed from the result indicated that ethanollic leaf extract of *R. officinalis* had antifungal efficacy on *A. flavus and F. oxysporum* as growth of the organisms on the plant extract were inhibited at different concentrations. This could be attributed to presence of bioactive compounds in the plant that have antifungal properties. This was in accordance with the findings of Centeno *et al.* (2010) who reported that a number of compounds contained in the extracts of *R. officinalis* such as borneol and other phenolics in the terpene fraction revealed antifungal properties. There was no significant difference in the ability of the plant extract to retard the growth of the text organisms which suggested that both the organisms are sensitive to the plant extracts at different concentrations. This finding agreed with the investigation performed by Rasooli *et al.* (2008) who established that fungal growth was suppressed by *R. officinalis* extract.

High percentage mycelia growth inhibition was recorded by *A. flavus* than *F. oxysporum* at 20% concentrations of the plant extract. Therefore the treatment of the ethanol leaf extract against *A. flavus* is more effective than *F. oxysporum*. The difference in the reduction of mycelial growth of the organisms by the plant extract may be directly related to differences in the fungal physiology, anatomy and the presence of secondary metabolites which is in consonance with the work of Iwu *et al.* (1992); Azu and Onyeagba, (2007); Omogbai and Eze, (2011) who reported on phytochemical screening and susceptibility of bacteria pathogens to extracts of *Evolvulus alsinoides*.

The inhibitory effect of the extract was proportional to their concentrations. The result of the study also revealed that the growth of the test organism is directly proportional to the increase of the extract concentration.

V. CONCLUSION

The study had revealed that ethanol leaves extract of *R. officinalis* had antifungal properties as growth of test organisms was inhibited. *A. flavus*, was found to be more sensitive to the plant extractthan *R. oxysporum.* The higher the concentrations of the extract, the more the inhibitory effect on the organisms.

VI. RECOMMENDATION

1. The plant is a potential source for production of drugs with a broad spectrum activity.
2. The result of the study also suggests that the ethanol extract possess compound with antifungal properties that can be used as antifungal agent in nowel drugs for the treatment of different fungal disease.

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