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THE ANTIFUNGAL ACTIVITIES OF THE METHANOLIC CRUDE EXTRACT OF THE LEAVES OF *Ocimum gratissimum* L., *Melanthera scandens* A. and *Leea guineensis* L. ON SOME PHYTOPATHOGENIC FUNGI

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ABSTRACT

In this study, the antifungal activities of methanolic crude extract of *Ocimum gratissimum* L., *Melanthera scandens* A. and *Leea guineensis* L were tested on their ability to inhibit the growth of phytopathogenic fungi. The radial mycelial growth and dry mycelial assay were used to test for activities against these phytopathogenic fungi: *Botryodiplodia theobromae, Phytophthora palmivora, Aspergillus flavus, Aspergillus niger* and *Aspergillus glaucus*. The antifungal activities of the plant extract at concentrations of 50, 100, 150, 200 mg/ml showed that *Leea. guineensis* exhibited strong antifungal activities while *O. gratissium* and *Melanthera scandens* exhibited moderate antifungal activities against the test fungi. The results of this study justify the claim of the traditional practitioners that the plants are used for the treatment of fungal diseases.

Keywords: Phytopathogenic fungi, Plant extract, Radial mycelia growth, Dry mycelial weight.

INTRODUCTION

Medicinal plants are plants that contain substances that could be used for therapeutic purposes or which are precursors for the synthesis of useful drugs **[1]**. The medicinal value of these plants lies in bioactive phytochemical constituents that produce definite physiological action on the human body **[2]**. *Ocimum gratissimum* belongs to the

family *Lamiaceae* and found mostly in the tropical countries including: Nigeria, India, North and South America, Mexico and Brazil where it is popularly known as alfavaca-cravo, alfavacao, alfavaca **[3]**. The local names are Efinrin, Efirin aaja, Erumaba (Yoruba), Daidoyatagida (Hausa), Esewon (Edo-Akoko), Nehonwu, Nchanwu (Igbo) and Menthesauvage (French). It is traditionally used to relief pains and also used in the treatment of rheumatism, diarrhea, high fever, convulsions, diabetes, eczema, piles and as a repellant **[4, 5, 6]**. The decoction of the stem is inhaled for the treatment of catarrh and bronchitis **[7]**.

Melanthera scandens belongs to the plant family, *Asteraceae* and found mostly in the tropical regions including Nigeria, Mexico, Central America, South America, Sub-saharan Africa **[3]**. The common local names are Aboyunrinyun and Ounje-ehoro (Yoruba), vine (English). The herbalists use the juice of leaves to stop dysentery. The plant is popularly used as further for animals **[7]**. It is used in the treatment of skin infections, gastroenteritis, stomachache, cuts, wounds, inflammation, diuretic and for hypertensive activities. It is also used against piles, diarrhea and hemorrhoid **[8]**. *Leea guineensis* is an evergreen shrub, up to 20ft high locally abundant in moist shaded places which belongs to the family *Leaceae* **[9]**. It has an English name called Red tree vine or Hansid-hapan. The local names are Kojiya, arigbokuta and Iya-kekere. The aqueous Methanolic extract of *Leea guineensis* exhibits potential in-vivo anti-tumor and antioxidant activity **[10]**. It is used in the treatment of enlarged spleen in children, pregnancy detection, purgative, toothache, gonorrhea, general weakness, skin lesions, skin rash, ulcer, herpes and boils. The plant is fungistatic and bacteriostatic **[7]**.

The aim and objective of this study was to test for the antifungal activities of the methanolic crude extract of the leaves of *O. gratissimum*, *M. scandens* and *L. guineensis* on phytopathogenic fungi.

MATERIALS AND METHODS

Collection of Plant Materials

The fresh plants were collected from a local farm in Ado-Ekiti, Ekiti State, Nigeria. Identification and authentication were carried out in the herbarium section of the Department of Plant Science, Ekiti State University, Ado-Ekiti, Ekiti State, Nigeria.

Processing of Plant Materials

The fresh leaves of the following plants *O. gratissimum*, *M. scandens* and *L. guineensis* were air dried at 28^{o}C for 30 days. They were

grounded into fine powder using an electric blender. Moreover, 50g of dried powdered leaves of the test plants were separately soaked in 1000 ml of methanol for 5 days. The extracts were filtered and evaporated using rotatory evaporator. The crude extracts were kept in the refrigerator at 4^{o}C until use.

Source of Microorganisms

Cultures of fungi used were *A. glaucus, A. flavus, A. niger* obtained from the Department of Microbiology, Ekiti State University, Ado-Ekiti, Nigeria and *B. theobromae, P. palmivora* obtained from the Cocoa Research Institute of Nigeria, Ibadan, Oyo-State, Nigeria.

Determination of Antifungal activities

The methods of **[11]** were used for the determination of antifungal activities.

Radial Mycelial Growth Assay

The following concentrations (50.0, 100.0, 150.0 and 200.0 mg/ml) of the extract were aseptically dispensed into different sterile petri-dishes. About 15 ml of sterilized molten malt extract agar was added to the plates and were swirled gently to mix content evenly. Mycelial discs (6 mm diameter) taken with a sterile cork-borer from the advancing edges of 3-5 days old cultures of the test fungi were placed centrally on the cooled seeded plates and incubated at 28^{o}C for 7days. The control experiments were carried out as described above, but only 1ml of the extracting solvent was added to each of the plates. The radial mycelial growth of both test and control plates were measured using an electronic caliper. The net growth was obtained by subtracting the diameter of the inoculum plugs from the test.

Dry Mycelial Growth Assay

Five mycelial discs each of the test fungi obtained as described radial mycelia growth were introduced into 120 ml Erlenmeyer flasks containing 25ml of sterile malt extract broth. Different concentrations (50.0, 100.0, 150.0 and 200.0mg/ml) of the induced extract were introduced into each of the conical flask containing inoculum plugs of the test fungi. The flasks were incubated at 28^{o}C for 7 days. For control, only 1ml of the extracting solvent was added to each of the flask and treated as above. At the end of the 7th day of incubation, the content of each flask was decanted into a funnel containing pre-dried and pre-weighed Whatmam filter paper. The content of the filter paper was dried at 50-60^{o}C in an oven until constant weight was obtained for each test fungi. The mycelial weight was determined by difference.

RESULT AND DISCUSSION

The antifungal activities of the methanolic extract of *O. gratissimum* on the radial mycelial growth of the test fungi are shown in **Table 1**. *B. theobromae* had a percentage inhibition that varied from 15.2-52.1% at concentration of 50-200 mg/ml, *P. palmivora* had a percentage inhibition that varied from 20-90% at concentration of 50-200 mg/ml. *A. flavus* had percentage inhibition that varied from 25-56.3% at concentration of 50-200 mg/ml. *A. niger* had a percentage inhibition that varied from 5.6-44.4% at concentration of 50-200 mg/ml. Similarly, *A. glaucus* had a percentage inhibition that varied from 2.6-81.60% at concentration of 50-200 mg/ml.

This is in agreement with the findings of **[12]** who reported the antifungal activities of the extract of neem plant (*Azadirachta indica*) and found that the extract caused a significant delay in sporulation and inhibited mycelial elongation in *Cercospora cruenta*, *Collectrichum truncatum* and *Fusarium oxysporium*. In addition, **[13]** also reported the antifungal activities of the solvent extracts of 49 different plants used in traditional medicine against *A. niger* and found 86% of the extracts to exhibit antifungal properties against *A. niger* by the inhibition of the mycelial elongation.

The antifungal activities of the methanolic extract of *M. scandens* and *L. guineensis* are shown in **Table 2 and 3** respectively. *B. theobromae* had a percentage inhibition that varied from 22.5-72.5% (*M. scandens*) and 43.6-65.5% (*L. guineensis*) at concentration of 50-200 mg/ml. *P. palmivora* had a percentage inhibition that varied from 28.6-100% (*M. scandens*) and 25-75% (*L. guineensis*) at concentration of 50-200 mg/ml. *A. flavus* had a percentage inhibition that varied from 30.8-69.2% (*M. scandens*) and 5.8-58.8% (*L. guineensis*) at concentration of 50-200 mg/ml. *A. niger* had a percentage inhibition that varied from 10-14% (*M. scandens*) and 26, 7-60% (*L. guineensis*) at concentration of 50-200 mg/ml. Similarly, *A. glaucus* had a percentage inhibition that varied from 29.4-70.6% (*M. scandens*) and 18.5-81.5% at concentration of 50-200mg/ml.

The antifungal activities of Methanolic extract of *Ocimum gratissimum, Melanthera scandens* and *Leea guineensis* on dry mycelial weight of test fungi are shown in **Table 4, 5 and 6**. *B. theobromae* had a percentage inhibition that varied from 2.5-34.7% (*Ocimum gratissimum*), 25.4-34.6% (*M. scandens*) and 4.2-47.9% (*L. guineensis*) at concentration of 50-200 mg/ml. *P. palmivora* had a percentage inhibition that varied from 7.2-61.9% (*O. gratissimum*), 10.2-51.7% (*M.*

scandens) and 52.8-71% (*L. guineensis*) at concentration of 50-200 mg/ml. *A. flavus* had a percentage inhibition that varied from 12.1-43% (*O. gratissimum*), 20.9-71.3% (*M. scandens*) and 7.4-36.1% (*L. guineensis*) at concentration of 50-200 mg/ml. *A. niger* had a percentage inhibition that varied from 16.5-81% (*O. gratissimum*), 27.8-68.1% (*M. scandens*) and 17.1- 81% (*L. guineensis*) at concentration of 50-200 mg/ml. Similarly, *A. glaucus* had a percentage inhibition that varied from 9.2-70.9% (*O. gratissimum*), 21.5-61.4% (*M. scandens*) and 10-72.9% (*L. guineensis*) at concentration of 50-200 mg/ml.

These results is in agreement to the findings of **[14]** who reported the antifungal activity of hexane extracts of Rosemary and Lavender leaves and found that the extract reduced the germination of the zoospores of *Phytophthora capsici*, *Phytophthora megakarya* and *P. palmivora* during the study. **[15]** also reported the antifungal activities of the ethanolic extract of *Alchornea cordifolia, Annona muricata, Allium sativum, Garcinia cola* and *Zingiber officinale* on the organisms causing potato root blight and found the extracts of the plants to inhibit the mycelial elongation and reduced the mycelial dry weight of *Botrydiplodia theobromae*, *Fusarium solani*, *Fusarium oxysporum*, *Aspergillus niger* and *Rhizopus stolonifer*.

[16] also reported the antifungal activities of the crude extracts of *Acorus calamus*, *Tinospora cordifolia* and *Celestrus paniculatus* and found that the extracts at varying concentrations of 50, 100, 150 mg/ml inhibited the radial mycelial growth and reduced the dry mycelial weight of *Aspergillus niger, Phytophthora palmivora, Botryodiplodia theobromae,* and *Aspergillus flavus.* From above results, *O. gratissimum, M. scandens, L. guineensis* may serve as a constituent of human diet supplying the body with minerals, protein, energy and as livestock and poultry feed. Also, the results of these studies justified the claim of the local medical practitioners that the plants are used for treatment of fungal diseases.

Table 1: Effects of the Methanolic Extract of *Ocimum gratissimum* L. on the Radial Mycelial Growth of the Test Fungi (mm)

TEST CONTROL	CONCENTRATION OF EXTRACT (mg/ml)								
	CONTROL	50		100		150		200	
		A	B	A	B	A	B	A	B
B. theobromae	46.0	39.0	15.2	29.0	37.0	28.0	39.10	22.0	52.1
P. palmivora	10.0	8.0	20.0	4.0	60.0	2.0	80.0	1.0	90.0
A. flavus	16.0	12.0	25.0	11.0	31.3	10.0	37.5	7.0	56.3
A. niger	18.0	17.0	5.6	13.0	27.8	11.0	38.9	10.0	44.4
A. glaucus	38.0	37.0	2.6	27.0	28.90	17.0	55.3	7.0	81.6

LEGEND: A = Net Radial Mycelial Growth (mm), B = % Inhibition of Radial Mycelial Growth

Table 2: Effects of the Methanolic extract of *Melanthera scandens* L. on the Radial Mycelial Growth of the Test Fungi (mm)

TEST CONTROL	CONCENTRATION OF EXTRACT (mg/ml)								
	CONTROL	50		100		150		200	
		A	B	A	B	A	B	A	B
B. theobromae	40.0	31.0	22.5	28.0	30.0	12.0	70.0	11.0	72.5
P. palmivora	7.0	5.0	28.6	4.0	42.9	2.0	71.4	0.0	100.0
A. flavus	13.0	9.0	30.8	8.0	38.5	6.0	53.8	4.0	69.2
A. niger	10.0	9.0	10.0	8.0	20.0	7.0	30.0	6.0	40.0
A. glaucus	17.0	12.0	29.4	11.0	35.3	10.0	41.2	5.0	70.6

LEGEND: A = Net Radial Mycelial Growth (mm), B = % Inhibition of Radial Mycelial Growth

Table 3: Effects of the Methanolic Extract of *Lea guineensis* L. on the Radial Mycelial Growth of the Test Fungi (mm)

TEST CONTROL	CONCENTRATION OF EXTRACT (mg/ml)								
	CONTROL	50		100		150		200	
		A	B	A	B	A	B	A	B
B. theobromae	55.0	31.0	43.6	28.0	49.1	21.0	61.8	19.0	65.5
P. palmivora	8.0	6.0	25.0	4.0	50.0	3.0	62.5	2.0	75.0
A. flavus	17.0	16.0	5.8	14.0	17.6	12.0	29.4	7.0	58.8
A. niger	15.0	11.0	26.7	8.0	46.7	7.0	53.3	6.0	60.0
A. glaucus	54.0	44.0	18.5	30.0	44.14	15.0	72.2	10.0	81.5

LEGEND: A = Net Radial Mycelial Growth (mm), B = % Inhibition of Radial Mycelial Growth

Table 4: Effects of the Methanolic Extract of *Ocimum gratissimum* L. on the Dry Mycelial Weight of the Test Fungi (mg)

TEST CONTROL	CONCENTRATION OF EXTRACT (mg/ml)								
	CONTROL	50		100		150		200	
		A	B	A	B	A	B	A	B
B. theobromae	121.0	118.0	2.5	106.0	12.4	90.0	25.6	79.0	34.7
P. palmivora	97.0	90.0	7.2	80.0	17.5	72.0	25.8	37.0	61.0
A. flavus	107.0	94.0	12.1	92.0	14.0	85.0	20.6	61.0	43.0
A. niger	158.0	132.0	16.5	74.0	53.2	71.0	55.1	30.0	81.0
A. glaucus	141.0	128.0	9.2	113.0	19.9	81.0	42.6	41.0	70.9

LEGEND: A = Dry Mycelial Weight (mg), B = % Inhibition of Dry mycelial Weight

Table 5: Effects of the Methanolic extract of *Melanthera scandens* L. on the Dry Mycelial Weight of the Test Fungi (mg)

TEST CONTROL	CONCENTRATION OF EXTRACT (mg/ml)								
	Control	50		100		150		200	
		A	B	A	B	A	B	A	B
B. theobromae	130.0	97.0	25.4	96.0	26.2	88.0	32.3	85.0	34.6
P. palmivora	118.0	106.0	10.2	95.0	19.5	84.0	28.8	57.0	51.7
A. flavus	115.0	91.0	20.9	84.0	26.9	79.0	31.3	33.0	71.3
A. niger	295.0	213.0	27.8	99.0	66.4	97.0	67.1	94.0	68.1
A. glaucus	233.0	183.0	21.5	172.0	26.2	147.0	36.9	90.0	61.4

LEGEND: A = Dry mycelial weight (mg), B = % Inhibition of Dry Mycelial Weight

Table 6: Effects of the Methanolic Extract of *Lea guineensis* L. on the Dry Mycelial Weight of the Test Fungi (mg)

TEST CONTROL	CONCENTRATION OF EXTRACT (mg/ml)								
	Control	50		100		150		200	
		A	B	A	B	A	B	A	B
B. theobromae	119.0	114.0	4.2	108.0	9.2	101.0	15.1	62.0	47.9
P. palmivora	290.0	137.0	52.8	128.0	55.9	90.0	70.0	84.0	71.0
A. flavus	108.0	100.0	7.4	99.0	8.3	88.0	18.5	69.0	36.1
A. niger	158.0	131.0	17.1	74.0	53.2	69.0	56.3	30.0	81.0
A. glaucus	140.0	126.0	10.0	111.0	20.7	82.0	41.4	38.0	72.9

LEGEND: A = Dry Mycelial Weight (mg), B = % Inhibition of dry Mycelial Weight

CONCLUSION

Plants have contributed immensely to the medical field. It has been the source of most drugs used for combating infections. The three plants used in this study were found to contain the important constituent needed to combat various kinds of infection in human.

However, the plants extract had a good antifungal effect on the test fungi and can be used to combat the infections caused by such phytopathogenic organisms on plants.

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