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Auntifungal Activity of Extracts of Euphorbia Kamerunica PAX

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Abstract: *Antifungal activities were detected in crude extracts of Euphorbia kamerunica plant using different organic solvents and water. Radial growth was used to measure the activity. Growths were observed only at concentrations of 2.5 mg/ml and 5 mg/ml and not at 10 – 100 mg/ml. Ethyl acetate extract inhibited the growth of Microsporum audouinii for the entire period of the experiment (9 days) even at 2.5mg/ml. There were decreases in radial growths with increases in concentration of extracts hexane, aqueous and methanol extracts on Aspergillus niger. Ethanol extract inhibited the growth of Microsporum canis var.distortum until day 7 at 2.5 mg/ml and 5 mg/ml with radial growth being 14.0±0.0g. Fungi used included Aspergillus niger, Aspergillus flavus, Microsporum audouinii, Microsporum canis var. distortum and Trichophyton mentagrophytes var. nodulare.*

Keywords: *Antifungal activity, crude extract, dermatophytes, Euphorbia kamerunica, organic solvents.*

I. Introduction

Euphorbia kamerunica plant belongs to the family Euphorbiaceace and plants in this family have been used to treat skin infections such as ulcers, warts, cancers, tumors [1,2,3,4]. The plant is known to contain skin irritants, co-carcinogenic principles and other toxic constituents responsible for health hazards to both human and grazing livestock [5] with its bioactivity on brine shrimp (Artemia salina) investigated [6]. Extract from Euphorbia species have been used in the treatment of skin diseases, migraine and intestinal parasites and warts [7]. Plants belonging to the family Euphorbiaceace have been found to contain diterpenes which have tiglane, ingenane and daphnane skeletons [8].

Medicinal plants have been used in the treatment of different diseases including skin diseases such as mycotic infections which is an old age practice in different parts of the world [9]. Compounds from plant extracts which are secondary metabolites serves as defence agents against invading microorganisms [10].

Researchers have conducted studies on antifungal substances from plants [11] which include terpenes, tannins, alkaloids, steroids, saponins, phenols, quinines and flavonoids. A great number of antimicrobial agents already exist for various purposes but the search for new antimicrobials ought to continue since the target microorganisms often evolve into new genetic variants which subsequently become resistant to existing agents with the current trend in the biotechnology of plant tissue cultures [12].

Dermatomycoses are infections produced by dermatophytes and may not be common as other microbes but when present, they could be difficult to eradicate especially in immunosuppressive situations. There are currently emergence of resistance strains and drug toxicity. Therefore, there is a distinct need for the discovery of new, safer and more effective antifungal agents [13]. New topical agents that will be effective are needed since fungal infections are difficult to treat even with the use of antifungal drugs [14] with drawbacks of major antibiotics being increase in incidence of opportunistic mycoses associated with AIDS.

Trichophyton, Microsporum and Epidermophyton are causative agents of dermatophytoses implicated in infections of the hair, skin and nails [15,16,17,18]. The ability to invade keratinized tissues and possession of several enzymes, such as proteinases, elastases, keratinases and other proteinases are the major virulence of these fungi [19] while Aspergillus flavus is implicated in invasive disease of immunosuppressed patients.

The investigation of plants used in traditional medicine for skin infections might also provide new topical antiseptic urgently needed in the third world countries. The actinomycotic activity of higher plants remained largely unexplored when compared with that of other microorganisms. In Nigeria, herbal remedies are still the preferred and sought method in the treatment of various ailments especially among the rural dwellers.
The objective of this present work is to determine the antifungal activity of this plant extracts on some pathogenic fungi.

II. Materials and Methods

2.1 Plant material and extraction

Euphorbia kamerunica plant was collected from the Botanical Gardens of the University of Ibadan, Oyo state, Nigeria and authenticated by Prof Biodun Ayodele of the Department of Botany, University of Ibadan. A voucher specimen with no 22278 was deposited at the herbarium. The plant was washed, cut and pounded into pulp using a mortar and pestle and extraction was carried out using the soxhlet apparatus. Solvents

used included hexane, dichloromethane, diethyl ether, ethyl acetate, methanol, ethanol, acetone and water. The extracts were then filtered and evaporated to dryness under reduced pressure and kept in the refrigerator until needed.

2.2 Microorganisms and medium.

Aspergillus niger and Aspergillus flavus were obtained from the Department of Botany and Microbiology, University of Ibadan and they were grown at 30^0C and maintained at 4^0C on Potato dextrose agar (PDA) slants. The dermatophytes used were Trichophyton mentagrophytes var.nodulare, Microsporum canis var. distortum and M. audouinii. They were grown at 30^0C and maintained at 4^0C on Sabouraud dextrose agar (SDA) slants.

2.3 Preliminary phytochemical studies.

Preliminary phytochemical studies were carried out using the methods described by [20] and [21]. Basic phytochemical screenings were performed on ethyl acetate and methanol extracts to test for alkaloids, phenols, chalcones, saponins, cardiac glycosides, flavonoids, tannins, anthraquinones and combined anthraquinones.

2.4 Antifungal assay

The methods of [22] and [23] were used. The extracts were tested on Microsporum audouinii, Microsporum canis var. distortum, Trichophyton mentagrophytes var.nodulare Aspergillus niger, and Aspergillus flavus. With the aid of a sterile 9.0 mm cork borer, mycelial discs were cut from 7- day old cultures of dermatophytes and fungi and aseptically transferred to the centre of Petri dishes containing Sabourand dextrose agar for dermatophytes and Potato dextrose agar for fungi impregnated with different concentrations of the extracts- 2.5, 5.0, 10.0, 25.0, 50.0 and 100.0mg/ml. Agar plates without extracts similarly inoculated with mycelial disc of test dermatophytes and fungi served as control. The plates were incubated in duplicates at 30^oC and radial growth was measured daily for nine days in two directions and the mean calculated to assess the antifungal property of the extract at different concentrations. The experiment was carried out in duplicates.

2.5 Statistical analysis

Results were expressed as means $\pm$ S.D of two separate experiments. Statistical significance was determined using SPSS 10 software after one-way variance analysis.

III. Results and discussion

Seven extracts of Euphorbia kamerunica were obtained using different solvents- hexane, ethyl acetate, acetate, acetone, aqueous, methanol, ethanol and dichloromethane. The activities of these extracts at different concentrations were tested on Aspergillus flavus, Aspergillus niger, Microsporum audouinii, Microsporum canis var. distortum and Trichophyton mentagrophytes var. nodular. Extracts of Euphorbia kamerunica when screened contained alkaloids, phenols, chalcones, saponins, cardiac glycosides, flavonoids and tannins. Phlobatannins, anthraquinones and combined anthraquinones were found to be absent in the plant extracts used (result not shown).

TABLE 1 shows the effect of crude extracts of E. kamerunica using different solvents on the radial growth of Aspergillus niger. At concentrations of 10.0, 25.0, 50.0 and 100.0 mg/ml, the extracts inhibited the growth of the fungus. At 2.5 mg/ml and 5.0 mg/ml, growths were observed. After 24 hours of incubation, the radial growth of the control was 16.5 $\pm$ 2.0 mm. Hexane extract at concentration of 2.5mg/ml and aqueous extract at concentrations of 2.5 mg/ml and 5.0 mg/ml had radial growths which were 13.5 $\pm$ 0.5 mm, 15.5 $\pm$ 5.0 mm and 15.0 $\pm$ 2.0 mm respectively. There were no significant differences in these values. The extracts were not effective. Dichloromethane extract at 2.5mg/ml and 5.0mg/ml inhibited the growth of A. niger till day 3 while growth was observed on day 4. At concentration of 2.5mg/ml on day 4, the growth was 21.5 $\pm$ 2.5 mm and 21.5 $\pm$ 2.5 mm at 5.0mg/ml and compared to the control, these values were significantly different.

Diethyl ether extract gave the least growth on day 9. At concentration of 5.0mg/ml, the growth was 39.5 $\pm$ 2.5 mm and 55.5 $\pm$ 1.5 mm at 2.5 mg/ml, while for methanol extract on the same day, at concentration of 2.5 mg/ml the radial growth was 48.0 $\pm$ 0.0 mm and 41 $\pm$ 1.0 mm at 5.0 mg/ml and these values were not significantly different from each other but to the control value, they were significantly different. The value of the radial growth of hexane extract at concentration of 2.5 mg/ml was 90.0 $\pm$ 0.0 mm at day 9 and this was significantly different to that of 5.0 mg/ml which was 48.0 $\pm$ 2.0 mm at day 9.

TABLE 2 shows the effect of crude extracts of Euphorbia kamerunica using different solvents on the radial growth of Aspergillus flavus. After 24 hours of incubation, only the control and aqueous extract had growth. The ethanol extract did not grow until day 3. At day 9, the least radial growth was observed for diethyl ether which was 31.5 $\pm$ 0.5 mm at concentration of 2.5 mg/ml and 33.5 $\pm$ 0.5 mm at 5.0 mg/ml and these values

were not significantly different from each other but significantly different from the control. TABLE 3 shows the effect of crude extracts of E. kamerunica plant using different solvents on the radial growth of Microsporum audouinii. For ethyl acetate extract, there was no growth recorded throughout the period of the experiment at concentrations of 2.5 mg/ml and 5.0 mg/ml. This showed that the extract inhibited the growth of the organism. For the ethanol extract, growth was not observed until day 8. At concentration of 2.5 mg/ml, the radial growth was 17.0± 0.0 mm and 14.5 ± 0.5 mm at 5.0 mg/ml. The values for the ethanol extract at these two concentrations were not significantly different on day 9 but they were significantly different to the value obtained for the control. The radial growth was 24.0 ± 0.0 mm at concentration of 2.5 mg/ml and 23.0 ± 2.0 mm at 5.0 mg/ml.

The effect of crude extracts of Euphorbia kamerunica plant using different solvents on the radial growth of Microsporum canis var. distortum is shown in TABLE 4. At day 4, there was no growth for methanol extract at 5.0 mg/ml and ethanol extract at concentrations of 2.5 and 5.0 mg/ml. At day 5, radial growth was seen at 5.0 mg/ml for methanol extract at 13.0 ± 0.0 mm. The ethanol extract had effect on the organism until day 7 when compared to the other extracts. For the control at day 9, the radial growth was 88.0 ± 2.0 mm and for ethanol extract at the same day, the values were 23.0 ± 0.0 mm and 21.5 ± 0.0 mm at concentrations of 2.5 mg/ml and 5.0 mg/ml respectively. This shows that compared to the control the values were significantly different. This indicates that the ethanol extract had effect on the radial growth of the organism, but the values were not significantly different at both concentrations.

TABLE 5 shows the effect of crude extracts of Euphorbia kamerunica plant using different solvents on the radial growth of Trichophyton mentagrophytes var. nodulare. There were no growths at concentrations of 10.0, 25.0, 50.0 and 100.0 mg/ml. No growth was observed until day 4 for ethanol extract. The values were not significantly different at concentrations of 2.5 mg/ml and 5.0 mg/ml which were 16.5 ± 0.5 mm and 14.0 ± 0.0 mm respectively but when compared to the control, they were significantly different.

At day 9, the dichloromethane extract showed the least radial growth values and these values were significantly different from each other and from the control. The values were 58.5 ± 1.5 mm at concentration of 2.5 mg/ml and 50.5 ± 0.5 mm at 5 mg/ml and for the control, it was 90.0 mm.

All the extracts used inhibited the growth of all the fungi at concentrations between 10.0mg/ml and 100.0mg/ml. Growth was observed at concentrations of 2.5 mg/ml and 5.0 mg/ml.

The extracts of Euphorbia kamerunica showed activities against the radial growth of fungi and dermatophytes used. There were variations in the activities of the different solvents used for extraction. Ethyl acetate extract of the plant totally inhibited the growth of Microsporum audouinii throughout the period of the experiment. [24] carried out a study on the plants used in Guatemala for the treatment of dermatophytic infection and their results were consistent with this study. [10] worked on 44 aqueous plant extracts and 22 out of them inhibited the growth of one or more dermatophytes including Microsporium canis and Trichophyton mentagrophytes which also in agreement with the work done here in that these extracts had acticities on dermatophytes. Study carried out by [25] on crude extracts of Erigeron floribundus showed broad spectrum against Microsporium canis, Microsporium gypseum, Trichophyton mentagrophytes, Epidermophyton floccosum. This is also in agreement with this study where antifungal activities were observed with extracts of Euphorbia kamerunica on Microsporum canis and Trichophyton mentagrophytes.

IV. Conclusion

From this experiment, some of the crude extracts of Euphorbia kamerunica plant were effective against the growth of some dermatophytes from day 6 of the experiment through to the end at concentrations of 2.5mg/ml and 5mg/ml. Further work needs to be carried out on the isolation of the compounds to know the active ones against these microorganisms, after which they might be used as treatment for infections caused by dermatophytes.

Table 1: Effect of crude extracts of Euphorbia kamerunica using different solvents on the radial growth (mm) of Aspergillus niger

	Radial Growth ± S.D/ Days								
Solvent/Concentration Mg/ml	1	2	3	4	5	6	7	8	9
Control	16.5±2.0*	32.±2.5 a †	44.0±2.5a	52.0±2.0ab	62.5±2.5a	66.0±2.0a	68.5±2.5ab	79.0±2.0a	87.5±2.5ab
HX: 2.5	13.5±0.5a	21.5±1.0bcd	35.0±0.5b	43.0±1.0bc	69.0±1.0a	71.5±1.5a	73.5±1.5a	80.5±2.5a	90.0±0.0a
5	-	11.5±0.5ef	18.0±1.0e	19.5±1.5fg	26.0±1.0fg	30.0±1.0g	33±2.0f	37.5±2.5fg	48.0±2.0fg
EA: 2.5	-	19.5±2.5cdef	31.0±1.5bc	38.5±1.5cd	56.0±1.5abc	59.0±1.0abc	63.5±1.5abc	66.5±1.5abc	73.5±2.5bcd
5	-	20.5±0.5bcd	31.5±2.5bc	37.5±2.5cd	55.5±2.5abc	57.5±2.5abcd	59.5±2.5abcd	62.5±2.5bcd	65.0±1.0cde
AC: 2.5	-	17.5±0.5def	23.5±2.0cde	29.5±2.0def	38.0±0.0def	40.5±1.5efg	42.5±0.5def	47.5±1.5defg	51.5±1.5efg
5	-	17.5±05def	28.0±2.0bcd	33.0±2.0cde	41.0±1.0ef	43.5±0.5defg	45.5±0.5def	50.5±1.5cdefg	53.0±1.0efg
AQ: 2.5	15.5±2.0a	30.5±0.5a	46.5±2.5a	55.5±0.5a	60.5±2.5ab	63.0±2.0ab	66.5±3.5ab	70.5±2.5ab	78.0±2.0abc
5	15.0±2.0a	28.0±2.0ab	31.0±2.0bc	33.5±2.5cde	38.5±2.5def	42.5±2.5efg	49.0±2.0cdef	54.0±2.0bcdef	62. 5±2.5cdef
MEOH: 2.5	-	15.0±2.0def	19.5±2.5e	26.0±1.0ef	29.0±1.0efg	33.0±1.0fg	38.0±2.0ef	40.5±1.5efg	48.0±0.0fg
5	-	-	10.5±1.5f	15.0±0.5g	21.0±1.0g	29.5±2.5g	34.0±0.0f	36.5±0.5g	41.0±1.0g
ETOH: 2.5	-	20.0±0.0bcde	30.0±0.0bc	33.5±2.5cde	46.5±1.5cd	49.0±1.0bcde	52.0±1.0bcde	54.0±0.0bcdef	60.0±2.0def
5	-	26.0±2.0def	32.0±2.0b	37.5±2.5cd	48.0±1.0bcd	49.5±2.5bcde	52.5±2.5bcde	56.0±2.0bcde	61.0±1.0def
DCM: 2.5	-	-	-	21.5±2.5fg	25.5±2.5fg	30.0±1.0g	39.0±2ef	44.0±2aefg	51.5±2.5efg
5	-	-	-	21.5±2.5fg	28.5±2.5efg	32.5±2.5fg	40.5±2ef	42.5±2.5efg	46.5±2.5fg
DEE: 2.5	-	13.5±1.5def	31..5±2.5bc	38.0±2.0cd	43.0±2.0cd	46.0±1.0cdef	48.5±0.5cdef	52.5±0.5cdefg	55.5±1.5efg
5	-	11.0±0.0f	21.0±1.0de	23.5±1.5efg	26.5±2.5fg	31.0±2.0g	33.0±2.0f	36.0±2.0g	39.5±2.5g

Hx = Hexane, EA= Ethyl acetate, AC= Acetone, AQ=Aqueous, MEOH= Methanol, ETOH= Ethanol, DCM= Dichloromethane, DEE= Diethyl acetate. * Means of two readings ± standard deviation. † Values in the same column followed by the same letter are not significantly different (p>0.05) from each other.

Diameter of cork borer = 9.0mm

Table 2: Effect of crude extracts of Euphorbia kamerunica using different solvents on the radial growth (mm) of Aspergillus flavus

	Radial Growth ± S.D/ Days								
Solvent/Concentration mg/ml	1	2	3	4	5	6	7	8	9
Control	16.5±1.5*	27.0±2.0†	40.5±0.5b	50.5±1.5b	58.5±2.5b	72.0±2.0a	82.0±2.0a	90.0±0.0a	-
HX: 2.5	-	16.5±0.5cd	21.5±0.5fg	31.0±2.0c	48.5±2.5ab	51.5±2.5bcde	55.0±1.5bcde	62.5±2.5bcd	68.0±2.0b
5	-	12.5±0.5ef	13.5±0.5i	19.0±1.0f	49.0±0.0ab	52.5±2.5bcd	56.5±2.5cdef	60.5±2.5bcde	65.0±1.5bc
EA: 2.5	-	18.5±0.0c	28.5±2.0bc	34.0±0.0d	42.5±2.5abc	50.5±0.5bcdef	52.5±0.5bcde	54.5±0.3cdef	59.0±1.0bcd
5	-	19.0±1.0c	32.5±0.5c	35.0±1.0d	41.5±0.5bc	59.5±1.5abc	66.0±2.0bcde	69.0±1.0bc	72.0±2.0b
AC: 2.5	-	18.0±1.0cd	27.5±2.5de	44.5±0.5d	48.5±0.5ab	56.5±0.5abc	66.0±2.0abc	69.0±1.0bc	73.0±2.0b
5	-	18.0±0.0cd	23.5±0.5ef	57.5±2.5a	48.5±0.5ab	56.5±0.5abc	66.0±1.0abc	69.0±1.5bc	71.5±1.5b
AQ: 2.5	15.0±0.0a	25.5±2.5ab	52.0±2.0a	58.0±2.0a	60.0±2.0a	65.5±1.5ab	68.5±2.5ab	73.5±1.5b	90.0±0.0a
5	14.0±1.0a	23.0±0.0b	32.5±2.5c	36.0±1.0d	52.5±2.5ab	55.0±2.0abc	60.5±0.5bcd	68.5±1.5bc	90.0±0.0a
MEOH: 2.5	-	14.5±1.5def	17.5±0.5ghi	18.5±0.5d	42.5±0.5bcd	45.0±1.0cdefg	48.0±1.0cdef	50.5±0.5defg	52.5±0.5cde
5	-	11.5±0.5f	13.5±0.5i	23.5±0.54f	24.0±1.0a	29.0±1.0gh	32.0±1.0fg	37.5±2.5fghi	42.0±2.0ef
ETOH: 2.5	-	-	18.5±0.5gh	18.5±0.5ef	30.5±1.5ab	35.0±1.0defgh	44.0±2.0def	46.5±1.5defgh	52.0±2.0cde
5	-	-	14.5±1.5hi	23.0±0.5f	26.5±0.5ab	31.0±1.0gh	40.5±0.5efg	44.5±2.5efghi	45.0±1.0def
DCM: 2.5	-	12.5±0.5ef	17.0±1.0ghi	23.0±1.0ef	29.5±2.5ab	34.0±2.0efgh	44.0±2.0def	47.0±2.0defgh	52.5±2.5cde
5	-	12.5±0.5ef	16.5±0.5hi	25.5±0.5e	30.0±0.0ab	32.5±1.5fgh	34.5±1.5fg	37.0±2.0ghi	40.5±1.5ef
DEE: 2.5	-	16.5±0.5cd	17.5±0.5ghi	19.0±0.0f	20.0±0.0a	21.0±1.0h	25.5±1.5g	29.0±1.0i	31.5±0.5f
5	-	15.5±1.5cde	16.5±1.5hi	19.0±0.0f	20.0±0.0a	20.5±0.5h	25.5±0.5g	30.0±2.0hi	33.5±0.5f

Hx = Hexane, EA= Ethyl acetate, AC= Acetone, AQ=Aqueous, MEOH= Methanol, ETOH= Ethanol, DCM= Dichloromethane, DEE= Diethyl acetate.* Means of two readings ± standard deviation. † Values in the same row followed by the same letter are not significantly different (p>0.05) from each other. Diameter of cork borer = 9.0mm

Table 3: Effect of crude extract of Euphorbia kamerunica plant using different solvents on the radial growth (mm) of Microsporum audouinii

	Radial Growth ± S.D/ Days							
Solvent/Concentration mg/ml	2	3	4	5	6	7	8	9
Control	17.0±0.0cd*	29.0±1.0bc†	40.0±1.0bc	43.0±0.0c	55.0±1.0cd	62.5±0.5b	68.5±1.5d	72.0±2.0c
HX: 2.5	29.0±1.0a	48.5±1.5a	65.5±0.5a	80.0±1.0a	90.0±0.0a			
5	31.0±0.0a	51.5±1.5a	63.0±2.0a	80.0±0.0a	90.0±0.0a			
EA: 2.5	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-
AC: 2.5	15.0±2.0cd	29.5±2.5bc	36.0±2.0cd	45.5±1.5c	49.5±1.5de	53.5±2.5d	56.5±2.5e	60.0±2.0d
5	-	11.0±0.0e	12.5±0.5f	22.5±2.5d	35.0±0.0g	37.5±0.5e	40.5±1.5f	44.5±1.5e
AQ: 2.5	18.0±0.0bc	31.5±0.5b	41.5±1.5bc	50.0±1.0bc	58.5±1.5c	64.5±2.5b	74.5±0.5c	81.0±1.0b
5	16.0±1.0bcd	31.5±1.5b	46.0±1.0b	57.5±.05b	68.5±1.5b	77.5±0.5a	81.0±1.0b	90.0±0.0a
MEOH: 2.5	-	25.0±1.0cd	36.0±1.0cd	51.0±1.0bc	67.5±2.5b	81.0±1.0a	90.0±0.0a	
5	-	-	15.0±0.0f	29.5±1.5d	40.0±2.0fg	55.0±2.0c	67.0±2.0d	88.5±1.5a
ETOH: 2.5	-	-	-	-	-	-	17.0±0.0h	24.0±0.0g
5	-	-	-	-	-	-	14.5±0.5h	23.0±2.0g
DCM: 2.5	14.0±0.0d	30.0±1.0bc	32.0±2.0d	45.0±0.0c	46.5±0.5ef	49.5±0.5d	52.5±0.5e	55.5±0.5d
5	14.0±0.0d	21.0±2.0d	22.0±2.0e	23.0±2.0d	25.0±2.0h	28.5±2.5f	32.5±2.5g	35.5±2.5f
DEE: 2.5	15.0±2.0cd	20.0±2.0d	24.0±2.0e	29.0±2.0d	38.0±2.0g	50.0±2.0d	57.5±2.5e	69.0±1.0c
5	19.0±1.0b	25.0±2.0cd	41.0±2.0bc	55.0±2.0b	58.0±2.0b	60.0±2.0c	67.0±1.0d	71.0±1.0c
Nystatin	-	10.5 ± 0.5e	11.5 ± 1.5f	13.5 ± 0.5e	13.5 ± 0.5i	14.5±0.5g	16.5±1.0h	17.5±1.0h

Hx = Hexane, EA= Ethyl acetate, AC= Acetone, AQ=Aqueous, MEOH= Methanol, ETOH= Ethanol, DCM= Dichloromethane, DEE= Diethyl acetate. * Means of two readings ± standard deviation.† Values in the same column followed by the same letter are not significantly different (p>0.05) from each other. Diameter of cork borer = 9. 0 mm

Table 4: Effect of crude extracts of Euphorbia kamerunica plant using different solvents on the radial growth (mm) of Microsporum canis var. distortum

	Radial Growth ± S.D/ Days							
Solvent/Concentration mg/ml	2	3	4	5	6	7	8	9
Control	25.5±0.5a*	36.0±0.0ab†	48.5±1.5a	55.5± 0.5a	63.0±1.0ab	70.0±0.0a	74.0±2.0a	88.0±2.0ab
HX: 2.5	17.5±0.5bc	18.0±0.0d	21.0±0.0c	29.0±1.0b	33.0±1.0cd	44.5±0.5cd	51.0±1.0cd	56.0±2.0d
5	17.0±0.0bc	20.0±1.0d	23.0±0.0cd	25.5±0.5bc	29.0±1.0cd	34.5±1.5d	39.5±0.5e	44±2.0e
EA: 2.5	-	21.0±1.0d	23.5±2.0c	24.0±1.0bcd	33.5±0.5c	45.5±0.5c	59.5±2.5bc	73.0±1.0c
5	-	17.5±1.5d	20.5±2.5cd	23.0±0.0bcd	27.5±2.5cdef	42.0±1.0dc	56.5±1.5bc	69.0±1.0c
AC: 2.5	21.5±2.5ab	31.5±2.5c	44.0±3.0ab	49.0±2.0a	67.0±0.0a	69.5±0.5a	73.0±1.0a	78.5±1.0bc
5	17.0±1.0bc	28.5±0.5c	41.0±1.0ab	48.0± 0.0a	56.5±0.5ab	64.5±0.5ab	67.5±2.5ab	79.0±0.0bc
AQ: 2.5	17.0±1.0bc	29.0±0.0c	40.0±0.0b	47.5±0.5a	59.0±1.0ab	63.5±1.5ab	67.5±2.5ab	90.0±0.0a
5	16.5±0.5c	29.5±1.5c	40.5±0.5b	48.0±0.0a	53.0±2.0b	55.0±3.0b	61.0±2.0bc	90.0±0.0a
MEOH: 2.5		-	16.0±0.0cd	18.5±0.5bcd	22.0±0.0def	23.0±1.0fg	27.5±0.5f	28.0±0.0f
5		-	-	13.0±0.0d	17.5±1.5f	19.0±1.0efg	22.0±1.0fg	24.0±0.0f
ETOH: 2.5		-	-	-	-	14.0±0.0fg	19.0±0.0fg	23.0±0.0f
5		-	-	-	-	12.0±0.0g	16.0±0.0g	21.5±0.0f
DCM: 2.5		-	17.5±0.5cd	24.0±1.0bcd	29.5±1.5cde	36.5±0.5cd	44.5±1.5de	51.0±1.0de
5		-	13.0±1.0d	16.0±2.0cd	20.0±1.0ef	24.5±1.5e	27.5±2.5f	41.5±2.5e
DEE: 2.5	17.5±0.5bc	35.0±0.0b	45.5±2.5ab	49.0±1.0a	58.0±2.0ab	62.0±2.0ab	66.0±2.0ab	69.5±2.5c
5	15.5±1.5c	39.0±1.0a	45.5±2.5ab	54.0±2.0a	60.0±1.0ab	62.5±2.5ab	65.5±2.5ab	69.0±2.0c
Nystatin	-	17.0± 0.0d	21.5 ± 1.0c	28.0 ± 1.0b	31.0 ± 1.0cd	35 .0±0.0cd	40.0 ± 0.0e	42 .0± 2.0e

Hx = Hexane, EA= Ethyl acetate, AC= Acetone, AQ=Aqueous, MEOH= Methanol, ETOH= Ethanol, DCM= Dichloromethane, DEE= Diethyl acetate. * Means of two readings ± standard deviation. † Values in the same column followed by the same letter are not significantly different (p>0.05) from each other. Diameter of cork borer = 9.0mm

Table 5: Effect of crude extracts of Euphorbia kamerunica plant using different solvents on the radial growth (mm) of Trichophyton mentagrophytes var. nodulare

Radial Growth ± S.D/ Days								
Solvent/Concentration mg/ml	2	3	4	5	6	7	8	9
Control	18.5±0.5bcde*	30.5±0.5bc†	41.5±0.5bc	49.5±0.5bc	54.5±2.5cde	64.0±1.0bc	74.5±0.5bc	90.0±0.0a
HX: 2.5	20.5±0.5abc	35.0±2.0a	45.0±2.0b	57.0±2.0a	75.0±2.0a	87.0±0.0a	88.0±0.0a	90.0±0.0a
5	23.5±0.5a	34.0±0.0ab	44.0±0.0b	53.0±0.0ab	63.5±0.5b	70.5±0.5b	78.0±0.0b	85.0±0.0ab
EA: 2.5	19.0±0.0bcd	28.5±0.0cd	41.5±1.5bc	43.0±1.0ef	50±2.0cdef	64.5±1.5bc	72.5±2.0bc	78.5±0.5c
5	-	21.0±1.0f	33.0±1.0de	43.5±0.5def	53.5±2.5cde	64.5±2.5bc	72.5±1.5bc	81.5±2.5bc
AC: 2.5	13.5±0.5f	23.0±0.0ef	23.0±0.0f	38.5±0.5fg	43.5±1.5fg	47.5±1.5e	51.5±2.5fgh	55.5±2.5gh
5	16.5±0.5cdef	25.5±0.5de	30.0±1.0e	37.0±0.0f	42.0±0.0fg	44.5±1.5e	48.0±1.0gh	51.5±1.5h
AQ: 2.5	20.0±0.0abc	31.0±0.0bc	43.0±0.0b	52.5±0.5ab	57.0±1.0bc	65.0±0.0bc	69.5±0.5c	89.0±1.0ab
5	20.0±0.0abc	33.0±2.0ab	43.0±0.0b	48.5±2.5bcd	52.5±0.5cde	59.0±1.0cd	62.0±0.0d	81.0±1.0bc
MEOH: 2.5	15.0±0.0ef	22.0±0.0ef	28.0±0.0ef	37.0±2.0g	46.5±2.5efg	57.5±1.5cd	71.5±0.5c	86.0±1.0ab
5	16.0±0.0def	24.0±0.0ef	30.5±0.5e	37.0±0.0g	46±0.0efg	58.0±1.0cd	63.0±1.0d	71.0±1.0d
ETOH: 2.5	-	-	16.5±0.5g	20.5±0.5h	23.5±0.5h	46.0±1.0e	52.5±2.5fg	59.0±1.0fg
5	-	-	14.0±0.0g	18.5±1.5h	21.0±0.0h	34.0±2.0f	51.0±1.0fgh	62.5±2.5ef
DCM: 2.5	20.0±2.0abc	35.5±0.5a	42.0±0.0bc	45.5±0.5cde	47.0±0.0defg	51.5±1.5de	54.0±2.0ef	58.5±1.5h
5	19.5±1.5bcd	31.0±1.0bc	36.5±0.5cd	39.0±0.0fg	40.5±0.5g	43.5±0.5e	46.5±0.5h	50.5±0.5ef
DEE: 2.	21.0±2.0ab	35.0±2.0a	45.5±2.5b	51.0±2.0b	53.5±2.5cde	56.5±2.5cd	59.0±2.0de	63.0±2.0e
5	18.5±2.0bcde	35.5±0.5a	51.0±1.0a	53.5±1.5ab	55.5±1.5bcd	57.5±1.5cd	61.5±1.5d	64.5±0.5d
Nystatin	-	10.5 ± 0.5g	14.0 ±0.0g	19.0 ± 0.0h	22.5 ± 0.5h	27.0 ±1.0g	32.0 ± 0.0i	35.5 ± 1.5i

Hx = Hexane, EA= Ethyl acetate, AC= Acetone, AQ=Aqueous, MEOH= Methanol, ETOH= Ethanol, DCM= Dichloromethane, DEE= Diethyl acetate. * Means of two readings ± standard deviation† Values in the same column followed by the same letter are not significantly different (p>0.05) from each other. Diameter of cork borer = 9.0 mm

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