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Formulation and Pharmaceutical Characterization of Antidermatophyte Creams of Methanol Leaf Extracts of *Euphobia Hirta, Jatropha Curcas* and *Acantus Montanus*

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Research Article

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Abstract

Background: The search for novel natural products from medicinal plants against multidrug fungal strains is very essential to overcome the resistance complication. Many researchers have focused on the antidermatophyte activity of crude extracts from naturally occurring plants.

Objective: This study investigates cream formulations of methanolic crude extracts of *E.hirta, J. curcas* and *A. montanus* for their pharmaceutical characteristics and antifungal potentials, especially against some drug resistant dermatophytes isolated from work equipment of local hair dressers.

Methods: Dermatophytes samples were inoculated into sterile Sabouraud dextrose agar (SDA) broth and incubated. Positive growths were examined macroscopically and microscopically and confirmed with the aid of a fungi atlas. The phytochemical constituents of the plants extracts were also determined. Antifungal susceptibility testing was also carried out using agar diffusion assay. Using the best extract combination ratios obtained from the checkerboard assay, creams were formulated with the methanolic crude extracts and the pharmaceutical properties of the creams were determined.

Results: MIC of the individual extracts showed that Trichophyton schoenleinii and Microsporon ferugenium were resistant to all the extracts except *J. curcas*. The checkerboard assay showed that the combination of *J. curcas* and *A. montanus* had the best anti-fungal activity for *T. schoenleinii*, while the combination of *J. curcas* and *E.hirta* also gave an excellent result. All the formulated creams exhibited good pharmaceutical characteristics.

Conclusion: The formulated creams showed good anti-fungal and pharmaceutical properties. Overall, the study scientifically validates that methanolic leaf extracts of A. montanus, *E. hirta* and *J. curcas* are potent antifungal agents.

Keywords: Dermatophytes; Euphorbia Hirta; Jatropha Curcas; Acantus Montanus; Cream Formulations

Abbreviations: SDB: Sabouraud Dextrose Broth; DMSO: Dimethyl Sulphoxide; FIC: Fractional Inhibitory Concentration

Introduction

Dermatophytosis is a fungal infection, which has been very frequent in occurrence among other fungal infections [1]. It is one of the most resistant infections, mainly due to environmental factors of temperature, moisture and phenotype [2]. It affects mainly the skin, scalp (*Tinea capitis*), fingernails, groin (*Tinea cruris*) and toe nails (*Tinea pedis*) and may also affects other parts of the body surfaces (*Tinea corporis*) [3]. Its prognosis is a high long-term impairment lesion that occurs again over time and progresses to a more complicated secondary infection. It can infect both individuals with high immune system and compromised immune system. The most common dermatophyte implicated belongs to the genera *Microsporium, Trichophyton* and *Epidermophyton*. Dermatophytes constitute a non-opportunistic class of fungi which are not life threatening and survive by obtaining nutrients from keratin, which are abundant in tissues of the nails, hair and skin [4]. They are referred to as keratinophillic infectious fungi because keratin is their source of nutrient. The enzyme keratinases, which are specialized keratinolytic/ proteolytic enzymes produced by dermatophytes to degrade the protein keratin found on the body surfaces, nails and hair scalp [5].

In view of the research for newer drugs due to increasing resistance to current ones, natural products continue to provide lead compounds for researchers to finding new drugs. Bioactive molecules from these natural herbs and plants have been chemically modified to produce new drug molecules with diverse activities. Thus, these naturally occurring plants and herbs have been the origin of so many drug molecules used in treatment of varying degrees of diseases and various illnesses owing to their therapeutic value. Many researchers have focused on the antidermatophyte activity of crude extracts from naturally occurring plants, because many plants have the vast ability to synthesize biologically active compounds with different functional groups. Hence, crude extracts provide good basis for the research of more potent, effective and safe to use antifungal agents, particularly dermatophytes which is more resistant to most existing antifungal agents [3]. In this respect, the *J. curcas, E. hirta* and *A. montaneus* have been investigated individually [6-9].

Additionally, plant extracts also contain secondary metabolites, which can target more than one cell, and this is the basis for using multiple plant extracts in disease management in which multiple targets are involved. These secondary metabolites are the defense mechanism used by plants, which are also very useful to humans in terms of nutrition [10]. Secondary metabolites are also involved in activation of signaling compounds that provides protection for the plants from the pathogens or environmental hazards and this aspect of nutritional value of these plant secondary metabolites has been experimented to also have high therapeutic index in treating many diseases and ailments [11]. Thus this study aims at investigating the methanolic crude extracts of *J. curcas, E. hirta,* and *A. montaneus* combinations as antidermatophyte, as well as the preparation of various cream formulations of these extracts, their pharmaceutical characterization, and the tests for antifungal properties of the creams using suitable clinical isolates.

Methods

Extraction of Plant Material

A mechanical grinder was used to pulverize air-dried plant samples and 2.5 kg each of the powdered material macerated, with intermittent shaking, in methanol (5 L) for two days. Using a combination of muslin cloth and filtered paper (Whatman No 1), the marcs were filtered, and concentrated using rotary evaporator. Resultant crude extracts were refrigerated until they were ready for further analysis.

Phytochemical Analysis

The standard procedure reported in Trease, et al. [12] was used to determine the phytochemical constituents of the plant extracts. Among the phytochemical screening tests conducted include tests for saponin, flavonoids, steroids, terpenoids (using Salkowski test), and cardiac glycosides (using Keller-Killani test). Other tests include tests for alkaloids, protein, carbohydrate, and reducing sugar.

Collection of Test Organism Samples

Sample collection was preceded by oral assent from the study participants. A total of 45 samples were aseptically collected using sterile swab sticks from the work equipment (combs of various sizes, scissors, hair pins, needles etc.) of the local hair dressers. Samples were transported to the laboratory without delay for mycological analysis and were maintained in the refrigerator at 4°C until they were ready to be analyzed. The antifungal drugs (clotrimazole, ketoconazole and miconazole) that were used for this study were bought from pharmacy premises, also the antidandruff solutions (hair shampoo and hair conditioner) that were used for this study were bought from Eke-Awka Market in Anambra State Nigeria, stored under appropriate conditions until they were ready to be used.

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Inoculation and Isolation

After aseptic collection of the samples with the sterile swab, the samples were inoculated in sterile Sabouraud Dextrose Broth (SDB) base (Oxiod, UK) and incubated at 25°C for 5 days. At the end of incubation, the test tubes were visually observed for turbidity (indicating growth). The growing samples were platted out on a freshly SDA (Oxoid, UK) supplemented with chloramphenicol 0.05 g/L (that serves as a broad spectrum antibiotic) and the cultures were incubated aerobically for 7 days at room temperature (25°C). Growing (positive) cultures were examined for both macroscopic and microscopic characteristics as described below. Isolates from the positive cultures were confirmed with the aid of an atlas. Pure colonies were then stored on slants at 4°C for further studies as described by Cheesebrough [13]. At the end of 4 weeks, the absence of any growth in the culture indicates negative. The frequency of occurrence of the isolates was also calculated.

Macroscopic (Colonial) Characterization of Fungal Isolates

The following features were used to macroscopically characterize the pure cultures: colour, texture (colony, velvety, powdery, granular and pasty) and pigment production (seen on the reverse of the medium).

Microscopic Examination and Fungal Culture

This involves the use of direct microscopy. The mixture of 10% KOH and 40% Dimethyl sulphoxide (DMSO), in equal proportion, was used to mount the samples collected and were screened for the presence of fungal elements. Scraped skin and plucked hair samples were placed in the KOH+DMSO drops on the slide and a clean cover slip placed over the samples, pressed to prevent the formation of air bubbles and observed after 8 minutes. The combination of low power (10x) and high power (40x) magnification were used to visualize each slide for the presence of hypae and/ or arthroconidia. Thereafter, the hair and skin scraped specimens were then inoculated into two Petri dishes containing SDA base and Dermasel agar base, both supplemented with chloramphenicol, which serves as a broad spectrum antibiotic, and cycloheximide (for the inhibition of saprophytic fungi). It should be noted that in the absence of heat, the DMSO increases susceptibility of the preparation and softens keratin more quickly than KOH alone. The above fungal cultures were subjected to selective media, which was prepared by adding one vial of Dermasel Selective Supplement SR0075 (Oxoid, UK) to each 500 ml of medium and the cultures incubated aerobically for 7 days at room temperature (25°C). Positive cultures were examined both macroscopic (color of the surface and reverse, topography, and texture) and microscopic characteristics, as before.

Antifungal Susceptibility Testing

The antifungal susceptibility was evaluated using three antifungal agents: clotrimazole (50 µg), miconazole (10 µg) and fluconazole (25µg). The tip of a sterile Pasteur pipette was used to probe dermatophytes colonies from Dermasel/ SDA agar cultures and the mixture of mycelium and conidia obtained were inoculate into a sterile tube containing 1mL distilled water and allowed to sediment for 30 minutes. The innocula suspensions, with the aid of swabs, were streaked uniformly over the surface of Mueller-Hinton (MH) agar (Oxoid, UK) plates, and the plates, with lids left ajar for 3 minutes – to prevent the absorption of excess surface moisture into the agar before the drug-impregnated disks were applied –incubated in a laminar flow cabinet at 25°C for 5 days. At the end of incubation, the zones of inhibition around the disks were measured.

Preparation of McFarland Turbidity Standard

Fungal growth in cultures was evaluated using McFarland turbidity standard, as described by Oli, et al. [14]. Briefly, a 0.5 McFarland standard was prepared by adding 0.5 mL of 0.048M $BaCl_2$ (1.17% w/v $BaCl_2 \cdot 2H_2O$) to 99.5ml of 0.18M H_2SO_4 (1% v/v) with continuous stirring. Using matches cuvettes and distilled water as a blank standard, the optical density of barium sulphate precipitate was determined. The standard was compared visually to a suspension of fungal growth in sterile saline. The approximate cell density corresponding to 0.5 McFarland is 1×10^6 cells/mL.

Standardization of Test Isolates

The test organisms (molds), which were previously grown on SDA for 4 days, were subjected to 10-fold serial dilutions. The 0.1ml of the spore suspension of each isolate was taken from the stock culture and serially diluted. 0.1mL of the suspension was plated out by spread plate method for semi-confluent growth. Dilutions which gave working inoculums of 10^6 CFU/mL were used. For the yeast isolates, the test organism was inoculated into Sabouraud Dextrose Broth (SDB) and incubated over-night.

Agar Well Diffusion Assay

Sterile SDA plates prepared in accordance with the manufacturer's specification were inoculated with the test culture by surface spreading method using cotton swab, and wells (8 mm in diameter) made on the plates with the aid of sterile cork borer. Aliquots of 80 µL of each extract

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diluted in DMSO at concentrations of (50, 25, 12.5 and 6.25 and 3.125) mg/mL were dispensed into each of the wells in the culture plates, and the culture incubated for 48 h at 25°C (clotrimaziole (50 µg/mL) served as the positive control against the fungal test organisms. On the other hand, the DMSO served as the negative control). The extent of zone of inhibition around each well was used to determine the antimicrobial potential of the crude extract and triplicate determinations were taken. Each of the sample extracts was tested against all the fungal isolates.

Synergistic Study

To determine the interactive synergistic inhibition between the methanolic crude extracts, the Checkerboard assay as describe by Oli, et al. [14] was used. Briefly, it involves preparing the stock solution of each of the extract using the individual MICs. Also, separate solutions of the crude extracts were prepared with DMSO, each solution containing twice the MIC of the extracts. Thereafter, continuous variations model was adopted, in which the solutions were combined in different ratios. Each combination was then diluted two-fold for up to 6 serial dilutions in sterile Pyrex test tubes. An aliquot of 60 µL of the diluted solutions was transferred into a corresponding well in a sterile agar plate, which was previously seeded with 0.5 McFarland standard of the test organism. The plates were incubated at 37°C for 24 hours. The fractional inhibitory concentration (FIC) of each extract is the minimal inhibitory concentration in the combination divided by the independent MIC of the extracts. The sum of the FICs of both extracts gives the FIC index. This is expressed as in Equation 1:

$$FIC\ Index = \frac{'A}{''A} + \frac{B}{B}$$

Where, and Ɓ represent minimal concentrations of extracts A and B having inhibitory effects when acting together, while ҆Ą and Ƀ stand for the respective MICs of the extracts. The FIC Index value of less than 1.0 indicates synergism; equal to 1.0 implies additive; value of more than 1.0 suggests indifference, and more than 2.0 indicates antagonism.

Method of Cream Formulation

The herbal creams were formulated by tritrating the crude extracts with a mixture of sorbitol and glycerin (1:5). Tragacanth served as a suspending agent in the presence of selected preservatives, which was added to the wetted crude-extract mass slowly. Finally, the final volumes of different batches of the preparations were made up using purified water.

Evaluation of the Formulated Creams

- **Physical evaluations:** The following preliminary evaluations of the herbal cream formulations were carried out:
- **pH:** To determine how acidic or basic the various formulations were, the Digital pH meter (Digital pH meter 335, Systronics, Noroda, Ahmedabad) was used. A 0.5 g of the weighed formulation was dispersed in 50 mL of distilled water and the pH was measured.
- **Homogeneity:** The formulated creams were visually checked for homogeneity. This was done by assessing the creams for the appearance of lumps.
- **Viscosity:** This as a measure of the cream formulation's resistance to motion under an applied force was assessed using Brookfield Viscometer (model LV-DV-II, Helipath spindle type S-96). The viscosity for each formulation was taken in triplicates and the value expressed as mean± standard deviation.
- **Spreadability:** The ability of the formulated polyherbal cream to spread was determined using the formula in Equation 2:

$$S = \frac{LM}{T}$$

Where S is spreadability, M is weight tied to upper slide, L is length of glass slides and T is time taken to separate the slides completely from each other.

Acute Skin Irritation Study

The ability of cream formulations to cause skin irritation as evidenced by signs of oedema and erythema was assessed using albino rats weighing between 150-200 g, which were obtained from Pharmacology department, Pharmacy school, Nnamdi Azikiwe University, Agulu. Required ethical approval for the use of the animals was obtained from the Animal Use and Care Ethical Committee, Ministry of Health of Anambra state. All the animals were provided standard animal feed and access to water *ad libitum*. A day prior to the commencement of the study, fur from different sites (e.g. head, tail, right hand (RH),and right leg (RL)), was removed, and the animals kept individually in cages to prevent contact with the other rats. The untreated skin areas served as control. The standard irritant used was aqueous solution of 0.8% formalin. Thereafter, a50mg of each of the formulation containing combinations of *J. curcas/A. montanus*, *E. hirta/J. curcas*, and *A. montanus/E. hirta*, respectively were evenly and gently applied on the test sites.

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Stability Studies

The ability of all cream formulations to retain and maintain cream characteristics within the original desirable properties were assessed at different temperature conditions (4, 25 and 37°C) over 90 days in a climatic chamber. The cream formulation properties evaluated at different time intervals (i.e., 15, 30, 60 and 90th day) include pH, viscosity, spread ability, consistency and phase separation.

Results

Percentage Yield of Plant Extracts and their Qualitative Phytochemistry

Table 1 shows the qualitative phytochemistry, as well as the percentage yield of methanolic extracts of the various plants used in this study. Percentage yields for *J. curcas*, *E.hirta* and *A. montanus* are 5.88, 3.57 and 7.92, respectively. The phytochemical analysis shows moderate quantity of tannin in *J. curcas* and *E. hirta*. While *J. curcas* is also rich in protein, *E. hirta* has appreciable quantities of saponin and flavonoid. Moderate quantities of alkaloid and flavonoid were found in *A. montanus.* There were no traces of terpenoid, cardiac glycoside, carbohydrate and steroid found in *A. montanus*. While *J. curcas* has traces of alkaloid, flavonoid and steroid, some other molecules such as saponin, terpenoid, cardiac glycoside and carbohydrate were completely absent. All the evaluated molecules were found in varied quantities in *E. hirta.*

Plants		*Jatropha curcas*	*Euphorbia hirta*	*Acantus montanus*
% yield of herbal extracts		5.88	3.57	7.92
Qualitative phytochemistry of the herbal extracts	Alkaloid	+	+	++
	Saponin	-	++	+
	Tannin	++	++	+
	Flavonoids	+	++	++
	Steroid	+	+	-
	Terpenoids	-	+	-
	Cardiac Glycoside	-	+	-
	Carbohydrate	-	+	-
	Protein	++	+	+

KEY: += Trace / mildly positive; ++ = Moderately positive; and -= Negative
Table 1: Percentage yield of herbal extracts and their Qualitative phytochemistry.

Identification of the Test Isolates

The dermatophytes used for this study were identified as follows: *Trichophyton schoenleinii, Microsporongypseum, Microsporonferungineum* and *Trichophyton mentagrophytes* (Table 2).

Code	Isolate
T2	*Trichophyton schoenleinii*
T3	*Microsporon gypseum*
T4	*Microsporon ferugineum*
T5	*Trichophyton mentagrophytes*

Table 2: The identified Dermatophytes.

Antimicrobial Susceptibility Test

Table 3 shows the antimicrobial susceptibility profiles of *J. curcas*, *E. hirta* and *A. montanus* against *T. schoenleinii, M. gypseum, M. ferungineum* and *T. mentagrophytes J. curcas* has the highest activity alone against *M. gypseum*, *T. mentagrophytes* and *M. ferugineum,* but has high resistance against *T. schoenleinii*; while *Euphorbia hirta* and *Acantus montanus* showed moderate activities against *M. gypseum*, *T. mentagrophytes* and *M. ferugineum*.

Minimum Inhibitory Concentration

Table 3 shows the minimum inhibitory concentrations of the various extracts against *M. gypseum* and *T. mentagrophytes*

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Plants	Dermatophytes used	Extract Concentration (mg/ml)					Control
		50 Inhibition zone diameter (mm)	25	12.5	6.25	3.13	
Jatropha curcas	*Trichophyton schoenleinii*	0	0	0	0	0	0
	Microsporon gypseum	11.5±0.7	9.5±0.7	6.5±0.7	6±0	5±0	15
	Microsporon ferugineum	5±0.7	3±0	2.5±0.7	0	1±0	10
	Trichophyton mentagrophytes	7.5±0.7	6.5±0.7	6±0	4±1.4	2.5±0.7	10
Acantus-montanus	*Trichophyton schoenleinii*	0	0	0	0	0	0
	Microsporon gypseum	8±0.7	6.5±0.7	5.5±0.7	5±0.7	4.5±0.7	15
	Microsporon ferugineum	0	0	0	0	0	7
	Trichophyton mentagrophytes	7±0	5.5±0.7	4±0	3±0	2±0	0
Euphorbia hirta	*Trichophyton schoenleinii*	0	0	0	0	0	0
	Microsporon gypseum	8±0	7±0	6±0	5.5±0.7	0	15
	Microsporon ferugineum	0	0	0	0	0	10
	Trichophyton mentagrophytes	4.5±0.7	4±0	3.5±0.7	2±0	2±0	0

Note: Control is 1% Clotrimazole

Table 3: Inhibition zone diameter (mm) of the plants extract against test dermatophytes.

Antidermatophyte Combination Assay

Tables 4 and 5 show the effect of combination of (*J. curcas* and *A. montanus)*; (*J. curcas* and *E.hirta)*; and (*A.montanus* and *E.hirta)* against drug resistant *M. ferugineum* and *T. schoenleinii,* respectively. At the ratios of 5:5 and 6:4 the combinations showed synergism against all test isolates, while at other ratios synergism, additive, indifference and antagonism effects were observed. The checkerboard assay carried out against *M. ferugineum* and *T. Schoenlenii* showed resistance to the individual extracts alone. The combination of *J. curcas* and *A. montanus* at equal concentration has the highest activity at the ratio of 8:2, 7:3, 6:4 and 5:5 against *T. schoenleinii* while the activity reduced when the concentration of *J. curcas* was reduced to 25mg. Combination of *J. curcas* and *E. hirta* at equal concentration (50mg) also gave an excellent result at the ratio of 8:2 and 7:3 against *T. schoenleinii.*

Combination Ratios	***M. ferugeniums***				
	JC : AM		JC : EH	AM : EH	
	FIC	Interpretation	Interpretation	FIC	Interpretation
10:00	-	-	-	-	-
9:01	1.83	Indifference > 1	Indifference > 1	1.44	Indifference > 1
8:02	1.46	Indifference > 1	Indifference > 1	2.16	Antagonism >2
7:03	0.76	Synergism < 1	Indifference > 1	1.41	Indifference > 1
6:04	0.62	Synergism < 1	Synergism < 1	0.89	Synergism < 1
5:05	0.89	Synergism < 1	Synergism < 1	0.78	Synergism<1
4:06	1	Additive =1	Indifference > 1	0.7	Synergism< 1
3:07	1.46	Indifference > 1	Indifference>1	1.38	Indifference > 1
2:08	1.97	Indifference > 1	Indifference >1	2.26	Antagonism >2
1:09	2.48	Antagonism >2	Antagonism> 2	1.24	Indifference >1
0:10	-	-	-	-	-

Table 4: Checkerboard assay of the extracts against *M. ferugenium*.

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Combination Ratios	T. schoenlein				
	JC : AM		JC : EH	AM : EH	
	FIC	Interpretation	Interpretation	FIC	Interpretation
10:00	-	-	-	-	-
9:01	1.48	Indifference >1	Indifference>1	1.4	Indifference>1
8:02	1.08	Indifference>1	indifference>1	2.24	Antagonism>2
7:03	1.24	Indifference>1	Indifference>1	1.3	Indifference>1
6:04	0.68	Synergism<1	Synergism<1	0.98	Synergism<1
5:05	0.92	Synergism<1	Synergism<1	1	Additive=1
4:06	0.84	Synergism<1	Indifference1>	1.41	Indifference>1
3:07	0.88	Synergism<1	Antagonism >2	1.33	Indifference>1
2:08	0.6	Synergism<1	Antagonism>2	2.36	Antagonism>2
1:09	1.14	Indifference>1	Indifference>1	2.27	Antagonism>2
0:10	-	-	-	-	-

Table 5: *In vitro* activity of combinations of the extracts against *T. schoenleinii*.

Inhibition Zone Diameter(IZD) of Polyherbal Formulations Against Test Isolates

The activities of the polyherbal combined extracts formulations in comparison with standard drug are clearly illustrated in Tables 4-6. All the combinations showed zero activity against *Trichophyton mentagrophytes*. The IZD of *J. curcas and A.montanus* against *M.gypseum* was larger than the standard drug (1 % clotrimazole).

	Inhibition zone diameter (mm)		
Test Isolates	*J. curcas + A. montanus*	*E. hirta+ J. curcas*	*A. montanus+ E. hirta*
Trichophyton mentagrophytes	7.3	4.5	4.5
Microsporon ferugineum	10.5	5	4.5
Microsporon gypseum	6.5	5	7
Trichophyton schoenleinii	0	0	0

Table 6: Inhibition zone diameter (mm) of the Polyherbal formulations against test isolates.

Pharmaceutical Characteristics of the Polyherbal Formulation

The Pharmaceutical characteristics of the polyherbal formulation are represented in Table 7. The average speed of all the 3 formulations is 6rpm. The viscosity ranges from 8467.50 mp as to 15494.67 mp as while percentage of torque is between 42.37 and 77.47 as shown in Table 7. The pH values of the combined extracts were fairly stable even three months after formulations. The average pH of the formulation is 6.5. The spreadability of the cream is 2.

Acute Irritation Study

Table 8 showed the result of the acute skin irritation test of the *J. curcas* and *A. montanus* combined formulation. There was mild irritation only on the day 3 of treatment. There was no other irritation with the other treatment as shown in Table 7.

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Evaluation Time	Formulations	Torque (%)		Viscosity (mpas)			pH			Spreadability (cm)	Speed (rpm)
1 hr. after formulation	A	41.3	50.7	8252.7	10138	7011.8	6.7	6.48	6.62	23.4	6
	B	65.2	53.4	13042	10671	11690	6.29	6.75	6.52	25.2	6
	C	72.8	85	14564	16992	14928	6.59	6.43	6.65	23.6	6
1 month after	A	66.8	65.6	1795.6	16801	1786.4	6.52	6.55	6.36	ND	6
	B	49.3	50.2	1524.6	1447.1	1425.7	6.62	6.37	6.73	ND	6
	C	64.9	72.3	1582.7	1615.3	1886.4	6.64	6.58	6.72	ND	6
3 months after	A	40.7	52.6	1462	9087	8048	5.73	6.42	5.68	ND	6
	B	63.1	52.7	12867	10692	11590	6.27	5.79	6.49	ND	6
	C	83.7	71.8	12046	14565	14899	6.34	6.72	5.8	ND	6

A = *J. curcas* and *A.montanus*; B = *E.hirta* and *J. curcas*; C = *A.montanus* and *E.hirta;* ND = Not done

Table 7: Pharmaceutical characteristics of the formulation.

Observation Time (hrs)	1		24	72		96		120		144		168	
	CL	Trt	CL	CL	Trt	CL	Trt	CL	Trt	CL	Trt	CL	Trt
	Diameter of inflammation (mm)												
Head (JC /AM)	**0**	**0**	**0**	**0**	**3**	**0**	**3**	**0**	**2.3**	**0**	**2**	**0**	**0**
Tail (EH/JC)	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Right hand (AM/EH)	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Right leg wasused as control (CL); Trt = Treatment; JC = *J. curcas*; AM = *A.montanus*; EH = *E.hirta*

Slight irritation was seen on the head on day 3 to 5 but started diminishing gradually

Table 8: Score of erythema and edema after application of the antidermatophyte polyherbal cream.

Discussion

Dermatophytosis is one of the most commonly occurring fungal infections. Also, it has a problem of increase in the level of resistance to antifungal drugs which could be attributed to several factors. This has led to various researches which are focused on the synthesis of newer, more potent, more effective and safer antifungal agents. Naturally occurring plans have been the source of many drug molecules which are used in the treatment of various diseases including fungal infections. The bioactive molecules which are obtained from these natural plants are modified to obtain newer drug molecules with various activities [15-19]. The assessment of the antifungal effect of *A. montanus*, *E. hirta* and *J. curcas*as seen in Table 3 showed that the methanolic leaf extracts demonstrated moderate antifungal activity against clinically isolated strains of *T. schoenleinii*, *M. gypseum* and *M. ferugineum*, the causal organisms mostly implicated in skin structure infections. The effect of the extract was higher against *M. gypseum,* followed by *T. schoenleinii* and *M. ferugineum* in a concentration dependent pattern. When used alone, J. curcas showed the highest activity against 3 of the organisms except T. schoelenleinii as compared to the other two extracts. On the other hand, E. hirta was active against T. mentagrophytes (Table 3) as a single extract. Synergistic effect of the combination of two extracts exhibited better result as compared to the use of a single extract. For instance, combining *A. montanus* and *J. curcas* showed a good activity against *T. schoenleinii*, and *J. curcas* and *E. hirta* even showed a better activity against *T. schoenleinii*. This could be as a result of the increase in the level and types of phytochemicals which are present in the combined extracts. Although the control drug (1% clotrimazole) showed strong activity against all the four isolates, none of the extract had effect against *T. mentagrophytes*.

Additionally, pronounced variability has been observed for the *in vitro* sensitivity of the three pathogenic fungi tested (*M. gypseum, T. schoenleinii* and *M. ferugineum)*, to various plant extracts (Table 5). At the concentration used, the

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order of effective control of the growth of *M. gypseum* by the methanolic extracts is as *J. curcas* > *A. montanus* > *E. hirta*. This finding is in agreement with Field, et al. [20]; Rongai, et al. [21]; Elad, et al. [22] and Giordani, et al. [23], indicating that the presence of different phytochemical compounds, either acting alone or in synergy, could be responsible for the observed antifungal effect of these crude extracts.

The result of the checkerboard assay (Table 4) showed that the combination of *J. curcas* and *A. montanus* is synergistic at the ratio of 7:3, 6:4, 5:5 and 4:6 while *J. curcas* and *E.hirta* have activity at 6:4 and 5:5, *A. montanus* and *E. hirta* showed activity at 6:4, 5:5 and 4:6. The cream formulations of these extracts showed promising antifungal activity that is comparable to the standard drug – 1% clotrimazole and they all have good pharmaceutical properties, with very mild irritation on third to fifth day of application which eventually weaned off gradually. The combination of *J. curcas* and *A. montanus* showed significant activity against *M. ferugineum,* while the combination *J. curcas* and *E. hirta,* and the combination of *A. montanus* and *E. hirta* are effective against *M. gypseum* and *T. schoenleinii,* respectively.

Conclusion

The methanolic extracts of the natural plants used in this study have been shown to be effective against the clinical isolates used. The implication of this finding is that these plants could serve as potential raw material for the development of plant-based products which could be used in the cure and management of the various dermatophytosis infections. All things taken together, this study scientifically validates the use of the methanolic leaf extracts of *Acantus montanus*, *Euphorbia hirta* and *Jatropha curcas* as potent antifungal agents, and their cream formulations have good pharmaceutical characteristics.

Recommendations

More research should be carried out on the elucidation of the bioactive components of natural plants and the assessment of their pharmaceutical abilities.

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