Phytochemical and Antifungal screening of *Jatropha curcas* leaf extract in ointment formulations- A Preliminary study

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ABSTRACT:

Background: Plants are rich sources of many natural products which have being extensively used for treatment of various infections. *Jatropha curcas*, a perennial plant belonging to Euphorbiacae family has been used medicinally to cure various diseases including skin infections. Toxicities and failure in treatment have been reported variously in literature to most synthetic dermatological formulations, hence there is the need to search for safe and effective alternatives. The present study aims at extracting *Jatropha curcas* leaves, formulating the extract into an ointment and evaluating the ointment. **Methods**: Pulverized leaves of *Jatropha curcas* were extracted using methanol and the extract was later formulated into an ointment with paraffin ointment BPC as a base. The extract and the ointment at concentrations 0, 2.5, 5, 7.5 and 10% w/w were tested against *Trichophyton tonsurans* and *Trichophyton interdigitale*, common dermatophytes often implicated in skin infections. Whitfield ointment was used as a reference standard.

Results: The results showed the presence of flavonoids, saponins and tannins as secondary metabolites. The plant extract had mean inhibition zones of 12.00, 16.25 , 18.35 mm against *Trichophyton interdigitale* and 12.25, 18.35 , 24.50 mm against *Trichophyton tonsurans* ($p=0.905$) respectively at concentrations 5, 7.5, and 10.0% used for ointment formulations. The ointment formulations at all concentrations did not show any activity while Whitfield ointment a reference standard had inhibition zones of 36 .50 and 38.50 mm against the tested organisms respectively.

Conclusion: Notwithstanding the inhibitory failure of the ointment formulations, the extract exhibited a promising antifungal potentials.

Key words: *Jatropha curcas* ; Antifungal; Inhibition zones; Extraction; Phytochemicals

INTRODUCTION:

The plant *Jatropha curcas* Linn is a drought resistant shrub, widely distributed in the wild or semi-cultivated areas in Central and South America, Africa, India, and South East Asia [1-3]. All parts of *Jatropha* (seeds, leaves, bark among others) have been used in traditional medicine and for veterinary purposes for a long time [4]. .The genus *Jatropha* belongs to tribe Joannesiae in the Euphorbiaceae family and consists approximately 170 known species. Linn [5] was the first to name the physic nut *Jatropha* L. in "species plantarum" and this is still valid today. Climatically *Jatropha* is found in the tropics and subtropics and adapts easily to heat, although it does well even in lower temperatures and can withstand light frost. It will grow under a wide range of rainfall regimes from 250 to over 1200 mm per annum [6]. In low rainfall areas and in prolonged rainless periods, the plant sheds its leaves as a response to drought, its water requirement is extremely low and it can stand long periods of drought by shedding most of its leaves to reduce transpiration loss. *Jatropha* is also suitable for preventing soil erosion and shifting of sand dunes. It grows on well drained soils with good aeration and is well adapted to marginal soils with low nutrient content [7]. On heavy soils, root formation is reduced. *Jatropha* is a highly adaptable species and its strength as a crop comes from its ability to grow on a very poor and dry sites.

Jatropha grows readily from seeds or cuttings, however, trees propagated by cuttings show a lower longevity and possess a lower drought and disease resistance than those propagated by seeds [8]. There are various methods to cultivate *Jatropha,* which vary from region to region and also on climatic conditions. These are direct seeding, pre-cultivation of seedlings (nursery raising), transplanting of spontaneous wild plants and direct planting of cuttings. Wider spacing (3 m x 3 m) is reported to give larger yield of fruits, at least in early years [8]. In different countries and regions, the seed yields of *Jatropha* may range from 0.1 to 1.5 tonnes/ha/year [9].

The leaves and other parts of the plant are used for the treatment of various diseases. Compounds that have been isolated from *Jatropha curcas* leaves include the flavonoid apigenin and its glycosides, vitexin and isoritexin, the sterols stigmasterol, B–D–sitosterol and its D-glucoside [10]. Furthermore, *Jatropha curcas* leaves were reported to contain steroid sapogenins, the triterpene alcohol, 1–

triacontanol and a dimer of a triterpene alcohol. Staubmann *et al*., [11] isolated a complex of 5-hydroxypyrrolidin-2,4-dione and pyrimidine-2, 4-dione from the leaves of *Jatropha curcas.* The leaves have been used in folk medicine to treat vaginal bleeding [12.] Members of rural communities of Churu district in the Thar desert, India used the juice from leaves to cure diseases such as dysentery and colic [13].The leaves extract were also applied to the breast to promote lactation [13]. In Southeast Asia and in some regions of Africa, the leaves are used as purgative while in the Philippines and Cambodia, the leaves are applied to the wounds [11]. In Cape Verde and Cameroon, a decoction is used internally and externally against fever. In Nigeria, it is used against jaundice, skin diseases [11]. Thomas [14] reported that ethanol extract of the leaves and twigs of *Jatropha curcas* have shown confirmed activity both *in vivo* and *in vitro* against P-388 lymphocytic leukemia. Fagbenro – Beyioku [15] investigated and reported the antiparasitic activity of the sap and crushed leaves of *Jatropha curcas*. The leaves are utilized extensively in West Africa ethno-medical practice in different forms to cure various ailments like fever, skin diseases, mouth infections, jaundice, guineaworm, sores and joint rheumatism [16].

Although there has been an extensive utilization of this plant ethno-medicinally, there has been little or no concrete scientific evidence to support various claims. Also there is no known delivery dosage design of the medicinal active content of the plant. The aim of the present work therefore is to explore the active secondary metabolites present in the plant and also engage in the design of dermatological delivery model.

MATERIALS AND METHODS:

Collection of the leaves: Fresh leaves of *Jatropha curcas* were obtained from different locations in Sagamu, Ogun State, Nigeria and it was authenticated at Forest Research Institute of Nigeria (FRIN), Ibadan, Oyo State, Nigeria with Registration No FHI. 109526.

Extraction of the leaves: The leaves were washed and air dried for 3 weeks. The air dried leaves were pulverized using a roller mill. The pulverized leaves (1000 g) was macerated with 2.5 litres of methanol and left for 72 hours at room temperature. The mixture was filtered through Whatman filter paper (No.1). The solvent was thereafter evaporated off using rotary evaporator and later transferred to heating mantle at 45 ^{o}C for the removal of any residual solvent.

Phytochemical Screening: Plant extract was screened for the presence of secondary plant metabolites using the method previously described by Sofowora [17] , Trease and Evans [18].

Test for anthraquinones: A 1 g weight of the extract was boiled with 2 ml of 10 % HCl for 5 minutes. This was filtered while hot and allowed to cool. The filtrate was partitioned with equal volume of chloroform and by means of pipette, the chloroform layer was transferred to a clear tube and then 10 % ammonia solution was added to it. Formation of bright pink coloration indicates presence of anthraquinones

***Test for alkaloids*:** To a 1 g weight of the extract was added 10 ml of 10 % HCl and heated on a water bath for 10 minutes. The mixture was filtered and the pH was adjusted to 7 with aliquots of ammonia solution. Small quantities of 2 ml each of the following reagents- Mayer's reagent,10% Picric acid,10% Tannic acid and Draggendorf's reagent were added to the filtrate and then mixed together in a test tube. The formation of white, or white precipitate indicated the presence of alkaloids .

Test for saponins: A 1 g weight of the extract was boiled with 10 ml of water for 10 minutes. The mixture was filtered while hot and the filtrate cooled, 5 ml of the filtrate was mixed with 2.5 ml of distilled water and shaken vigorously for a stable persistent froth. The froth was mixed with 3 drops of olive oil, shaken vigorously and then observed for the formation of emulsion.

Test for tannins: A 0.5 g weight of the extract was boiled in 20mls of water in a test tube and then filtered. A few drops of 0.1%ferric chloride was added and observed for brownish-green or a blue-black coloration

Test for flavonoids: **A 0.**5 g of magnesium metal was added to 2 g of the powdered leaves extract in concentrated hydrochloric acid. A reddish coloration indicates a positive result for the presence of flavonoids.

Preparation of the ointment: The ointment formulations of *Jatropha curcas* leaf extract were prepared with paraffin ointment BPC (1979) as a base using fusion method as shown in Table 1.

The ingredients in the base were melted together in the order of increasing melting point while different weights of the extract were incorporated at the molten stage.

Table 1*: Jatropha curcas* leaf extract ointment formulations:

Ointment formulations	Extract (g)	Base (g)	Concentration %w/w)
1	0.00	25.00	0.00
2	0.63	24.37	2.50
3	1.25	23.75	5.00
4	1.88	23.12	7.50
5	2.50	22.50	10.00

Antifungal assessment of the extract and ointment formulations: Overnight cultures of *Trichophyton tonsurans* and *Trichophyton interdigitale* were prepared. Sterile molten Sabouraud Dextrose Agar (SDA) was aseptically poured into eight sterile plates and were allowed to set for 45 minutes. The plates were seeded with 0.2 ml of 1:100 dilution of the organisms by spreading on the surface using a sterile glass spreader. The set plates were dried in hot air oven. After drying, a sterile cork borer (8mm in diameter) was used to bore five equidistant holes on the set plates .A 1 ml volume of different concentrations of the extract 0%, 2.5%, 5%, 7.5% and 10% w/v were introduced into the holes. This procedure was repeated for 1 mg each of the ointment formulations at 0%, 2.5%, 5.0%, 7.5% and 10.0% w/w concentrations respectively. Whitfield (1 mg) was used as a control formulation. All experiments were carried out in duplicates.

Statistical Analysis:

Student t-test paired independent (two-tailed) at significant level of 0.05 was used to confirm the significance statistically of the sensitivities of the two organisms to *Jatropha curcas* leaf extract across concentrations range.

RESULTS:

The calculated yield was found to be 2.4 %w/w.

The result of phytochemical screening of powdered leaves for the presence of secondary metabolite is as presented in Table 2.

Table 2: Results of phytochemical screening of methanol extract of *Jatropha curcas* leaves

Phytochemical constituents	Remarks
Alkaloids	-
Anthraquinone	-
Flavonoids	+
Saponins	+
Tannins	+

KEY: + = Present; - = Absent

The result of the antifungal effect of the crude extract of *Jatropha curcas* leaves against test organisms is presented in Table 3.

Table 3: Inhibition zones of *Jatropha curcas* leaf extract against test organisms in (mm)

Sample	Concentrations (%w/w)	Organisms *Trichophyton tonsurans* *Trichophyton interdigitale* Inhibition Zones	
		Mean (mm)	Mean (mm)
1	0.00	0.00	0.00
2	2.50	0.00	0.00
3	5.00	12.25±0.01	12.00±0.01
4	7.50	18.35±0.01	16.25±0.01
5	10.00	24.50±0.02	18.35±0.01

Whitfield

Ointment		36.50±0.10	38.50±0.10

The result of antifungal assessment of *Jatropha curcas* leaf extract ointment against test organisms is presented in Table 4

Table 4: Inhibition zones of *Jatropha curcas* leaf extract ointment against test organisms in (mm)

Samples	Concentrations (%w/w)	Organisms	
		Trichophyton tonsurans	*Trichophyton interdigitale*
		Inhibition Zones	
		Mean (mm)	Mean (mm)
1	0.00	0.00	0.00
2	2.50	0.00	0.00
3	5.00	0.00	0.00
4	7.50	0.00	0.00
5	10.00	0.00	0.00
Whitfield Ointment		36.50±0.10	38.50±0.10

DISCUSSIONS:

The calculated yield was found to be 2.4% w/w. This yield appears not to be adequate to support herbal formulation on commercial scale. Although the plant can easily be cultivated and it is even drought resistant [2], a large quantity of the plant material will be required for extraction at every turn of activity. This may turn out to be tedious and not viable economically. The isolation and development of the bioactive compounds present in the plant may be realistic in supporting commercialization of this product. The secondary metabolites found present in the leaf extract were flavonoids, saponins and tannins as can be seen in Table 2. Igbinosa *et al.*, [19] earlier reported presence of saponins, steroids, tannins, glycosides, alkaloids, flavonoids in the stem bark of *Jatropha curcas* but absence of steroids, glycosides and alkaloids in the leaves which is in agreement with the present work. Rigid and extensive cellular wall structure of the stem bark as compared with that of the leaves may account for more varied bioactive molecules present in the stem. The presence of chlorophyll scattered all around the leaves does not support the storage of bioactive compounds like alkaloids, steroids among others. All the bioactive molecules present in the plant whether in the leaves or in the barks have been found to exert their medicinal activities synergistically when used in crude form [20] .

The antifungal activity of the *Jatropha* leaf extract against test organisms is as shown in Table 3.

The present study also shows that reference product (Whitfield ointment) had 36 mm inhibition zone, higher than that of 10% concentration of the extract used, it is not unlikely that further increase in the concentration of the extract may give a better antimicrobial outcome. This is because as concentrations of the extract was increasing the lethal effect of the extract against test organisms was also increasing

($p<0.05$). Furthermore, inhibition zones for *Trichophyton interdigitale* and *Trichophyton tonsurans* are similar as can be seen in Table 3 while that of reference agent (38 mm) is highest. The lethal mechanisms of the extract against the two test organisms appears similar, may be because of the similarity in the cellular structure of these organisms. The compounds present in the plant, have been found to exert antimicrobial activities through different mechanisms [19]. Thomas [14] and Shimoda [21] reported that tannin a secondary metabolite found in the leaf extract have been found to form irreversible complexes with proline rich protein resulting in the inhibition of cell well synthesis thereby leading to death of the organisms.

The ointment formulations did not show activity against the test organisms as can be seen in Table 4. For a drug formulated in a semi-solid preparation to be effective, it must be able to diffuse out of the base. Paraffin ointment BPC (1979) used as the base in the formulation is a type of hydrocarbon base that is occlusive in nature. Affinity between the bioactive compounds found in the leaf extract and the hydrocarbon base may be one reason responsible for the non-availability of the compounds at the site of action. Also it has been found out that lipophylic drug substances formulated with non-aqueous base often have difficulty in migrating out of the base to exert its action, whereas lipophobic substances will not present with such difficulty.

CONCLUSION:

The crude extract of *Jatropha curcas* powdered leaves exhibited a promising antifungal effect as compared with reference compound (Whitfield ointment) against the dermatophytes investigated especially at higher concentrations. Bioactive compounds present in the leaves of this plant, if isolated and screened may show better antimicrobial activity than the crude extracts. The ointment formulations did not show any activity against the test organisms. This may probably be due to release of the extract from the base. A further work may require a design in a modified semi-solid base like a vanishing cream.

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