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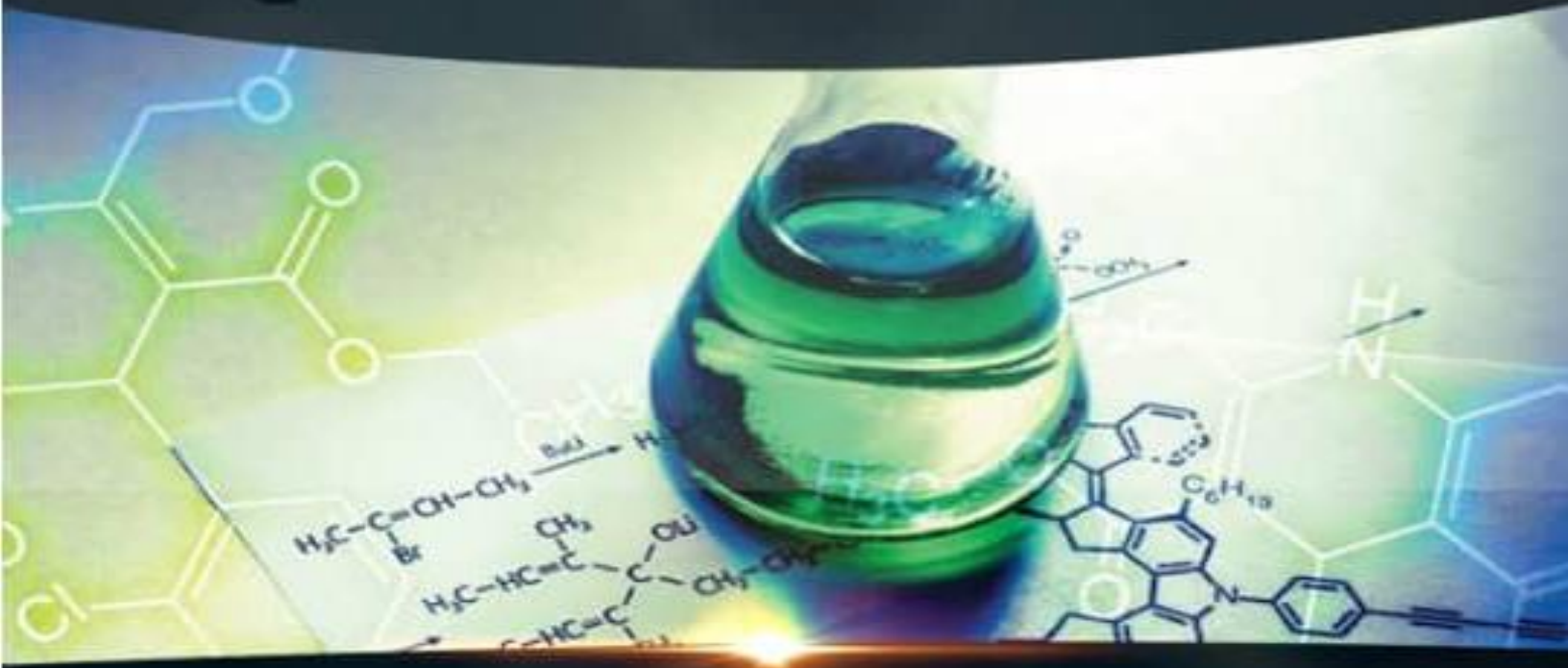

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CATALYZING NATIONAL ECONOMIC RECOVERY IN POST-COVID-19 ERA THROUGH INNOVATIVE RESEARCH

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Phytochemical Composition and Antifungal Activity of Aqueous and *N*-Hexane Extracts of *Calotropis procera* Leaf

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ABSTRACT

Natural products play important roles in drug discovery and development process, particularly in the field of infectious diseases. The aim of this study is to determine the antifungal activity of aqueous and n-hexane leaf extracts. The qualitative phytochemical study was performed according to standard methods. Antifungal activity of aqueous and n-hexane leaf extracts of *Calotropis procera* against dermatophyte (*Microsporum spp* and *Trichophyton spp*) at different concentrations (20mg/Ml, 30mg/mL, 40mg/mL, 50mg/mL and 60mg/mL) was carried out using agar incorporation method. The result of the qualitative phytochemical studies showed the presence of steroids, tannins, glycosides, phenols, terpenoids, flavonoids, alkaloids and saponins in aqueous extract of *C. procera*, while the n-hexane leaf extract showed absence of alkaloids, flavonoids and cardiac glycosides. There was complete inhibition of the growth of *Microsporum spp* and *Trichophyton spp*, after 14 days of culture with the aqueous extracts of *Calotropis procera* and at different concentrations (1.33mg/mL and 2.66mg/mL) of *n*-hexane leaf extract, there was complete inhibition of the growth. The result showed higher antifungal activity when compared to a conventional drug; fulcin.

KEYWORDS: *Calotropis procera*, Antifungal, *Microsporum spp* and *Trichophyton spp*

INTRODUCTION

The World Health Organization reported that 70–90% of the world's population depends chiefly on traditional medicine (WHO, 2004). Natural products play important roles in drug discovery and development process, particularly in the field of infectious diseases, where 75% of these drugs are of natural origin (Newman *et al*., 2003). The local use of natural plants as primary health remedies is due to their pharmacological properties. Many plant extracts owe their potency to the presence of metabolites (Kinghorn, 1994). Plants provide abundant resources of antimicrobial compounds and have been used for centuries to inhibit microbial growth (Zhang *et al*., 2006). The increasing incidences of fungal infections and gradual rise in azole resistance and available antibiotics had highlighted the need to find more alternative antifungal agents from other sources (Fostel and Lartey, 2000).

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For many years now, interest in new safer, cheaper and more effective antifungal agent has grown with the increasing incidences of fungal infections, in the science of natural products the antimycotic activity of higher plants remains largely unemployed compared with other microorganism. Fungal related diseases may not be common as other microbes but when present, they could be difficult to eradicate especially in immuno-suppressive situations (Bertram, 1984; Alexopoulos *et al*., 1994). Research on bioactive substances from plant sources has great scope and could lead to the provision of value - added economic return, and the establishment of natural plant provision (Adekunle, 2000). In Nigeria the crude extract of some local plants are used by the natives to cure fungal diseases in human. Some of these plants include *Brachystigia eurycomahams* (*Caesalpinaceae*), *Richardia Brasiliensis* (Gomez) *Rubiaceae* and *Calotropis procera* Sodom apple.

Calotropis procera (family *Asclepiadaceae*) is commonly called Sodom apple. It is un-branched with soft wooden trunk, yellowish brown stem bark and the slash exudes caustic latex that turns yellow on exposure to air (Aliero *et al*., 2001). In northern Nigeria the leaves, roots and stem barks of *Calotropis procera* are used in indigenous practice to treat fungal diseases (for topical application), convulsion, asthma, cough and inflammation. Aliero *et al*. (2001) reported the use of fresh follicles of the plant (soaked in cold water) for the treatment of asthma, the burnt dry stem for **wound healing** and the plant is also used in the treatment of several diseases of domestic animals.

The anti-fungal activity of *Calotropis procera* has been speculated by local people that it cures a number of contagious skin diseases caused by certain parasitic fungi e.g. ringworm infection and eczema (Joseph, 1970). Dermatophytes are fungi that require keratin for growth. These fungi can cause superficial infections of the skin, hair, nails skin and internal organs, respectively. Dermatophytes are spread by direct contact from other people (anthropophilic organisms), animals (zoophilic organisms), and soil (geophilic organisms), as well as indirectly from fomites. This research work will emphasis on the leaves of *C. procera* to ascertain the phytochemical constituents and antifungal properties as claim by traditional medicine practitioner against a fungal infection called ringworm.

METHODOLOGY

Collection of Plant Materials

The plant; *calotropis procera* were collected around new examination hall, Usmanu Danfodiyo University, Sokoto. This was done after confirmation from some traditional healers who claim that the plant is a cure for ringworm infection. The plant was identified in the herbarium of Biological Science Department. The plant leaves collected were room-dried and pulverized into powder and the powdered sample was subjected to aqueous and n- hexane solvent extraction (Matawalli *et al*., 2004).

Source of Infectious Fungi

Trichophyton rubrum (*Trichophyton spp.*) and *Microsporum gypseum* (*Microsporum spp,*) were clinical isolates obtained from Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria. They were maintained on Sabouraud Dextrose Agar (SDA) medium and re-identified by microscopic examination of a portion at mycology laboratory of Biological Sciences Department, Usman Dan Fodiyo University of Sokoto.

Materials and Reagents

Materials

Beakers(250 mL and 500 mL), Separating funnel, Volumetric flask(250 mL,500 mL and 1000 mL), Funnel, Measuring cylinder,(10 mL and 250 mL) and Petri dishes are made by Pyrex England, Weighing balance (Sartarius 2351), Metlee Balance-p163, Hot plate (Precision scientific U.S.A.), Autoclave (potable) (Arnold and sons ltd), Incubator (Gallenkamp,England).

Reagents

Malt extract agar, Ethanol, Formaldehyde, Methylated spirit, n-hexane BDH chemical, England. All reagents were of analytical grade. Distilled water, Antibiotic (streptomycin), Dana pharmaceuticals India and Fulcin tablet M& B Ltd.

Phytochemical Screening

Plants extracts were qualitatively screened using standard techniques for the detection of alkaloid, flavonoids, glycosides, steroids, tannins and saponins (Sofowora, 1993; Evans, 1995).

PREPARATION OF PLANT EXTRACTS AND MEDIA

Extraction and Fractionation of Sample (n-hexane) Extract

Five different concentrations (20 mg/mL, 30 mg/mL, 40 mg/mL, 50 mg/mL and 60 mg/mL) were prepared by dissolving 10g of the *Calotropis procera* (*C. procera*) leaves in 500 mL, 333 mL, 250 mL, and 166.5 mL of distilled water respectively to obtain aqueous extract.

Fifty grams (50 g) of dried sample was weighed and dissolved into 500 mL distilled water in a conical flask for 24 h. The solution was then filtered to obtain aqueous extract. Equal volume of n-hexane was then mixed with aqueous extract and then fractionated using separating funnel. The n-hexane extract were evaporated and the dried sample weighed. Two different concentrations (1.33 mg/mL and 2.66 mg/mL) were prepared from the dried samples according to modified method of Hassan *et al*. (2006).

PREPARATION OF MALT EXTRACT MEDIA

Thirty grams (30 g) of malt extract powder was dissolved in 1000 mL of distilled water in a conical flask. The solution was shaken gently to allow complete dissolution. The solution was then sterilized using an autoclave at 121^{o}C for 30 minutes and was allowed to cool for 45 ^{o}C. Fifteen milliliters (15 mL) of the malt extract agar was dispense into 10 conical flask and autoclaved using the same procedure described above.

BIOLOGICAL SCREENING BY AGAR INCOPORATION METHOD

Agar and Plant Extract Incorporation and Subsequent Inoculation of the Organism

Five milliliters (5 mL) of the water extract was added to conical flasks containing malt extract agar. The solution was shaken and transferred into the petri dishes in the incubator room. Five milliliters (5 mL) of the last water extract, obtained after fractionation was added to a conical flasks of leaf extract. Five milliliters (5 mL) of n-hexane extract were also added to the conical flasks and transferred into the petri dishes in the incubator.

Pure culture of *Trichophyton spp* and *Microsporum spp* were inoculated into different petri dishes at the middle using 2 × 2 mm inoculum i.e., by diffusion on a solid medium (agar incorporation method) fulcin was used as a standard and two petri dishes that do not

contain the plant extract were used as mycological control. The growth of the organisms were observed daily for two weeks and their diameter measured.

RESULTS

Percentage Yield of Extracts

The extracts yield obtained for the different solvent extracts evaluated (Table 1). *C. procera* aqueous extracts had the highest extract yield (%), while *C. procera* n- hexane extracts had the least extract yield (%).

Table 1: Percentage (%) Yield of *C. procera* Extracts

***C. procera* Extracts**	**Leaf powder (g)**	**Extract yield (%)**
Aqueous	50.00	0.21
n-Hexane	50.00	0.50

Qualitative Phytochemical Screening of *C. procera* Leaf Extracts

The qualitative phytochemical constituents of *calotropis procera* leaf extract revealed the presence of tannins, alkaloids, flavonoids, phenols and glycosides in aqueous extract while flavonoids and glycosides were absent in n-hexane fraction of *calotropis procera*.

Table 2: Qualitative Constituents of Aqueous and n-hexane *C. procera* Leaf Extracts

Phytochemicals	***C. procera* Aqueous leaf extracts**	***C. procera* n-hexane leaf extracts**
Alkaloids	**+**	**-**
Flavonoids	**+**	**-**
Saponins	**+**	**+**
Steroids	**+**	**+**
Phenols	**+**	**+**
Terpenoids	**+**	**+**
Tannins	**+**	**+**
Glycosides	**+**	**-**

Note: - = not dictated, += dictated

ANTIFUNGAL SCREENING RESULTS

Effect of *C. procera* Aqueous Leaf Extracts on Diameter of Growth of *Microsporum spp*

Table 3 shows the results of effect of *C. procera* aqueous leaf extract on the diameter of growth of *Microsporum spp* for 14 days (2 weeks). The growth of *Microsporum spp* was completely inhibited by the extract at all the concentrations when compared with the negative control (inoculated with *Microsporum spp* but not treated), while the positive control (inoculated with *Microsporum spp* treated with standard drug; fulcin) was only able to suppress *Microsporum spp* till day 5 where growth was observed up to day 14.

Table 3: Effect of *C. procera* Aqueous Leaf Extracts on Diameter of Growth of *Microsporum spp*

Concentration	***Days***													
(Mg/ml)	1	2	3	4	5	6	7	8	9	10	11	12	13	14
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	-	-	-	-	-	-	-	-	-	-	-	-	-	-
50	-	-	-	-	-	-	-	-	-	-	-	-	-	-
60	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-ve control	5	9	15	18	22	27	30	35	40	44	50	65	79	90
60 Fulcin	-	-	-	-	5	10	15	20	32	40	35	30	24	12

Effect of *C. procera* Aqueous Leaf Extracts on Diameter of Growth of *Trichophyton spp*

Table 4 shows the results of effect of *C. procera* aqueous leaf extract on the diameter of growth of *Trichophyton spp* for 14 days (2 weeks). The growth of *Trichophyton spp* was completely inhibited by the extract at all the concentrations studied when compared with the negative control (inoculated with *Trichophyton spp* but not treated), while the positive control (inoculated with *Trichophyton spp* treated with standard drug; fulcin) was able to inhibit the growth *Trichophyton spp* up to day 14.

Table 4: Effect of *C. procera* Aqueous Leaf Extracts on Diameter of Growth of *Trichophyton spp*

Concentration	***Days***													
(Mg/ml)	1	2	3	4	5	6	7	8	9	10	11	12	13	14
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	-	-	-	-	-	-	-	-	-	-	-	-	-	-
50	-	-	-	-	-	-	-	-	-	-	-	-	-	-
60	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-ve control	5	9	15	18	22	27	30	35	40	44	50	65	79	90
60 Fulcin	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Effect of *C. procera* n-Hexane Leaf Extracts on Diameter of Growth of *Microsporum spp*

Table 5 shows the results of effect of *C. procera* n- hexane leaf extract on the diameter of growth of *Microsporum spp* for 14 days (2 weeks). The growth of *Microsporum spp* was completely inhibited by the extract at all the concentrations tested when compared with the negative control (inoculated with *Microsporum spp* but not treated), while the positive control (inoculated with *Microsporum spp* treated and with standard drug; fulcin) was only able to suppress *Microsporum spp* till day 5 where growth was observed up to day 14.

Table 5: Effect of *C. procera* n-Hexane Leaf Extracts on Diameter of Growth of *Microsporum spp*

Concentration	***Days***													
(Mg/ml)	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2.66	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-ve control	5	9	15	18	22	27	30	35	40	44	50	65	79	90
30 Fulcin	-	-	-	-	5	10	15	20	32	40	35	30	24	12

Effect of *C. procera* n-Hexane Leaf Extracts on Diameter of Growth of *Trichophyton spp*

Table 6 shows the results of effect of *C. procera* aqueous leaf extract on the diameter of growth of *Trichophyton spp* for 14 days (2 weeks). The growth of *Trichophyton spp* was completely inhibited by the extract at all the concentrations tested when compared with the negative control (inoculated with *Trichophyton spp* but not treated), while the positive control (inoculated with *Trichophyton spp* treated with standard drug; fulcin) was also able to inhibit the growth *Trichophyton spp* up to day 14.

Table 6: Effect of *C. procera* n-Hexane Leaf Extracts on Diameter of Growth of *Trichophyton spp*

Concentration	***Days***													
(Mg/ml)	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2.66	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-ve control	5	9	15	18	22	27	30	35	40	44	50	65	79	90
30 Fulcin	-	-	-	-	-	-	-	-	-	-	-	-	-	-

DISCUSSION

In this study difference in extract yield was obtained for the plant extracts, which could be due to solvent difference, polarity and affinity. The phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, steroids, triterpenoids, saponins and glycosides in *C. procera* extracts with the exception of flavonoids and glycosides which were absent in the n-hexane extract. This could be due to difference in solvent used in the analysis. Solvent polarity is an important parameter that affects the yield of a plant material. Thus the higher the polarity, the better the solubility of compounds such as phenols. This result is similar to the study reported by Hassan *et al*. (2006). The presence of bioactive compounds could be the reason for the antifungal activity and the use of this plant for medicinal purposes.

Medicinal plants are potential source of new drugs. Since they contain a enormous quantity of molecules with a great variety of structures and pharmacological activities. Several well established human antiprotozoal drugs have their origins in nature, such as quinine an alkaloid from *Cinchona* sp. (Rubiaceae) and artemisinin, a sesquiterpene lactone from *Artemisia annua* (Asteraceae) used to treat malaria or emetine, an alkaloid from *Cephaelis*n *ipecacuanha* (Rubiaceae) used to treat amoebiasis. Additionally, these antiprotozoal plant-derived compounds have been used as leads to develop other semi-synthetic or synthetic drugs with better efficacy, safety or pharmacokinetic profiles (Tagboto and Townson, 2001).

The biological screening carried out on *C. procera* is a preliminary study to confirm the antifungal property on *Microsporum spp* and *Trichophyton spp.* Results revealed that aqueous extract of *Calotropis procera* had complete inhibitory effect on the growth of *Microsporum spp* at all the various concentration tested. While 60 mg/mL of Fulcin did not show complete inhibitory effect on the growth of *Microsporum spp*.at the 5thday, growth of 5mm was noticed which increased to 40mm at the 10th day and later decreased to 12mm at the 14thday.

The n-hexane leaf extracts, showed complete inhibitory effect on the growth of *Microsporum spp* and *Trichophyton spp*. when compared with the control, it shows that the crude extracts are more effective that the conventional drug (Fulcin), since 1.33 mg/mL of n-hexane completely inhibited the growth of *Microsporum spp* and *Trichophyton spp*. while about 30 mg/mL of Fulcin was required to inhibit the growth of *Microsporum spp*. The extract completely inhibited the growth of *Trichophyton spp*. while 1.33 mg/mL growth in diameter was observed at the 6thday which increased to 54.5mm at the 14thday. This shows that 2.66 mg/mL of root n-hexane extract is more effective on *Trichophyton spp*. than *Microsporum spp.* Therefore, it can be deduced that *C. procera* has a dose dependent activity on the organisms.

At this point it can be suggested that all these phytochemicals observed had contributed to the antifungal activity of the plant. Tannins have been reported to inhibit growth of microorganisms by precipitating microbial protein and making nutritional protein unavailable to them (Idu, 2007); while the antimicrobial effects of flavonoids have been attributed to their ability to form complex with extra cellular, soluble protein and with bacterial cell wall proteins (Musa *et al*., 2008). The flavonoids have been known to be synthesized

by plants in response to microbial infection (Al-Bayati and Al-Mola, 2008).

CONCLUSION

This research work has been able to establish some medicinal fact about the plant used. Therefore, the medicinal uses and claims of these plants in the treatment of ringworm infection by traditional practitioners should not be discouraged. Therefore, the inhibitory activity of the *C. procera* extract was verified in this study and thus established the medicinal use of the plant by local folks to treat fungal infection.

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