# Phytochemical and antifungal screening of *Acalypha wilkesiana* Mull Arg (Euphorbiaceae) leaf extract in cream formulations

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**ABSTRACT**

The plant *Acalypha wilkesiana* (Euphorbiaceae) has been ethnomedicinally reported to have antifungal activities. There is the need to have convenient dosage formulations that would be therapeutically useful to patients .The present study seeks to screen for the presence of secondary metabolites present in the plant and then evaluate the extract and cream formulations against certain selected fungal organisms. The extract was obtained from pulverized dried leaves with ethanol 95%v/v at powdered leaves to solvent ratio 1:10.The plant extract was screened phytochemically using standard procedures.The extract was formulated into cream at concentrations 0%w/w, 2.5%w/w, 5.0%w/w, 7.5%w/w and 10.0%w/w respectively. The cream formulations were evaluated against *Tricophyton tonsurans* and *Epidermophyton floccosum* as test organisms using ketoconazole cream as reference. The extraction gave a yield of 10.68%w/w. The phytochemical screening indicated presence of saponin, tannins and alkaloids. The extract showed activity against *Tryhophyton tonsurans* only with mean inhibition zones of 10.00mm to 16.00mm for all the concentrations tested. Cream formulations gave favourable physical characteristics and zones of inhibition of 12.00mm and 15.00mm against *Tricophyton tonsurans* at 7.5%w/w and 10.0%w/w concentrations respectively. This compares favourably with ketoconazole cream with inhibition zone of 18.00mm. The results of this work show that *Acalypha wilkesiana* leaf extract cream has potential in treating fungal infection of the skin due to presence of *Tricophyton tonsurans*.

**Keywords:** *Acalypha wilkesiana*, Inhibition, *Tricophyton tonsurans*, *Epidermophyton floccossum*

## INTRODUCTION:

Plants are great source of medicine, especially in traditional medicine, which are useful in the treatment of diseases (Bako *et al*,2005). According to World Health Organization (WHO,2001), 80% of the world population use medicinal plants in the treatment of diseases. In African countries the usage is even higher. It has been estimated that up to 90% of the population in developing countries rely on the use of medicinal plants to help meet their primary health care needs (WHO,2005). *Acalypha wikesiana* Mull Arg (Copper leaf) is a plant from the family Euphorbiaceae. The genus Acalypha comprises about 570 species (Ikewuchi and Ikewuchi, 2009), a large proportion of which are weeds while the others are ornamental plants. The plants are found all over the world especially in the tropics of Africa, America and Asia. *Acalypha wilkesiana* is an evergreen shrub. It grows 3m high and spreads 2m across. The stem is erect with many branches. The branches have fine hairs. It has a closely arranged crown. The leaves are coppery green with red splashes of colour. This gives a mottled appearance. The leaves are large and broad with teeth around the edge.

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They can be 10-20cm long and 15cm wide. Some of the species are well known in traditional medicine and a few have actually appeared in the homeopathic pharmacopoeia of United States of America (1941) and India(1971). Few studies have reported the phytochemical constituents of *Acalypha wilkesiana* . The presence of sesquiterpenes, monoterpenes, triterpenoids and polyphenols have been reported (Akinde,1986). Adesina *et al*(2000) reported the presence of garlic acid, corilagin, geranin, quercetin, 3-o-rutinoside and campherol in the leaves of *Acalypha wilkesiana* (AW). The medicinal properties of AW include antimalarial activity, gastrointestinal effect (Cowan,1999.), antihypertensives, antifungal (Cowan, 1999), antibacterial ( Birk and Petri, 1980), antihelminthic (Oyelami *et al*,2003; Oladunmoye, 2006; Bimark,2010). The expressed juice or boiled decoction was ethnomedicinally used in the treatment of *Pityriasis versicolor, Impetigo contagiosa, Tinea versicolor* and other similar skin infections. The present study seeks to screen the leaf extract of AW for its phyto-constituents, formulate into creams and then evaluate the antifungal properties.

**MATERIALS AND METHODS:**

Collection of *Acalypha wikesiana* leaves: The leaves were collected from various locations in Ode-Remo in Ogun state of Nigeria between the hours of 5-6pm. The plant species from which leaves were collected was authenticated at Forest research Institute of Nigeria (FRIN) with Voucher No 109531. The leaves were washed to remove impurities and air –dried till a constant weight of the dried leaves was obtained. The dried leaves were comminuted using blender model Mx-738 (Nakai, Japan) and stored in air tight containers. Ethanol 95%v/v was used as solvent for extraction at leaf weight: solvent of 1 to 10. The milled leaves of about 250µm size was macerated in the solvent for 10 days at ambient room temperature and filtered through a whatman filter paper (No.1) to remove the coarse leaf materials into pre-weighed sterile containers. The solvent was evaporated using rotary evaporator (Buchi Rotary evaporator,Germany) The extract was concentrated into mucilage form by removing any residual solvent using heating mantle. The extract was kept in air- tight containers and stored in a refrigerator.

**Phytochemical screening of pulverized *Acalypha wikesiana* leaves**:

The powdered leaves of *Acalypha wikesiana* were screened for the presence of the following secondary metabolites using standard procedures.

**Alkaloids**: To 1.0g of the powdered sample was added 10mls of 10%HCL, then heated on a water bath for 10 minutes. This was filtered with the pH adjusted to 6-7. About 0.5mls each of the following reagents: Wagner's reagent, 10%Tannic acid, Mayer's reagent, Drangendorf's reagent were added to the filtrate. Precipitate formation is an indication of the presence of alkaloids (Sofowora, 1993).

**Tannins:** One gram (1.0g) of the sample was boiled with 20mls of water for 5 minutes, cooled and then filtered. About 5 drops each of Ferric chloride solution, Lead acetate, Potassium dichromate were added to 1.0mls of the filtrate already diluted to 5.0mls with distilled water. Formation of green precipitate is an indication for the presence of tannins (Tease and Evans, 2002).

**Saponins**: Ten milliliters (10mls) of distilled water was added to 1.0g of powdered sample. The mixture was heated for 10 minutes, filtered while hot and the filtrate was allowed to cool. The following tests were carried out on the filtered extract:

I. Frothing activity: Ten milliliters of distilled water was added to 2.5mls of the filtrate and then shaken together for 2 minutes. The formation of frothing persisting for about 15 minutes is an indication of the presence of saponins.

ii. Emulsifying property: Two drops of olive oil was added to 5mls of the filtrate. This was vigorously shaken together. Formation of stable emulsion is an indication of the presence of saponins (Sofowora, 1993)

**Anthraquinones:**

i.Test for free anthraquinones: Five milliliters (5mls) of chloroform was added to 0.5g of powdered sample in a test tube and shaken for 5 minutes. The mixture was later filtered. Five milliliters of 10% ammonia solution was later mixed with the filtrate. The formation of bright pink colour in the aqueous layer is an indication of the presence of free anthraquinones (Sofowora,1993).

ii. Test for combined anthraquinones: One gram of the sample was boiled with 2mls of 10%HCL for 5 minutes. The extract was filtered while hot and then cooled. Two milliliters of chloroform was added to the filtrate. The chloroform layer was later transferred into a clean test tube and then 10% ammonia solution was added to it and shaken together. The formation of pink colour is an

indication of the presence of combined anthraquinones (Sofowora, 1993).

**Cardiac glycosides:**

One gram of the sample was boiled with 10mls of 80% alcohol for 5 minutes on a water bath. The filtrate was diluted with equal volume of water. Two milliliters of Lead acetate was added to the filtrate and then filtered after standing for a few minutes. Two milliliters of concentrated sulfuric acid was added along the side of the test tube to the filtrate. The formation of a light reddish brown colour at the interface with a green colour in the acetate layer indicates the presence of cardiac glycosides (Trease and Evans,2002).

**Assessment of antifungal effect of *Acalypha wikesiana* leaf extract**:

*Acalypha wikesiana* leaf extract with ketoconazole cream as reference was tested against clinical isolates of *Tricophyton tonsurans* and *Epidermophyton floccosum* common organisms implicated in fungal infections using agar pour plate method The clinical isolates obtained from Olabisi Onabanjo University Teaching Hospital were cultured on Sabouraud dextrose agar and incubated at 25$^{o}$C for 10 days. Thereafter a loopful of the culture was transferred into 40% sucrose solution and then mixed thoroughly. One milliliter of each organism dispersed was added to 19mls of sterilized agar solution, mixed thoroughly and later poured into petri dishes for setting. The set plates were dried in hot air. After drying, 2 cups were bored using a sterile cork borer (5mm in diameter) on each plate ensuring that the cups were far apart as much as possible and not too close to the edge of the plate. Using sterile Pasteur pipettes, different concentrations of the extract (0.0%w/w,2.5%, 5.0%, 7.5% and 10.0%) in duplicate were introduced into the labeled cups and the plates were incubated at 25$^{o}$C for 48hrs. At the end of incubation period, the plates were observed for clear zones of inhibition and recorded as mean ± SD.

**Formulation of *Acalypha* leaf extract cream:**

*Acalypha* leaf extract was incorporated into aqueous cream base to get the desired test concentrations of 0%w/w, 2.5% w/w,5.0% w/w, 7.5% w/w and 10% w/w using fusion method. Twenty-five gram (25g) of each sample contained 25.00g, 24.37g, 23.75g, 23.12g and 22.50g of the base respectively.

**Evaluation of drug release**:

Melted cream (0.25mls) was measured into a 25ml volumetric flask and made up to 25ml with phosphate buffer and then mixed thoroughly. Sterilized nutrient agar was poured into plates and allowed to solidify, the surface of each plate was flooded with ferric chloride solution 50% w/v and the excess solution was drained off. Three cups were bored in these plates using cork borer. 0.5ml of different concentrations (0.0% w/w, 2.5% w/w, 5.0% w/w, 7.5% w/w and 10.0% w/w) of the cream samples were placed in the holes. This was done in duplicate. The plates were then placed on a laboratory bench for 30mins for diffusion to take place before incubation at 25$^{o}$C and 37$^{o}$C. The zones of colour changes were measured for each sample at time intervals of 1, 2, 3, 12 and 24 hours respectively.

**Assessment of antifungal effect of *Acalypha* leaf extract cream formulations**:

Different concentrations of *Acalypha* leaf extract cream formulations were evaluated against clinical isolates of *Tricophyton tonsurans* and *Epidermophyton floccosum* in duplicate using pour plate method as in the case of pure extract. The leaf extract was replaced with cream formulations.

**RESULTS:**

Percentage yield of the extract is 10.68%w/w.The outcome of the phytochemical screening is presented in Table 1. The results of the diameter of the zones of inhibition of the leaf extract of *Acalypha wikesiana* against *Triochophyton tonsurans* and *Epidermophyton floccosum* is shown in Table 2.

**Drug release:**

The result of drug release of the cream samples at 25$^{O}$C and 37$^{0}$C measured in the diameter of zones of colour change in cm is presented in Table 3. The diameter of the zones of inhibition in mm of the cream formulations at various concentrations is shown in the Table 4.

Table 1: Results of the phytochemical screening of the leaf of *Acalypha wikesiana*

| Qualitative Test | Observation |
|---|---|
| Saponin | +++ |
| Cardiac glycosides | - |
| Tanin | +++ |
| Combined anthraquinone | - |
| Free anthraquinone | - |
| Alkaloid | +++ |

KEY : +++ = Presence - = Absence

Table 2: Assessment of the Antifungal properties of *Acalypha wikesiana* leaf extract showing inhibition zone in (mm) at various concentrations

| Sample | Organisms | | | | | |
|---|---|---|---|---|---|---|
| | *Trichophyton tonsurans* | | | *Epidermophton floccosum* | | |
| | Value 1 (mm) | Value 2 (mm) | Mean (mm) | Value 1 (mm) | Value 2 (mm) | Mean (mm) |
| 0.0%w/w | 0.00 | 0.00 | 0.00 | | | |
| 2.5%w/w | 11.00 | 9.00 | 10.00±0.10 | - | - | - |
| 5.0%w/w | 12.00 | 12.00 | 12.00±0.20 | - | - | - |
| 7.5%w/w | 13.00 | 15.00 | 14.00±0.40 | - | - | - |
| 10.0%w/w | 15.00 | 17.00 | 16.00±0.80 | - | - | - |
| Ketoconazole cream | 18.00 | 18.00 | 18.00±0.80 | 28.00 | 28.00 | 28.00±1.05 |

Table 3: Drug release of *Acalypha wilkesiana* cream formulations.

| Samples | Time (hour) at 25°C | | | | | Time(hour) at 37°C | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 12 | 24 | 1 | 2 | 3 | 12 | 24 |
| ALC1 | - | - | - | - | - | - | - | - | - | - |
| ALC2 | - | - | - | 0.6cm | 1.5cm | - | - | - | 0.7cm | 1.8cm |
| ALC3 | - | - | 0.4cm | 0.9cm | 2.0cm | - | - | 0.2cm | 1,2cm | 2.3cm |
| ALC4 | - | - | 0.5cm | 1.1cm | 2.2cm | - | - | 0.4cm | 1.5cm | 2.5cm |
| ALC5 | - | - | 0.7cm | 1.3cm | 2.5cm | - | - | 0.5cm | 1.6cm | 2.8cm |

**Keys:** ALC1 = Acalypha leaf extract cream (0.0%w/w). ALC2 = Acalypha leaf extract cream (2.5%w/w). ALC3 = Acalypha leaf extract cream (5.0%w/w). ALC4 = Acalypha leaf extract cream (7.5%w/w). ALC5 = Acalypha leaf extract cream (10.0%w/w).

**DISCUSSION**:

The calculated yield of *Acalypha wikesiana* leaf extract was found to be 10.68%w/w. This yield is able to support the herbal formulation of this extract since the plant is abundantly available everywhere especially in the tropics. This yield is also able to give rise to high quantities of isolated bioactive compounds whenever necessary. The secondary metabolites found present in the leaf extract were alkaloids, saponins and tannins as can be seen in Table 1. 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 (Hodek *et al*,2002). Phytoconstituents present in plants namely alkaloids, tannins, triterpenoids, flavonoids and saponins are producing exciting opportunities for the expansion of modern chemotherapies against wide range of microorganisms (Lutterodt *et al*,1999; Marjorie, 1999).

Table 4: Inhibition zones of *Acalypha wikesiana* leaf extract cream against test organisms in (mm)

| Formulations | *Trichophyton tonsurans* | | | *Epidermophyton floccosum* | | |
|---|---|---|---|---|---|---|
| | Diameter (mm) | | | Diameter (mm) | | |
| ALC1 | - | - | - | - | - | - |
| ALC2 | - | - | - | - | - | - |
| ALC3 | - | - | - | - | - | - |
| ALC4 | 12 | 11 | 10 | - | - | - |
| ALC5 | 15 | 13 | 11 | - | - | - |
| Ketoconazole cream | 18 | | | 30 | | |

The antifungal activity of the *Acalypha* leaf extract against test organisms is as shown in Table 2. The extract showed activity against *Tryhophyton tonsurans* only with mean inhibition zones of 10.00mm to 16.00mm for all the concentrations tested. Surprisingly, there was no activity against *Epidermophyton floccosum*. The difference in the antifungal activity between these two organisms may be attributable to the difference in their cellular structure. It is suspected that cellular wall structure of

*Tricophyton tonsurans* is more susceptible to the lethal effect of the phytoconstituents present in the leaf extract than the rigid cell wall of *Epidermophyton floccosum* . It was observed that the zones of inhibition against *Tricophyton tonsurans* is of increasing order as the concentration of leaf extract was increasing from 0% w/w to 10-% w/w. This is because as concentrations of the extract was increasing the sensitivity of the organism was also increasing .

The result of drug release from the different concentrations of cream prepared using the extract is as shown in Table 3. The zones of colour change increased in diameter with increase in concentration and time for all concentrations studied. The amount released and the rate of release of a drug suspended in a vehicle such as cream may be related to time and to variables of the system (Aulton,1996). The rate of release of *Acalypha* extract is greatly influenced by diffusion co-efficient, concentration and solubility of the extract in the cream base. Employing the following, equation (Aulton,1996).

$$\frac{d_m}{d_t} \cong \left(\frac{AD_vC_s}{2t}\right)^{\frac{1}{2}} \quad \text{..... eqn (1)}$$

Where $d_m/d_t$ is rate of release, A is total amount of cream base, $D_v$ is the diffusion co-efficient in the base, $C_s$ is the solubility of the drug in the vehicle and t is time. It may be possible to predict *in-vitro* availability of medicament from vehicle base.

Temperature also had effect on the rate of release of the extract at various concentrations, higher at 37$^o$C than 25$^o$C as can be seen in Table 3. This is possibly so because temperature is able to alter diffusive tendency of drugs in cream base at elevated temperature due to increase in fluidity of the base.

The antifungal activity of *Acalypha wikesiana* leaf extract cream formulations is as presented in Table 4. It was observed that there was inhibition against *Trychophyton tonsurans* at concentrations of 7.5%w/w and 10.0%w/w with zones of inhibition 10.00mm and 11.00mm respectively. Cream formulations had lesser activity as compared with reference sample, ketoconazole that showed a remarkable activity against test organisms with zones of inhibition 18.00mm and 24.00mm for *Trichophyton tonsurans* and *Epidermophyton floccosum* respectively . The rate of release of the extract from the base which is a function of time, concentration, diffusion co-efficient and temperature of storage may account for absence of inhibition at concentrations 0.0%w/w, 2.5%w/w and 5.0%w/w. The minimum inhibition concentration becomes a critical factor at the level of formulations as compared with crude extract since other factors as highlighted above come into play for the availability of the extract to exert its activity. The non -activity of the cream formulations against *Epidermophyton floccosum* could be explained from the point of view of the cellular structure of this organism which appear more rigid than that of *Trichophyton tonsurans*. Further increase in the concentrations of the extract in the formulations may lead to increase in better availability and higher inhibition zones that

will compare favorably or even perform better than the reference sample.

**CONCLUSION**

Although *Acalypha wikesiana* leaf extract and cream formulations did not show activity against *Epidermophyton floccosum*, it however exhibits promising antifungal potentials against *Trichophyton tonsurans* which follows therefore that the herbal formulation of the leaf extract may be useful in specific diagnosis where *Trichophyton tonsurans* has been shown to be the dermatophyte involved in the skin disease condition.

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