

Formulation Of *Acalypha Wilkesiana* Muell. Arg. Ethanol Leaf Extract into Creams for the Treatment of Microbial Skin Infections.

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ABSTRACT : *Acalypha wilkesiana* Muell. Arg leaf has been used by the local populace for the treatment of skin diseases for ages. Ethanol extract of the dried leaves of *Acalypha wilkesiana* Muell. Arg was investigated for in vitro antimicrobial activities by agar diffusion and broth dilution techniques using *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi* and a dermatitis fungus, *Candida albicans*. Streptomycin and nystatin were used respectively for the bacteria and fungus as standard drugs. The various concentrations of the extract, 6.0, 6.2 and 6.4g were formulated into cetomacrogol, zinc creams and emulsifying wax ointment and the testing of the efficacy of the creams and ointment was carried out. The zones of inhibition (mm) of the extract on the growth of the microorganism were *Staphylococcus aureus* (30.0 ± 0.1), *Bacillus subtilis* (16.0 ± 0.2), *Escherichia coli* (25 ± 0.1), *Salmonella typhi* (no zone of inhibition), *Candida albicans* (32.2 ± 0.2). *Acalypha wilkesiana* cetomacrogol cream showed better antimicrobial activity than the zinc and emulsifying wax ointment. When compared with the standard drugs in vitro both cetomacrogol and zinc creams were effective on *Staphylococcus aureus*, *Bacillus subtilis* and *Candida albicans* except the emulsifying wax ointment which had the least zone of inhibition because of the occlusive effect of paraffin on its diffusion rate. This study revealed that the dried leaves of ethanol extract of *Acalypha wilkesiana* possesses antimicrobial activity and can be formulated into a suitable cream for the treatment of skin infections to confirm its folkloric use.

KEYWORDS: *Acalypha wilkesiana*, Antimicrobial activity, Ethanol extract, Antibacterial activity, Diffusion rate

I. INTRODUCTION

The use of natural substances as medication by man can be said to be as old as human race [1]. Recently, there had been an increased interest in the use of medicinal plants in developing countries. This is because herbal medicines have been reported safe and without any adverse side effect especially when compared with synthetic drugs [2]. Also, there has been little or no report of any form of microbial resistance during the use and administration of herbal medicines. More importantly in Africa, particularly West Africa, new drugs (synthetic drugs) are often unaffordable to the poor therefore, there is need for improving and formulation of our surrounding useful medicinal plants into dosage forms for treatment of diseases; topical, subcutaneous and systemic [2]. *Acalypha*, *A. wilkesiana* Muell Arg leaf has been reported to have medicinal properties for the treatment of malaria, dermatological and gastrointestinal disorders [3],[4] antihypertension properties [5], and for its antimicrobial activities [6], [7], [8], [9], [10], [11], [12], [13]. *Acalypha wilkesiana* (Copperleaf, Jacob's coat, fire dragon) (Family: Euphorbiaceae) is an ornamental plant commonly planted in the gardens or surroundings in Southern Nigeria, although it can also be grown indoors as a container plant. It is native to Fiji Island and propagated by stem cuttings at any time of the year [14]. The genus "*Acalypha*" comprises about 570 species. Agu [15] and Adesina *et al.* [16] reported that the essential oils distilled from leaves of the plant possess phytochemical and microbiological activities against *Staphylococcus aureus* and *Klebsiella aerogenes*. According to Akinde [3] and Ogundaini [9], the expressed juice or boiled concoction is used for the treatment of gastrointestinal disorders and fungal skin infections such as *Pityriasis versicolor*, *Impetigo contagiosa*, *Candida intetrigo*, *Tinea versicolor*, *Tinea corporis*, and *Tinea pedis*. The leaves of this plant are eaten as vegetables in the management of hypertension in Southern Nigeria. Seeds from *Acalypha wilkesiana* are essential components of a complex plant mixture used by traditional healers in southwest Nigeria in the treatment of breast tumors and inflammation [3].

Adesina *et al.*[7] reported a seasonal variation in the distribution of the three natural antimicrobial phenols (geraniin, corilagin and gallic acid) in the genus *Acalypha*. Information obtained from local communities in Ado town in Ekiti State and Ilorin city in Kwara State of Nigeria also revealed that the local populace use leaf of *A. wilkesiana* as a herbal remedy for the undefined skin infection in neonates and children of a year old [17]. The leaf is boiled in water to yield a dark red liquid which is added to bathing water. A portion of the boiled liquid is also given to the baby to drink. The present study therefore investigated the in-vitro antibacterial effect of various extracts of *A. wilkesiana* leaf on some gastrointestinal tract pathogens and bacteria causing skin infections in neonates. The aim of this study was to compare the antimicrobial activity of *Acalypha wilkesiana*'s ethanol extract on bacterial and fungal isolates.

II. MATERIALS AND METHODS

Collection of plant materials : Fresh leaf of *A. wilkesiana* was collected by hand – plucking from parent plants sported within the premises of the Faculty of Pharmacy, University of Uyo, Uyo, Nigeria. The leaf was identified in the herbarium unit of the Department of Pharmacognosy and Natural Medicine of the same institution. After authentication, the leaf was pulverized and weighed. The dried leaf was ground into fine powder and stored in air-tight container until use.

Extraction procedure : The extracts of *A. wilkesiana* were obtained using different solvents: aqueous, methanol, ethanol, ethylacetate, n-hexane, petroleum ether. The ethanol, methanol and petroleum ether reagents were products of Oxoid Ltd, Hants, United Kingdom. One hundred grams of the powdered leaf was weighed separately into four 250ml conical flasks containing 250ml of distilled water (aqueous extraction), 250ml of 96% ethanol (ethanolic extraction), 250ml of 100% methanol (methanolic extraction), 250 mL of ethylacetate, (ethylacetate extraction), 250mL of 100% of n-hexane (n-hexane extraction) and 250ml of 100% petroleum ether (petroleum ether extraction). The mixtures were covered and stirred every 24h using a sterile glass rod for 3 days for aqueous extraction and 5 days for ethanol, methanol, ethylacetate, n-hexane and petroleum ether extractions. The mixtures were filtered through Whatman filter paper No. 1 (Whatman Limited, England). The resulting yellowish-green filtrates were then concentrated at 40°C and subsequently lyophilized to dryness. The final products were sticky dark-brown substances which were stored in universal bottles and refrigerated at 4° C prior to testing.

Phytochemical screening : Simple standard chemical tests were employed for detecting the presence of some phytochemical components such as saponins, tannins, alkaloids, terpenoid, flavonoid, phlobatannin and cardiac glycosides in the plant extract.

Determination of Saponins : The ability of saponins to produce frothing in aqueous solution was used as screening test for saponins. About 0.5g of each plant extract was shaken with distilled water in a test tube, frothing which persisted on warming was taken as evidence for the presence of saponins [18].

Determination of Tannins : Plant extract (5g) of each portion was stirred with 100ml of distilled water, filtered and Ferric chloride reagent was added to the filtrate. A blue-black green precipitate indicated the presence of tannins [19], [20]. Ten grams of the plant samples were extracted with 60ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper. The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed [21].

Test for alkaloids: Extract (0.5) was diluted with 10ml of acid alcohol boiled and filtered. Two milliliter of diluted ammonia was added to 5ml of the filtrate. Five milliliter of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10ml of acetic acid. This was divided into two portions. Meyer's reagent was added to one portion and Dragendorff's reagent to the other. The formation of a cream (with Meyer's reagent) or reddish brown precipitate (with Dragendorff's reagent) was taken as positive for the presence of alkaloid [19].

Test for steroids : Extract (0.1) was dissolved in 2ml of chloroform and sulphuric acid was carefully added to form a lower layer. A reddish brown color at the interphase is indicative of the presence of steroidal ring [22].

Test for flavonoids : Powdered sample of *A. wilkesiana* (2g) was de-tanned with acetone. The sample was placed on a hot water bath for all traces of acetone to evaporate. Boiling distilled water was added to the de-tanned sample. The mixture was filtered while hot. The filtrate was cooled and 5ml of 20% Sodium hydroxide was added to equal volume of the filtrate. A yellow solution indicates the presence of flavonoids [18], [22].

Determination of cardiac glycosides : This was carried out using Keller - Killani test as described by Trease and Evans (1978). Extract (0.5g) was dissolved in 2ml of glacial acetic acid containing one drop of ferric chloride, then 1 ml of concentrated sulphuric acid was added gently by the side of the test tube into the mixture. A brown ring obtained at the interface indicated the presence of deoxy-sugar characteristic of cardenolides.

Determination of Phlobatanins : This was carried out as described by Aiyelaagbe and Osamudiamen [19], and Egwaikhide *et al.* [20]. Deposition of a red precipitate when an aqueous extract of the plant part was boiled with 2% hydrochloric acid was taken as evidence for the presence of phlobatanins.

Test for Terpenoids : This was done using Sakowski test as described by Sofowora [18]. Each extract (5mL) was mixed with 2ml of chloroform and 3ml concentrated H₂SO₄ was added to form a layer. Formation of a reddish brown coloration at the interphase indicated the presence of terpenoids.

Test for deoxy-sugar : The plant extract (0.5g) was dissolved in 2mLs of glacial acetic acid containing one drop of Ferric Chloride, it was diluted with 1mL of conc. H₂SO₄.

Preparation of various concentrations of extracts. : Extract 2g was reconstituted in distilled water to obtain 100ml of a 20 mg/ml solution. A portion of the 20mg/ml solution was diluted with an equal volume of distilled water to obtain a 10mg/ml solution. The double dilution procedure was continued to obtain lower concentrations of the extract.

Preparation of creams : The methods used were obtained from the Pharmaceutical Codex, 1994 and the British Pharmacopoeia depending on the formula employed.

Preparation of cetomacrogol cream PC with ethanol extract of *A. wilkesiana* : *Acalypha wilkesiana* ethanolic extract (6g) was dissolved in 2mL of purified water while propylbenzoate (0.02g), methyl hydrobenzoate (0.03) were dissolved in 2mL of water and transferred to the extract solution properly mixed with the addition of 0.3mL of benzyl alcohol. The same procedure was done for 6.2g and 6.4g of the ethanol extract.

Preparation of Zinc cream PC, with ethanolic extract of *A. wilkesiana* .: *Acalypha wilkesiana* ethanolic extract (6.0g) was weighed and dissolved in 5ml of distilled water and the solution mixed with 0.01 g of calcium hydrochloride. Oleic acid (0.1mL) and arachis oil (6.4mL) were added to the mixture and well triturated to form a smooth paste. Wool fat (1.6g) was incorporated gradually with continuous stirring. Sufficient amount of purified water was added to produce the required volume. This same procedure was made for 6.2 and 6.4g of ethanol extract of *A. wilkesiana*.

Preparation of cream using the formula of emulsifying wax ointment BP, with ethanol extract of *A. wilkesiana* : *Acalypha wilkesiana* ethanol leaf extract (6g) was weighed and dissolved in 5mL of distilled water in a glass mortar. Emulsifying wax (4g), white soft paraffin (8g) and liquid paraffin (2g) were weighed respectively and melted together in a crucible and transferred into the mortar containing the extract. The mixture was properly mixed anti-clock wisely until cold and transferred into an already weighed and labeled bottle. The bottle was covered and properly labeled. The same procedure was used to prepare 6.2 and 6.4g of the extract of the same emulsifying ointment cream.

Test microorganisms : Four broth cultures of bacterial isolates of *Staphylococcus aureus* NCTC 6571, *Bacillus subtilis* NCTC 8853, *Escherichia coli* NCTC 10418, and *Salmonella typhi* NCTC 8571 and a fungus *Candida albicans* of clinical isolate used in this study were obtained from Pharmaceutical Microbiology Laboratory, Faculty of Pharmacy, University of Uyo. Streptomycin (0.004 mg/ml) was used as standard for the bacteria, while nystatin was for the only fungi *Candida albicans* (1000 i.u.).

Standardization of Inoculums : All the test organisms were sub-cultured on nutrient agar for 24h and 5 colonies were transferred into 5ml of sterile nutrient broth in test tubes and incubated for 3h at 37° C. The growth of bacterial suspension obtained was compared to that of freshly prepared Barium sulphate solution {0.5ml of a 1% Barium in Chloride to 99.5ml of 1% H₂SO₄ (0.36 Normal)}. The turbidity was adjusted by adding more sterile nutrient broth to match 0.5 McFarland standards (10 cfu/ml).

Preparation of nutrient molten agar : Mueller Hinton agar (7.6g) was dissolved in 200ml of distilled water and heated to boiling to dissolve the medium completely. The solution then was sterilized by autoclaving at 121°C for 15 minutes.

Antimicrobial sensitivity of *A. wilkesiana* ethanol extract : Sterile petri dishes were properly labeled as plate 1, 2, 3, 4, and 5. Broth culture (0.01 mL) of *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi* and *Candida albicans* were transferred (inoculated) into the plates respectively with different sterile Pasteur pipettes for each. Freshly prepared Mueller Hinton agar (20mL) was poured into the different plates and swirled to mix with the previously inoculated broth culture of the microorganism. They were allowed to solidify. A cork borer properly flamed each time was used to bore two sets of hole (one for the standard drug and the other for extract) on each plate. A single Pasteur pipette properly flamed was used to transfer nystatin to the plate 5 containing *Candida albicans*. A different sterile pipette was used to transfer 100mg/mL of the extract to each of the holes in the plates. The plates were allowed to stand for 1 hour on the bench to allow for diffusion of the agents into the media, before the growth of the microorganisms. After an hour the plates were incubated at 37°C for 48 hours. The plates were observed for 24 hours and 48 hours and the zones of inhibition across the holes were measured for each plate.

Determination of Antimicrobial Activity : The antibacterial activities of the various extracts of the leaf were determined using agar diffusion technique [23]. Sterile solid Mueller Hilton agar (Himedia, Vadhani Ind. Est., LBB Marg Mumbal-400 086) plates were aseptically streaked with the standardized bacterial culture (1.5×10^8 cfu/ml). Then, a sterile cork borer (6mm in diameter) was used to bore four holes and one in the centre of the petri dish. Varied concentrations (10mg/ml, 30 mg/ml, 60 mg/ml) of the extracts were introduced into the holes. The hole in the centre was used as negative control in which few drops of the re-constituting reagent were introduced. The inoculated Petri dishes were left for a few minutes for the extract to diffuse into the agar. The plates were then incubated at 37°C for 24h. The zones of inhibition were measured in millimeter.

Determination of Minimum Inhibitory Concentration : Broth dilution technique was used for this purpose. Varying concentrations of the extracts were prepared as 10mg/ml, 30mg/ml and 60mg/ml in a universal bottle. About 50µl of the extract was introduced into the nutrient broth inoculated with standardized bacterial suspension (1.5×10^8 cfu/ml) and incubated at 37°C for 24h. The lowest concentration of the extract which inhibited the growth of each inoculum was considered as minimum inhibitory concentration.

Statistical analysis : All experimental measurements were carried out in triplicate and were expressed as an average of three analyses \pm standard deviation. Statistical analyses were performed by the t-test.

III. RESULTS AND DISCUSSION

Sensitivity test of the ethanol extract in 24 and 48 hours : The sensitivity test results of the ethanolic extract for 24 hours and 48 hours are presented in Table 1 while the representative plots of square of zone of inhibition versus log of quantity of extract for cetomacrogol, emulsifying ointment and zinc creams are respectively presented in Figures 3.1, 3.2, 3.3. The result showed that *A. wilkesiana* possesses antibacterial and antifungal activity though the activity is more pronounced against *Candida albicans* than the bacteria used for the test. The ranking of sensitivity test is *Candida albicans* > *S. aureus* > *E. coli* > *B. subtilis*. There was no effect on *S. typhi*.

Table 1: Sensitivity results of extract on microorganisms

Duration of sensitivity test	24 hour		48 hour	
	Extract	Standard	Extract	Standard
	Zone of inhibition (mm)			
Organism	Extract	Standard	Extract	Standard
<i>S. aureus</i>	30.0 \pm 0.1	30.2 \pm 0.1	30.0 \pm 0.1	32.2 \pm 0.1
<i>B. subtilis</i>	15.2 \pm 0.1	25.1 \pm 0.1	16.2 \pm 0.2	28.3 \pm 0.2
<i>E. coli</i>	25.1 \pm 0.2	22.1 \pm 0.3	25.1 \pm 0.1	24.2 \pm 0.1
<i>S. typhi</i>	-	10.3 \pm 0.1	-	11.3 \pm 0.1
<i>c. albicans</i>	20.0 \pm 0.2		3.0 \pm 0.2	35.2 \pm 0.1
		35.2 \pm 0.2		

Candida albicans is a known skin pathogens causing problems in the body. It is common especially in the presence of agravatory factors such as obesity, hot humid tropical climate, diabetes mellitus and other cases of reduced body immunity. This therefore supports its traditional use in treating skin infections. *Acalypha*

wilkesiana shows significant antibacterial activity against gram positive bacteria such as *S. aureus* and *B. subtilis*. *S. aureus* is known to give rise to skin infections like impetigo, boils, carbuncles and rashes of the beard. Therefore, herbal preparations of the extract can be effective in the treatment of such skin diseases as traditionally claimed. The extract had also shown significant antibacterial activity against gram negative bacteria used such as *E. coli* but no effect on *S. typhi*.

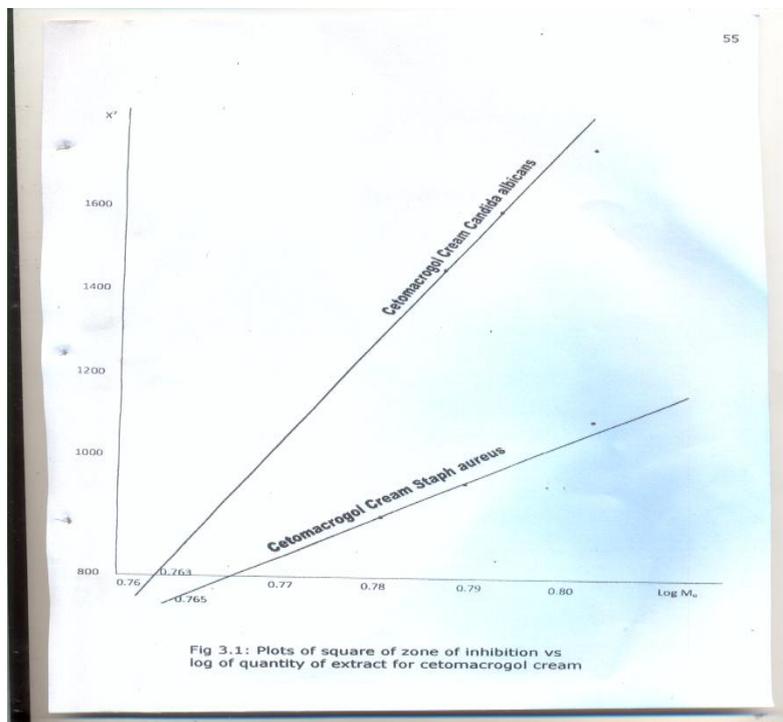


Fig. 3.1: Representative plots of square of zone of inhibition vs log of quantity of extract for cetomacrogol cream formulated with *Staphylococcus aureus* and *Candida albicans*

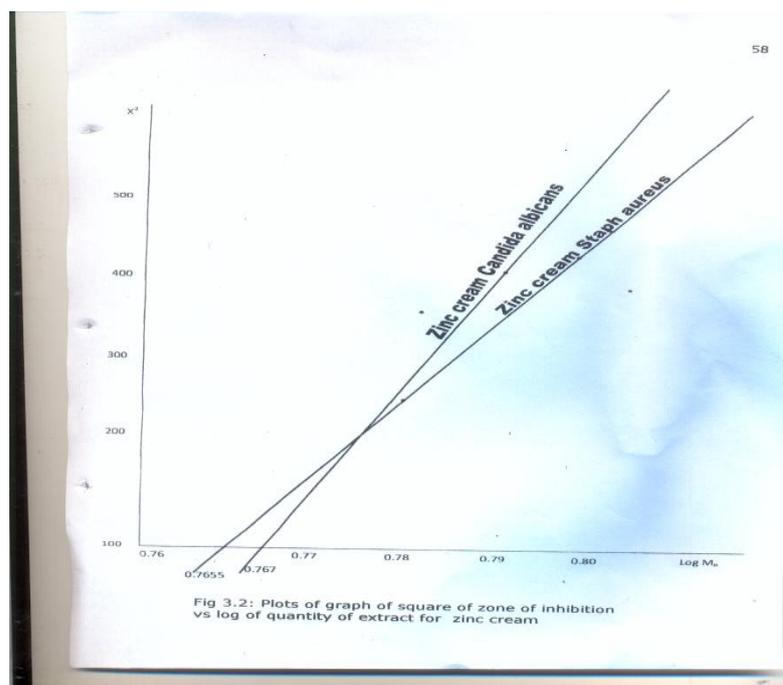


Fig. 3.2: Representative plots of square of zone of inhibition vs log of quantity of extract for zinc cream formulated with *Staphylococcus aureus* and *Candida albicans*

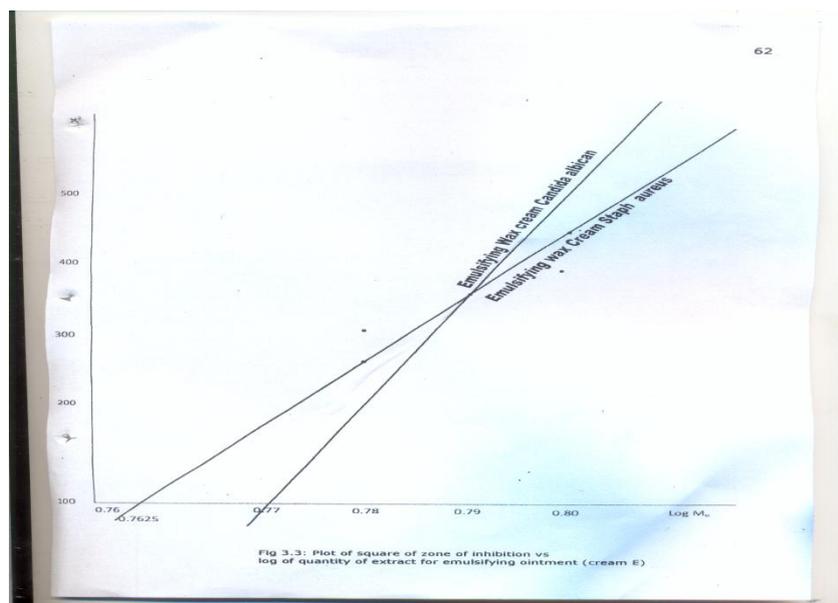


Fig. 3.3: Representative plots of square of zone of inhibition vs log of quantity of extract for emulsifying ointment formulated with *Staphylococcus aureus* and *Candida albicans*

Diffusion rate of the various creams in agar gel for 24 and 48 hours : The results of the diffusion rate of the various creams for 24 and 48 hours are presented in Table 2. The three creams (E) prepared using the emulsifying ointment B.P formula had a dull appearance compared with cream Z and C. They were also the thickest with a very low diffusion rate. Diffusion being the systematic movement of molecules from regions of higher concentrations to regions of lower concentrations indicate the extent at which the various creams can be absorbed or penetrate the skin. The low diffusion rates of these creams indicate low degree of absorption into the skin which can be attributed to paraffin in the formula. Paraffins are poorly absorbed and are used as basis for ointments which are not to be absorbed [24]. The three cetomacrogol creams produced have a shiny appearance, are less thick compared to creams E but produced greatest diffusion rate. The greatest diffusion rate shows that the creams will be absorbed into the skin which implies that they can elicit pharmacological action superficially as well as combating the microbes underneath the skin. The parabens in the formula are used as preservatives against fungi growth. The propyl paraben is effective at the oily phase while the methyl paraben is effective at the aqueous phase [25]. This resulted in a synergistic antifungal effect. Creams Z (zinc cream) were moderately thick with a shiny appearance and darker colouration, much darker than the others. The darker coloration can be attributed to the wool fat in the formula [24]. Zinc creams had moderate diffusion rate but less than those of creams C which implies that they will be absorbed moderately thereby extending its antimicrobial effect underneath the skin.

Extract (g)	Diffusion rate (mm)					
	24 hours			48 hours		
	Creams (mm)					
	C	Z	E	C	Z	E
6.0	30.2 ±0.1	10.1±0.3	15.3±0.1	40.3±0.2	16.1±0.1	20.3±0.1
6.0	32.3 ±0.1	10.2±0.2	15.4±0.3	40.4±0.1	16.4±0.2	20.4±0.3
6.4	32.2 ±0.2	10.3±0.1	15.4±0.1	40.2±0.2	16.2±0.3	20.3±0.1

Table 2: Diffusion rate results of the various creams in agar gel for 24 and 48 hours

Efficacy test results of the various creams : Agar diffusion test is a means of measuring the effect of antimicrobial agent against microbial growth in culture. It is a means of measuring the effect of the various

creams against the test organism. The effectiveness of these creams is measured by the zone of inhibition, which is the region where the concentration of the antimicrobial agent (the creams) inhibits the growth of tested microorganism. No colonies will be found in the zone of inhibition. Thus the zone of inhibition is a measure of effectiveness: the larger the clear area around the well the more effective the compound. From the results obtained, it can be said that the cetomacrogol cream has the highest zone of inhibition. Therefore, it can be said to be the most effective compared with others. This is due to the synergy of the constituents. The low zone of inhibition of the emulsifying wax ointment could be attributed to the effect of the paraffin present which according to the Pharmaceutical Codex it is said to be poorly absorbed, can inhibit its diffusion rate via the agar

gel. Also the zone of inhibition varies in the various creams with respect to the different quantity of the extract incorporating in them. Therefore the graphs have been drawn to assess the critical concentration of the various

creams, just sufficient to stop (inhibit) the growth of the test organism. The results deduce the critical concentration of the extract that will inhibit microbial growth. In Fig. 1, the critical concentrations of the extract that can inhibit microbial growth are 5.78g and 5.82 for *C. albicans* and *S. aureus* respectively. In Fig. 2 the critical concentration of the extract that can inhibit growth are 5.78g and 5.89g for *C. albicans* and *S. aureus* respectively. Fig. 3 for emulsifying ointment shows the highest critical concentration which implies that when formulating emulsifying ointment, more of the extract should be incorporated as its releasing rate is impeded by the by the presence of paraffin in the formula [24].

TABLE 3 IN THE LANDSCAPE PLEASE

Efficacy test Result Zone of inhibition (mm)												
Quantity of extract	24 hours						48 hours					
	C		Z		E		C		Z		E	
	Sa	Ca	Sa	Ca	Sa	Ca	Sa	Ca	Sa	Ca	Sa	Ca
6.0	30.1 ±0.1	31.4 ±0.2	15.3 ±0.1	10.3 ±0.2	15.4 ±0.1	16.4 ±0.2	3 0	38.5± 0.1	17.4 ±0.2	19.5 ±0.2	17.3 ±0.2	18.0± 0.1
6.2	31.2 ±0.2	32.3 ±0.1	16.5 ±0.2	15.4 ±0.2	17.3 ±0.2	16.4 ±0.1	3 2	40.4± 0.2	19.4 ±0.1	20.4 ±0.1	19.3 ±0.1	19.5± 0.2
6.4	32.4	32.4	18.3	15.6	18.5	17.3	3	41.4±	20.3	20.3	20.4	21.0±

Table 3: Efficacy test results of the various creams

Sa: *Staphylococcus aureus*; Ca: *Candida albicans*; C: Cetamacrogol cream, Z: Zinc cream; E: Emulsifying ointment Percentage yield and phytochemical analysis

The percentage yields of the extracts in methanol, ethanol, ethylacetate, n-hexane and petroleum ether were 25%, 21%, 5%, 2% and 0% respectively. Methanol and ethanol are polar substances. Ethylacetate is not as polar as methanol and ethanol while n-hexane and petroleum ether are non-polar. There were good yields in methanol and ethanol. Ethanol extract was chosen for this investigation because of its safe handling and environment friendly nature unlike methanol which is very volatile and toxic. However, the yields were poor. According to Vogel's textbook of Practical Organic Chemistry, yields around 100% are called quantitative; yields above 90% are called excellent, yields above 80% are very good; yields above 70% are good, yields above 50% are fair and yield below 40% are called poor. This poor yield can be attributed to limitations in measurement accuracy and some external factors such as: temperature which affects the rate of the reaction, container used, equilibrium used in weighing, the medium (polar or non polar) and the nature of the substance involved [26]. The phytochemical testing of the ethanol extract gave positive reactions to tannins, flavonoids, steroids, saponin and cardiac glycosides, alkaloids at varying degrees and negative reactions for sugar and anthraquinones as shown in Table 4. The medicinal properties of *Acalypha wilkesiana* can be attributed to its constituents. Tannins are water soluble polyphenols that are commonly found in higher herbaceous and woody plants [27]. They have been reported to possess antimicrobial activity which depends on its astringent property [28].

Table 4: Phytochemical screening of *A. wilkesiana*

Components	Results
Tannins	++
Flavonoids	+
Cardiac glycosides	++
Anthraquinones	-
Sugar	-
Alkaloids	++
Saponins	+

++ = moderate

+ = trace amount

- = absent

Alkaloids are groups of naturally occurring chemical compounds that contain mostly nitrogen atoms which include some related compounds with neutral [29] even weakly acidic properties [30]. Alkaloids are soluble in organic solvents but not water [31]. The presence of alkaloids in plant plays a role in the medicinal qualities of the plant. This can be proven based on the use of alkaloids in medicine. Based on these findings, it can be said that alkaloids in *Acalypha wilkesiana* contribute to its acclaimed medicinal effect and antimicrobial property. Flavonoids have been said to belong to a group of natural substances with variable phenolic structures and are found in fruit, vegetables, grains, bark, stem roots, flowers, tea and wine [32]. It has also been said that the presence of flavonoids in plants is responsible for the attractive colours in flowers [33]. Therefore the characteristic colour of *Acalypha wilkesiana* leaf can be attributed to the presence of flavonoids. According to the American journal of clinical nutrition, scavenging of oxygen derived free radicals has been said to be an important effect of flavonoids and has been the mechanism by which the plant exert its acclaimed anti-inflammatory, antiviral and anti-allergic properties, hence its use in treating skin infections [34]. Saponins are natural surfactants or detergent (natural soapy substances) with distinct foaming characteristics [35]. They are found in many plants and are the plants immune system. Many plants produce natural soaps (saponins). These chemicals are toxic to bacteria and fungi and so form parts of the plants protection against disease [1]. Therefore, the presence of saponin in *Acalypha wilkesiana* leaves also contributes to its antibacterial and antifungal property used in treating skin infections. Cardiac glycosides are secondary metabolites created by plants and animals [36]. Cardiac glycosides are toxic but have drug like therapeutic effects when used appropriately [2]. Cardiac glycosides are found in many medicinal plants and contain digitoxin, digoxin and ditoxin. They have a strong and direct action on the heart, helping to support its strength and rate of contraction when it is failing. Therefore, its presence in *Acalypha wilkesiana* conforms to its traditional use in treating heart disease.

III. CONCLUSIONS

The ethanol extract of *Acalypha wilkesiana* has shown antimicrobial properties from the results of the tests carried out. It has shown greatest antimicrobial property to *Candida albicans* and none to *Salmonella typhi* which means *A. wilkesiana* cannot be used to treat any salmonella related ailment but will be very effective in *S. aureus* and *B. subtilis* related infections. *Acalypha wilkesiana* creams formulated with cetomacrogol have shown the greatest antimicrobial properties while the least antimicrobial properties was reflected by emulsifying ointment. This is due to the impeding effect of paraffin present in the formulation. From the graphs, critical concentration of the extract that can inhibit microbial growth can be said to be almost the same for all the creams except the emulsifying cream that require more of the extract which also can be attributed to the impeding effect of paraffin. In consequence, the leaf of *A. wilkesiana* possesses antimicrobial activity to treat skin infections as claimed by the traditionalists and can be used in formulating herbal skin creams to treat some skin infections.

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