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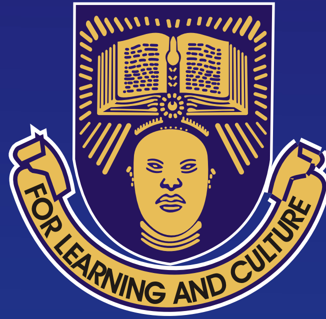

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ANTIBACTERIAL EFFECT OF EXTRACTS OF *ACALYPHA WILKESIANA* ON GASTROINTESTINAL TRACT PATHOGENS AND BACTERIA CAUSING SKIN INFECTIONS IN NEONATES.

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ABSTRACT

In-vitro antibacterial effects of aqueous, ethanolic, methanolic, and petroleum ether extracts of *Acalypha wilkesiana* leaf on some gastrointestinal tract pathogens (*Staphylococcus aureus, Bacillus cereus, Klebsiella pneumoniae, Salmonella typhi, Escherichia coli and Shigella dysenteriae)* and bacteria causing skin infection in neonates (*Staphylococcus aureus, Streptococcus pyogenes* and *Klebsiella aerogenes*) were investigated. The phytochemical components present in the leaf were also assessed.

The antimicrobial activity and minimum inhibitory concentrations of the extracts were determined using agar diffusion and broth dilution techniques respectively. The aqueous, ethanolic and methanolic extracts inhibited the growth of the test bacteria in varying degrees while the petroleum ether extract did not show any inhibitory effect on all the test bacteria. The observed antibacterial effects of the *A. wilkesiana* extracts was in direct proportion to the concentration of the extracts used. Minimum inhibitory concentration of the extracts ranged from 10-30μg/ml. The antibacterial activity of the various extracts of the leaf was comparable to the reference antibiotics, though the antibiotics showed better inhibitory property ($p<0.05$) in some cases on the test isolates than the leaf extracts The study showed that *A. wilkesiana* leaf possessed inhibitory properties, thus justifies the folklore uses of the leaf for the treatment of gastroenteritis and skin infections in neonates.

Keywords: Antimicrobial activity, *Acalypha wilkesiana*, skin infections, neonates, pathogens

INTRODUCTION

Since antiquity, man has used plant to treat common infectious diseases and today traditional medicines still includes their usage as part of the habitual treatment of various maladies.

In the past few decades, the search for new anti-infectious agents has occupied many research groups in the field of ethnopharmacology and ethnomedicine. Medicinal plants have been used as sources of medicine in virtually all cultures (Baquer, 1995). During the last decade, the use of traditional medicine has expanded globally and is gaining popularity. It has continued to be used not only for primary health care of the poor in developing countries but also in countries where conventional medicine is predominant in the national health care system (Lanfranco, 1999). According to World Health Organization, herbal medicine services the health needs of about 80% of the world's population, especially in developing countries (WHO, 1978).

The use of plants for therapeutic purposes in Yoruba land of Nigeria dates back to centuries where they first applied the use of plant parts in the cure of different ailments. Since then, medicinal plants were used in curing diseases (Sofowora, 1983). Today however, mainstream medicine is increasingly receptive to the use of antimicrobial agents rather than crude extracts from perceived healing plants. Traditional medicine using plants has thus almost been replaced by modern medicine in civilized and industrialized countries but remains dominant in health care of developing countries especially in rural areas. In Nigerian ethnomedicine, one plant is often used for the treatment of a variety of diseases. Plant parts such as stems, leaves, barks and roots are chopped into extractable pieces and allowed to extract for a period of time. The techniques used to administer herbal medicine are quite varied. A common method of preparation is a pot of cold water, brought to a boil and then left to stew for a while. Such decoctions could be taken orally until the perceived acceptable concentration is achieved (Iwu, 1986). If required, applications could be done externally to wash wounds and other types of skin infections. Therapeutic bathing of wounds using various herbal concoctions for their medicinal properties

is also a common practice. Herbal plasters are also usually applied directly to the painful area (Sofowora, 1983).

It is noted that there are indications of possible synergies between traditional and western medicine. In Nigeria today, therapy with medicinal plants is of great importance in conjunction with western medicine in the health care system. Similarly, at the global level for instance, during the international conference on primary health care of 1978, the Alma Ata Declaration built the historical basis of the official politics of the traditional medicine programme, thus opening a dialogue between two distinct systems of health care which are the traditional and the modern one. In contrast to western medicine which is technically and analytically based, traditional medicine is believed to take a holistic approach to good health as it perceives success and misfortune to be based on the actions of individuals and ancestral spirits according to the balance or imbalance between the individual and the social environment.

Like other species of *Acalypha*, *A. wilkesiana* Muell Arg has been reported to have medicinal properties for the treatment of malaria, dermatological and gastrointestinal disorders (Akinde, 1986; Akinyemi *et al*., 2006), antihypertension properties (Ikewuchi *et al.,* 2009), and for its antimicrobial activities (Adesina *et al.,* 1980; 2000; Kabir *et al.,* 2005; Ogundaini, 2005; Oladunmoye, 2006; Erute and Oyibo, 2008; Onocha and Olusanya, 2010; Akpomie and Olorungbon, 2011). *Acalypha wilkesiana* (Copperleaf, Jacob's coat, fire dragon) which belongs to the 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 (Al-Attar, 2010). The genus "*Acalypha*" comprises about 570 species. Agu (1980) and Adesina *et al.* (1980) 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 (1986) and Ogundaini (2005), 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. Adesina *et al.* (2000) 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 (Alade and Irobi, 1993). 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.

MATERIALS AND METHODS

Collection of Plant Materials

Matured fresh leaf of *A. wilkesiana* was collected by hand-plucking from parent plants spotted at different locations at the University of Ado-Ekiti, Nigeria. The leaf was identified in the herbarium unit of Plant Science Department of the same institution. The leaf was air-dried at room temperature for three weeks. The dried leaf was ground into fine powder and stored in air-tight plastic container till use.

Extraction Procedures

The extracts of *A. wilkesiana* were obtained using different solvent (methanol, ethanol, petroleum ether and distilled water). 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 70% ethanol (ethanolic extraction), 250ml of 100% methanol (methanolic 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 ethanolic, methanolic 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.

Source of Test Organisms

The test isolates were all clinical strains. Clinical human strains which are gastrointestinal tract pathogens were all locally isolated organisms (LIO) obtained from the stock culture collection of the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria. These include *Staphylococcus aureus* (LIO)*, Bacillus cereus* (LIO)*, Klebsiella pneumoniae* (LIO)*, Salmonella typhi* (LIO)*, Escherichia coli* (LIO) and *Shigella dysenteriae* (LIO). The bacteria causing skin infections in neonates were isolated during the study. Subjects sampled include neonates or infants between age zero and one year old with observable skin infections. Using sterile swab sticks, swabs of portion of skin with observable epidermal infections were collected from neonates at the immunization unit of the University of Ado-Ekiti Teaching Hospital, Ado-Ekiti, Nigeria. The swabs were cultured on nutrient agar plate, enriched and further plated out on appropriate selective media, Mannitol Salt Agar for isolation of *Staphylococcus aureus* and MacConkey agar for *S. pyogenes* and *K. aerogenes*. The entire agar used were products of Oxoid Ltd, Hants, United Kingdom.

The identity of isolates were confirmed using standard biochemical tests described by Olutiola *et al.* (1991) as *S. aureus, S. pyogenes* and *K. aerogenes*.

Phytochemical Analyses

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 (Sofowora, 1982).

Determination of Tannins

Five grams of each portion of plant extract was stirred with 100ml of distilled water, filtered and Ferric chloride reagent added to the filtrate. A blue-black green precipitate indicated the presence of tannins (Aiyelaagbe and Osamudiamen, 2009; Egwaikhide *et al.,* 2007)

Determination of Flavoniods

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 (Edeoga *et al.,* 2005).

Test for alkaloids

A 0.5g of extract 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. Meryer's reagent was added to one portion and Draggendorff's reagent to the other. The formation of a cream (with Meryer's reagent) or reddish brown precipitate (with Draggendorff's reagent) was taken as positive for the presence of alkaloid (Aiyelaagbe and Osamudiamen, 2009).

Test for steroids

About 0.1g of the extract 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 (Trease and Evans, 1989).

Test for flavonoids

2g of powdered sample was detanned 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 detanned 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 (Trease and Evans, 1989; Sofowora, 1983).

Determination of Cardiac Glycosides

This was carried out using Keller - Killani test as described by Trease and Evans (1978). About 0.5g of extract 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 (2009) and Egwaikhide *et al.* (2007). 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 (1993). Five milliliter of each extract was mixed with 2ml of chloroform and 3ml concentrated H_2SO_4 was added to form a layer. Formation of a reddish brown colouration at the interphase indicated the presence of terpenoids.

Antimicrobial Assays

Standardization of Inoculum

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% H_2SO_4 (0.36 Normal)}. The turbidity was adjusted by adding more sterile nutrient broth to match 0.5 McFarland standard (10^6cfu/ml).

Determination of Antimicrobial Activity

The antibacterial activities of the various extracts of the leaf were determined using agar diffusion technique (Chen *et al.*, 1997). Sterile solid Mueller Hilton agar (Himedia, Vadhani Ind. Est., LBB Marg Mumbal-400 086) plates were aseptically flooded with the standardized bacterial culture ($1.5x10^6$cfu/ml). Then, a sterile cork borer (6mm in diameter) was used to punch four holes along the sides and one in the centre of the Petri dish. Varied concentrations (10mg/ml, 30mg/ml, 60mg/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.

Preparation of various concentrations of extracts.

The extract was reconstituted in distilled water to obtain various concentrations of the extract. Two gramme of extract 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.

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 ($1.5x10^6$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.

Antibiotic Sensitivity Test

Antibiotic disk diffusion technique described by Bauer *et al.* (1966) and NCCLS (2003) was used for the determination of the susceptibility of the isolates to the reference standard antibiotics. About 2ml of standardized inoculums of the test bacteria was used to flood the surface of sensitivity test agar (Oxoid Laboratories, U.K.) plates and commercially prepared antibiotic discs were carefully and firmly placed on the seeded plates with the aid of sterile forceps. The commercially prepared antibiotics employed as positive control include; amoxicillin (30µl), ciprofloxacin (10µl), gentamicin (10µl) and erythromycin (10µl) The plates were then incubated at 37°C for 24h. The diameters of zones of inhibition produced were measured using a ruler calibrated in millimeter. Isolates were classified as either sensitive or resistant using the NCCLS (2003) Antibiotic disk Manual for interpreting zone diameter.

Statistical analysis

Data from three independent replicates on the antimicrobial activities of *A. wilkesiana* were subjected to one-way analysis of variance at $p<0.05$ level of significance for comparison of the extracts activities. The Statistical Package for Social Sciences (SPSS) version 10 was employed in the statistical analysis of the data.

RESULTS

All the leaf extracts except petroleum ether extract showed antimicrobial activity on the test organisms in varying degree. All the skin pathogens were susceptible to the aqueous, ethanolic, and methanolic extracts of the leaf at all extracts concentrations (10mg/ml, 30mg/ml, 60mg/ml) while the gastrointestinal tracts pathogens were susceptible to these extracts at the concentration of 20mg/ml and above (Table 1). However, all the leaf extracts did not produce any zone of growth inhibition on *B. cereus* (LIO). In all, the zones of growth inhibition increased with increasing concentration of the extracts. Ethanolic extract exhibited antibacterial activity on *S. pyogenes and K. aerogenes* with zones of growth inhibition varying from 1 to 4mm, while the methanolic extract produced zones of growth inhibition of 4mm each at 10mg/ml and 30mg/ml of the extract on *S. dysenteriae* (LIO).

Klebsiella aerogenes was susceptibile to the aqueous and ethanolic extracts of the leaf with zones of growth inhibition ranging between 1 to 5mm in aqueous extract and 1 to 4mm in ethanolic extract (Table 1). The aqueous, ethanolic, and methanolic extracts showed appreciable antibacterial activity on *S. aureus* pathogen from neonates' skin as the extracts all inhibited the growth of pathogen with considerable zones of growth inhibition varying from 8 to 15mm in aqueous extract, 12 to 16mm and 5 to 15mm in ethanolic and methanolic extracts respectively at the various concentrations.

Similarly, the inhibitory property of the leaf extracts on the gastrointestinal tract pathogens varied with the extract type and concentration. All were susceptible to the aqueous, ethanolic and methanolic extracts of the leaf at all concentrations with diameter of zones of growth inhibition ranging from 5 to 10mm with sensitivity increasing with concentrations. *Salmonella typhi* was susceptible to the extracts at 30mg/ml and above. Although the efficacy of the extracts increased with increasing extract concentration, the increase recorded was not significant with the gastrointestinal tract pathogens at the level of significance ($p<0.05$), However, the corresponding increase in concentration and inhibition zone was significant with the aqueous, ethanolic and methanolic extracts of the leaf on the skin pathogens at $p<0.05$.

The various solvents (distilled water, ethanol, methanol) used as negative control did not produce any zone of growth inhibition with the entire test organism.

Minimum inhibitory concentration (MIC) of the leaf extracts on all the test organisms ranged from 10 to 30µg/ml (Table 2). The minimum inhibitory concentration of aqueous, ethanolic, and methanolic extracts of *A. wilkesiana* on the skin pathogens was 10µg/ml, except ethanolic extract which had a MIC of 30µg/ml on *S. pyogenes.* The MIC of the leaf extracts on *E. coli, S. aureus, K. pneumoniae, S. typhi and B. cereus was* 20µg/ml, meanwhile aqueous and ethanolic extracts had MIC of 30µg/ml on *S. dysenteriae.*

The diameters of zones of inhibition recorded with the reference standard antibiotics which were used as positive control ranged from 12 to 18mm with amoxicillin, 10 to 17mm with gentamicin and 20 to 36mm with ciprofloxacin (Table 3)

Qualitative analyses of the phytochemicals present in the leaf extracts showed that it contained 12.85% saponin, 10.16% flavonoid, 7.14% tannin, and 0.6% phenols, while alkaloids steroid, phlobatanin, terpenoid were absent (Table 4).

Table 1. Antibacterial activities of Extracts of *Alcalypha wilkesiana* on gastrointestinal tract and skin pathogens

Concentration of extracts (mg/ml)
Diameter of zones of growth inhibition (mm)

Test organisms	Aqueous extract				Ethanolic extract				Methanolic extract				Petroleum ether extract			
	10	30	60	[a]Contr-ol	10	30	60	[b]Contr-ol	10	30	60	[c]Cont-rol	10	30	60	[d]Cont-rol
Staphylococcus aureus	8	13	15	0	12	13	16	0	5	10	15	0	0	0	0	0
Streptococcus pyogenes	5	8	10	0	0	2	4	0	2	5	10	0	0	0	0	0
Klebsiella aerogenes	1	3	5	0	1	2	4	0	5	10	13	0	0	0	0	0
*Escherichia coli (*ATCC2592)	0	6.3	10	0	0	6	7	0	0	5	6	0	0	0	0	0
Staphylococcus aureus (LIO)	0	6.5	10	0	0	6.4	10	0	0	6.6	10	0	0	0	0	0
Salmonella typhi (LIO)	0	6	9	0	0	6	10	0	0	5	10	0	0	0	0	0
Shigella dysenteriae (LIO)	0	6.6	8	0	0	6.4	8.3	0	0	4	4	0	0	0	0	0
Klebsiella pneumoniae (LIO)	0	6.4	8	0	0	5	8	0	0	3	6	0	0	0	0	0
Bacillus cereus (LIO)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

[a]Control: distilled water for the aqueous extract
[b]Control: ethanol for the ethanolic extract
[c]Control: methanol for the methanolic extract

Table 2: Minimum Inhibitory Concentration of *Acalypha wilkesiana* extracts

Test organisms	Aqueous Extract	Ethanolic Extract	Methanolic Extract	Petroleum ether extract
Staphylococcus aureus	10	10	10	ND
Steptococcus pyogenes	10	30	10	ND
Klebsiella aerogenes	10	10	10	ND
Escherichia coli (LIO)	20	20	20	ND
Staphylococcus aureus (LIO)	20	20	20	ND
Salmonella typhi (LIO)	30	30	20	ND
Shigella dysenteriae (LIO)	20	20	20	ND
Klebsiella pneumoniae (LIO)	20	30	20	ND
Bacillus cereus (LIO)	ND	ND	ND	ND

* ND = Not determined

Table 3. Zones of Inhibition of Standard Reference Antibiotics

Test organisms	Diameter of zones of growth inhibition (mm)			
	Amoxicillin(30µg)	Erythromycin(10µg)	Gentamycin(10 µg)	Ciprofloxacin(10 µg)
Staphylococcus aureus	R	15	17	20
Streptococcus pyogenes	R	R	15	23
Klebsiella aerogenes	R	R	17	22
Escherichia coli (LIO)	15	R	15	24
Staphylococcus aureus (LIO)	12	R	16	23
Salmonella typhi (LIO)	18	26	10	36
Shigella dysenteriae (LIO)	13	R	10	*20*
Klebsiella pneumoniae (LIO)	15	R	11	31
Bacillus cereus (LIO)	13	R	12	27

LIO: Locally isolated organism; R: Resistance

Table 4. Phytochemistry of *Acalypha wilkesiana*

Bioactive Constituent	% composition
Tannin	7.14
Saponin	12.85
Flavonioid	10.6
Cardiac glycosides	-
Phlobatannin	-
Phenol	0.6
Alkaloid	0.36
Steroid	-
Terpenoid	-

-: not present

DISCUSSION

All the leaf extracts except petroleum ether extract showed some degree of antibacterial activity against the test organisms, hence they possess inhibitory properties. The antibacterial activity exhibited by the aqueous, ethanolic, and methanolic extracts was comparable as they all showed considerable activity at all extracts concentrations. *Klebsiella aerogenes* appeared to show the least susceptibility to the aqueous and ethanolic extracts of the leaves as compared to the appreciable growth inhibition zones produced by the methanolic extract. The ethanolic extract was highly effective on *S. aureus* but had a low inhibition on *S. pyogenes and K. pnemoniae.* Petroleum ether extract had no inhibitory effect on any of the test organisms. This may be due to insolubility of the active ingredients of *A. wilkesiana* in petroleum ether solvent. The aqueous extract of the leaf showed an appreciable inhibitory effect on both *S. aureus* and *S. pyogenes* and the inhibition increased with increased extract concentrations.

The findings of the study agree with the earlier studies of other investigators (Adesina *et al.* 1980; 2000; Kabir *et al.,* 2005, Ogundaini, 2005;

Oladunmoye, 2006; Erute and Oyibo, 2008; Onocha and Olusanya, 2010; Gotep *et al.,* 2010, Akpomie and Olorungbon, 2011). Alade and Irobi (1993) reported that aqueous and ethanolic extracts of *A. wilkesiana* possess in-vitro antimicrobial activities against some microorganisms incriminated in the pathogenesis of human infection. Moreover, the study corroborates with the work of Jekayinfa *et al.* (1997) who demonstrated *in-vitro* antimicrobial effect of water extract of *A. wilkesiana o*n dermatitis. On the contrary, Gotep *et al.* (2010) and Akpomie and Olorungbon (2011) recorded higher zones of growth inhibition with *K. aerogenes* and *S. typhi* as against the low inhibition observed in this present study with these organisms. The variation observed may be due to environmental factors which may include climatic conditions; geographical locations, extraction techniques and solvents. Previous studies (Alade and Irobi, 1993; Iwu, 1986) have demonstrated the antimicrobial activities of the constituent of some flowering plants. These studies however elucidate the major problem with this type of research, namely the lack of uniformity in the criteria selected to study the activity. This has in the past led to relevant contradictions between the results obtained by different researchers on the same type of plant. Authors study the same sample with different methods. To try to solve this problem, Rios *et al.* (1998) published a review of the experimental methods used for studying the activity of both plant extracts and essential oils to date. They proposed the use of diffusion methods for studying polar compounds of small or medium molecular sizes and for determining the antimicrobial spectrum because this method allows researchers to test different compounds against one microorganism. The solid dilution method was recommended for studying polar and non-polar substances as well as all types of complex extracts (Rios *et al.* 1998). This method is especially good for determining the relative potency of extracts or essential oils and for establishing their antimicrobial spectrum as it facilitates the use of different strains against the extracts on the same plate (Rios *et al.* 1998). Finally, liquid dilution method is the best way to establish the real potency of a pure compound, but solubility is an obvious requisite (Rios *et al.* 1998).

The MIC of the leaf extracts ranged from 10 to 30μg/ml, indicating its efficacy and potency on the test organisms thus suggesting that the extracts may act as bactericidal agents to these microorganisms. The diameters of zones of inhibition recorded with the reference standard antibiotics were comparable to those observed with the various extracts of the leaf, though the antibiotics showed better inhibitory property ($p<0.05$) on the test isolates than the leaf extract (Table 3). The wider zones of growth inhibition recorded with some reference antibiotics against the test organisms as compared to the leaf extracts may be ostensibly due to the chemical synthesis of the pure compounds of the antibiotics used (Table 2). It is worth knowing that all the test organisms except *S. aureus* and *S. typhi* developed resistance to erythromycin while *S. aureus*, *Streptococcus pyogenes* and *Klebsiella aerogenes* were resistant to amoxicillin as seen in Table 3, whereas these organisms were apparently susceptible to the aqueous, ethanolic and methanolic extracts of the leaf. This indicates that the extracts of the leaf are as effective as the commercially prepared antibiotics thus confirming its potency in the treatment of infections particularly gastroenteritis and skin dermatitis. Moreover, since the leaf extracts exhibited inhibitory activity on the test organisms, the antibacterial activity appears to be broad spectrum. Al-Attar (2010) investigated the protective effect of *A. wilkesiana* leaf on lowering the levels of serum glucose, triglycerides and cholesterol levels and its ameliorative influences on serum Sodium (Na), Potassium (K) and Calcium (Ca) disturbances in STZ-induced diabetic mice. He reported that *A. wilkesiana* represents an effective antihyperglycemic, antihyperlipidemic adjunct and ameliorative role on electrolytes disturbances for the treatment of diabetes and a potential source of discovery of new therapeutic agent.

The bioactive compounds responsible for the inhibitory effects of the leaf extracts were detected in its phytochemical screening, some of which were reported in literature as antimicrobial constituents. The qualitative and quantitative analysis of the leaf of *A. wilkesiana* revealed that it contains flavonoid, saponin and tannin in varying proportions with traces of cardiac glycosides and phenol, while phlobatannin, alkaloid, steroid and terpenoid were absent. The antimicrobial activities observed in this study may be attributed to the presence of these phytochemicals in the leaf (Table 4). Plants that are rich in a wide variety of secondary metabolites have been found In-Vitro to have antimicrobial properties. The previous studies on *A. wilkesiana, A. hispida* and *A. communis* identified gallic acid, corilagin, geraniin

and triterpenoids of cycloartane-type, flavonoids like quercetin- and kaempferol-derivatives as compounds responsible for the observed antimicrobial activity (Adesina *et al.*, 2000; Gutierrez-Lugo *et al.,* 2002).

CONCLUSION

The study has further revealed that the leaf of *A. wilkesiana* possesses antimicrobial activity. Owing to its abundance and demonstrated effectiveness, it is therefore recommended that medicinal plants like *A. wilkesiana* be used as cheap and readily available sources of skin medication and therapy for gastrointestinal disorder in developing settings in Africa. Further work on the isolation and characterization of the active ingredients in the crude extract of *A. wilkesiana* is however advocated.

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Ife Journal of Science
Table of Contents: December 2011 Edition; Vol. 13, No. 2

Authors	Title	Page
Jegede, O.I. and Fawole, O.O. ,	Fecundity and Egg Size Variation in *Tilapia Zillii* (gervais) and *Tilapia Mariae* (boulenger) from Lekki Lagoon, Nigeria.	219
Bayowa, O.G., Ilufoye, D.T. and Animasaun, A.R. .	Geoelectric Investigation of Awba Earth Dam Embankment, University of Ibadan, Ibadan, Southwestern Nigeria, for Anomalous Seepages.	227
Adedeji A.A., Aduwo A. I., Aluko O. A. and Awotokun F.	Effect of Chicken Droppings as Organic Fertilizer on Water Quality and Planktonic Production in an Artificial Culture Media	239
Aborisade, Abiola T and Adebo, Cosmas T	Effect of Curing on the Shelf Life of Ambersweet Oranges (*citrus Sinensis* Osbeck) Stored at Ambient Tropical Condition	251
Ogungbesan G.O. and Akaegbobi I.M.	Petrography and Geochemistry of Turonian Eze-aku Sandstone Ridges, Lower Benue Trough, Nigeria Implication for Provenance and Tectonic Settings.	263
Ayinde F.O. and Asubiojo O.I.	Micellar Catalysis of the Hydrolysis of Aryltrifluoroacetates	279
Eze, U.N., Okonji, R.E., Ibraheem, O. and Shonukan, O.O.	Isolation And Characterization Of A Bacterial Thermostable Protease From Poultry Dung.	289
Badejo M.A, Owojori O.J., and Akinwole P.O.	A Survey of the Population of the African Weaver Ant, *Oecophylla Longinoda* (hymenoptera:formicidae) in Contrasting Habitats in Ile-Ife, South-Western Nigeria.	299
S. A. Opeloye	Depositional Environment of Lamja Sandstone in the Upper Benue Trough, Northeastern Nigeria	309
Okunlola, A.O., Akinola, O.O. and Olorunfemi, A.O.	Petrochemical Characteristics and Industrial Features of Talcose Rock in Ijero-Ekiti Area, Southwestern Nigeria.	317
Adekoya, J.A., Aluko, A.F. and Opeloye, S.A.	Sedimentological Characteristics of Ajali Sandstone in the Benin Flank of Anambra Basin, Nigeria	327
Jimoh, M.A., Saheed, S.A. and Botha, C.E.J.	Response of Barley Cultivars to Infestations of the Two South African Biotypes of the Russian Wheat Aphid	339
Omafuvbe, B.O. , Feruke-Bello, Y.M. and Adeleke, E.O.	Aerobic Mesophilic Bacteria Associated with Irish Potato (*solanum Tuberosum* L.) Spoilage and their Susceptibility Pattern to Lactic Acid Bacteria and Antibiotics.	347
Oluyemi, E.A. and Olabanji, I.O.	Heavy Metals Determination in Some Species of Frozen Fish Sold at Ile-Ife Main Market, South West Nigeria	355
Oketayo, O.O. and Ojo, J.O.	Anthropometric Predictive Equations for Percentage Body Fat in Nigerian Women Using Bioelectrical Impedance as Reference	363
Oluduro A. O., Bakare M. K., Omoboye O. O., Dada C.A. and Olatunji C. I.	Antibacterial Effect of Extracts of *Acalypha Wilkesiana* on Gastrointestinal Tract Pathogens and Bacteria Causing Skin Infections in Neonates.	371
Adeleke, E.O., Omafuvbe, B.O., Adewale, O.I. and Bakare, M.K.	Screening and Isolation of Thermophilic Cellulolytic Bacteria from Cocoa Pod and Cassava Peel Dumpsites in Ile-Ife, Southwest Nigeria	381
Akinola, A. P. Borokinni, A. S. Fadodun, O. O. Olokuntoye, B. A.	Finite Element Modeling of Deformation in a Composite Bar	389