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Comparative Study of Phytochemical Constituents and Antimicrobial Activities of *Acalypha wilkesiana* and *Acalypha godseffiana* Extracts

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Authors' contributions

This work was carried out in collaboration between all authors. Author AOA designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors KMD and OJO managed the analyses of the study. Author OCA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of the investigation was to evaluate and compare the phytoconstituents and antimicrobial activities of leaf extracts of two species of *Acalypha* (*A. wilkesiana* and *A. godseffiana*).
Methodology: The methods employed include manual pulverization of the air-dried leaves and solvent (ethanol) percolation for 72 hrs. The crude extracts were kept in sterile Mc Cartney bottles and stored in the refrigerator 4±2℃. Thereafter, th ey were screened for phytochemical components. Moreover, they were investigated for antimicrobial activities against *Bacillus subtilis, Escherichia coli, Enterococcus faecalis, Klebsiella pneumoniae, Streptococcus pneumoniae, Staphylococcus aureus, Aspergillus flavus, Candida albicans,* and *Cryptococcus neoformans.*
Results: From the results obtained during the study, flavonoid, saponin, phobatanin, tannin and alkaloid were found in both *A. wilkesiana* and *A. godseffiana*, steroid was found only in

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A. wilkesiana while terpenoid and anthraquinones were not detected in the two species. The quantitative screening showed that *A. wilkesiana* extract possess more of flavonoid (7.32 µg/g), tannin (4.21 µg/g), alkaloid (4.19 µg/g) and steroids (1.18 µg/g) compared to the extract of *A. godseffiana,* whereas, saponin (5.23 µg/g) and phobatanin (4.32 µg/g) were found to be present in higher quantities in *A. godseffiana* than the extract of *A. wilkesiana.* The antimicrobial activity assay revealed that at the highest concentration used (50 mg/ml) the zones of inhibition ranged from 9.00 ± 0.00^{b} mm against *A. fumigatus* to 16.00 ± 0.00^{d} mm against *S. aureus*. Moreover, there was a significant difference between the susceptibility of Gram positive bacteria and Gram negative bacteria. The extract of *A. godseffiana* was more active against the selected pathogens compared to the extract from *A. wilkesiana.*

Conclusion: The results obtained suggest that both species of *Acalypha* possess comparable phytochemicals however, *A. godseffiana* was more potent as antimicrobial agent than *A. wilkesiana* this support their folkloric use as remedy for several disease conditions and new antimicrobial agents may be developed from them.

Keywords: Phytochemical; antimicrobial; Acalypha; wilkesiana; godseffiana.

1. INTRODUCTION

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 [1]. A medicinal plant is any plant which one or more of its organs contain substances that can be used for therapeutic purposes or serve as precursor for the synthesis of useful drugs. Medicinal plants contain biologically active chemical substances (phytochemicals) such as saponins, tannins, essential oils, flavonoids, alkaloids, and other compounds which have preventive or curative properties. These complex chemical substances generally occur as secondary plant metabolites in these plants and are useful to humanity [2]. Higher and aromatic plants have traditionally been used in folk medicine as well as in the extension of the shelf life of foods in the case of those with antimicrobial activity [1].

All over the world, hundreds of plants have been identified as good sources of medicinal agents and are used in traditional medicine for different purposes, including the treatment of bacterial and fungal infections [3]. Ethnopharmacological uses of plants feature strongly among Nigerian peoples. It has been pointed out [4], that plants continue to play a prominent role in primary health-care of about 80% of the world's population. Over the years, there have been alarming reports of multiple drug resistance in medically important strains of bacteria and fungi [5].

The persistent increase in the incidence of such antibiotic resistant strains of organisms have led to the development of more potent antibiotic such as the 3rd and 4th generation cephalosporins by pharmaceutical companies [6]. It has long been recognized that some plant materials exhibit antimicrobial properties. The use of these plant materials as preservatives capable of preventing the development of microorganisms in foods has become the subject of extensive studies [7]. In particular, the inhibitory effects of extracts of many kinds of herbs and spices against food-borne bacteria and other pathogens have been reported; among these are cassia, clove, garlic, sage and thyme [8].

Many countries have maintained research programmes to screen traditional medical preparations for their efficacy, India and America are good examples [9]. Medicines by local populations include the treatment of several tropical diseases including schistosomiasis, leishmaniasis, malarial, fungal and bacterial infection [1]. However, despite the abundance of such flora, analytical data are available only from a few plants-both native and exotic species. There is thus a glaring need to fill the scientific gap created by the lack of data on many Nigerian proven medicinal plants. According to World Health Organization, herbal medicine services the health needs of about 80% of the world's population, especially in developing countries like Nigeria [9].

Like other species of *Acalypha*, *A. wilkesiana* and *A. godseffiana* Müll. Arg has been reported to have medicinal properties for the treatment of malaria, dermatological and gastrointestinal disorders respectively, antihypertension

properties, and for its antimicrobial activities [10]. *Acalypha wilkesiana* and *A. godseffiana* (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.

According to [11], the expressed juice or boiled concoction of these plants are used for the treatment of gastrointestinal disorders and fungal skin infections such as *Pityriasis versicolor, Impetigo ontagiosa, Candida intetrigo, Tinea versicolor, Tinea corporis, and Tinea pedis*. 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 [12]. 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. It is widely used in southern Nigeria as a remedy for the treatment of undefined skin infections in children [10,13,14]. The present study is designed to compare the phytochemical content and antimicrobial activities of the two species of *Acalypha*.

2. MATERIALS AND METHODS

2.1 Collection of Plant Samples

Matured fresh leaf of *A. wilkesiana* and *A. godseffiana* was collected by hand-plucking from parent plants at different locations within Rufus Giwa Polytechnic, Owo, Ondo State, Nigeria and were authenticated at the Environmental Biology unit of Science Laboratory Department of the same institution and voucher specimen (AWL2619-*A. wilkesiana*, AWL2620-*A. godseffiana*) was deposited at the Department of Forestry Resources Technology, Rufus Giwa Polytechnic, Owo, Ondo State before they were transferred to the laboratory for analysis.

2.2 Source of Test Organisms

The microorganisms employed in the study were clinical isolates collected from the Federal Medical Center, Owo. They were *Bacillus subtilis, Escherichia coli, Enterococcus faecalis, Klebsiella pneumoniae, Streptococcus pneumoniae, Staphylococcus aureus, Aspergillus flavus, Candida albicans,* and *Cryptococcus neoformans.*

2.3 Reagents/Chemicals

All reagents and chemicals used were of analytical grade and were obtained from the Department of Microbiology, Federal University of Technology, Akure, Ondo State, Nigeria.

2.4 Experimental Design

2.4.1 Preparation of samples

The freshly collected leaf of *A. wilkesiana* and *A. godseffiana* were thoroughly washed with tap water followed by distilled water. The pulp were separated and air-dried to avoid possible break down of the active components and grinded using a mechanical grinder and then passed through a sieve. The resultant powder was packed and labeled accordingly.

2.4.2 Extraction of samples

The powdered samples were extracted with organic solvent (ethanol). Twenty-five gram of the powdered sample was steeped in 100 ml of the solvent (25% w/v) for 72 hours after which the mixture was filtered by using Whatman No 1 filter paper. The filtrate was left to air dry. The obtained dried crude solvent extracts of the samples were kept in an air tight container to avoid contamination [15]. They were later reconstituted in 0.01% Tween-20 prior to use.

2.4.3 Phytochemical screening

The extracts of the different plant parts were subjected to qualitative and quantitative phytochemical analysis for the presence of tannins, saponins, flavonoids, alkaloids, anthraquinones, phobatanin, steroid and terpenoid were carried out on the extracts using standard procedures as described by [16,15].

2.4.4 Evaluation of antimicrobial activity

The agar well diffusion method was used. One milliliter of standardized inoculum (1.5×10^8 cfu/ml) of each bacterium was separately added to 20 ml of sterile molten nutrient agar in sterile Petri-dishes. The contents were mixed and allowed to solidify. Wells of 5mm diameter were bored using a sterilized cork borer; thereafter 0.1 ml of the extracts at varying concentrations prepared by diluting 0.10 g, 0.20 g, 0.30 g, 0.40 g

and 0.50 g of the extracts in 100 ml of 0.01% Tween-20 to obtain concentrations of 10 mg/ml, 20 mg/ml, 30 mg/ml, 40 mg/ml and 50 mg/ml respectively as well as 0.1 ml of antibiotic (chloramphenicol, 100 g/ml for bacteria and myconazole, 100 µg/ml for fungi) which served as controls were separately dispensed into individual wells. The plates were allowed to stand at room temperature for 10 minutes to allow for the diffusion of the extracts into the agar [17]. The plates were then incubated at 37℃ for 24 hours for bacteria and yeasts, moulds were incubated at 25℃ for 72 hrs. After incubation, the diameters of the zones of inhibition around the wells were measured in mm [15].

2.5 Statistical Analysis

Unless otherwise indicated, results are expressed as means ± SD of three replicates. Data were subjected to one–way analysis of variance (ANOVA) using SPSS version 15.0. The Duncan's Multiple Range test was used to separate the means at the 5% level of probability.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Constituents of two *Acalypha* Species

The result of the quantitative phytochemical screening of the two species of *Acalypha* is presented in Table 1 where flavonoid, saponin, phobatanin, tannin and alkaloid were found in both *A. wilkesiana* and *A. goddsefiana*, Steroid was found only in *A. wilkesiana* while Terpenoid and anthraquinones were not detected in the two species of *Acalypha* screened. Earlier report of differences in the phytochemical has been reported by [18] who carried out a phytochemical and morphometric Analysis of the Genus *Acalypha* Linn. (Euphorbiaceae).

The quantitative phytochemical composition analyses of the two *Acalypha* species (powdered sample) revealed that *A. wilkesiana* extract possess more of flavonoid (7.32 µg/g), tannin (4.21 µg/g), alkaloid (4.19 µg/g) and steroids (1.18 µg/g) compared to the extract of *A. godseffiana,* whereas, saponin (5.23 µg/g) and phobatanin (4.32 µg/g) were found to be present in higher quantities in *A. godseffiana* than the extract of *A. wilkessiana* (Fig. 1). This has been observed earlier by [18,19] and may be due to differences in their species chemical constituents.

Table 1. Qualitative phytochemical composition of two *Acalypha* species

Phytochemical	*A. wilkesiana*	*A. godseffiana*
Flavonoid	+++	++
Saponin	++	++
Phobatanin	++	++
Tannin	++	+
Alkaloid	++	++
Terpenoid	-	-
Steroid	+	-
Anthraquinones	-	-

Key: +++ = Present in abundance, ++ = Present in moderate amount, + = Present in trace amount, - = Not detected

The presence of various secondary metabolites in the plants' extract justify their medicinal use. Some of these compounds are known for their large spectrum of pharmacological properties, which include antimicrobial (alkaloids and saponins) and antioxidant (tannins) activities [20].

3.2 Antimicrobial Activities of two *Acalypha* Species

The antimicrobial activities of the leaf extract of *A. wilkesiana* and *A. godseffiana* against the test human pathogens is presented in Tables 2 and 3. It is shown from the tables that the activity of the extracts varied with concentration gradient as the zones of inhibition (mm) increases with increase in concentration of the plant extracts.

It can also be deduced from the tables that the Gram positive bacteria (*B. subtilis, S. aureus* and *S. pneumoniae*) were more susceptible to the extracts than the Gram negative bacteria (*E. coli* and *K. pneumoniae*). This has been reported earlier by [21] may be attributed to the differences in their cell wall make ups, earlier observation has been made in the susceptibility of Gram positive and Gram negative bacteria to antibacterial agents [22].

Furthermore, it was observed that the pathogenic yeasts were more susceptible to the extracts than the mould. This may be due to the differences in their morphology and physiological properties including mode of feeding. This has been reported earlier by [23].

Table 2. Antimicrobial activity of ethanol extract of *Acalypha wikesiana* leaf on selected pathogens

Organisms	10 mg/ml	20 mg/ml	30 mg/ml	40 mg/ml	50 mg/ml	Chl(100 µg/ml)	Myz(100 µg/ml)
	Zones of inhibition (mm)						
B. S	6.00±0.00^{a}	8.33±0.58^{b}	11.00±0.00^{c}	13.00±0.00^{d}	13.00±0.00^{d}	11.00±0.00^{c}	N.A
S. A	7.00±0.00^{a}	9.67±0.58^{b}	12.67±0.58^{c}	14.67±0.58^{d}	16.00±0.00^{d}	18.00±0.00^{e}	N.A
S. P	7.33±0.67^{a}	10.67±0.33^{b}	13.67±0.33^{b}	15.33±0.33^{cd}	15.67±1.20^{d}	20.33±0.88^{d}	N.A
E.F	NI	6.00±0.00^{a}	8.67±0.58^{b}	11.33±0.58^{c}	12.67±0.58^{d}	12.67±1.15^{d}	N.A
E. C	NI	NI	7.67±0.58^{a}	10.33±0.58^{b}	11.00±0.00^{b}	14.33±0.58^{c}	N.A
K. P	NI	NI	8.33±0.33^{a}	10.67±0.33^{b}	11.67±0.33^{c}	16.33±0.33^{d}	N.A
A. *F*	NI	NI	7.33±0.58^{a}	9.00±0.00^{b}	9.00±0.00^{b}	N.A	14.33±0.58^{c}
C. A	6.33±0.58^{a}	9.00±0.00^{b}	11.67±0.58^{c}	13.67±0.58^{d}	14.67±0.58^{d}	N.A	12.00±0.00^{c}
C. N	3.67±0.58^{a}	6.67±0.58^{b}	8.33±0.58^{c}	10.33±0.58^{d}	11.67±0.58^{e}	N.A	16.33±0.58^{f}

Values are Mean±S.E.M (mm), Values followed by different alphabet along the rows are significantly different at p≤0.05, NI = No inhibition, N.A = Not applicable, Chl = Chloramphenicol, Myz = Miconazole, B.S = Bacillus subtilis, S.A = Staphylococcus aureus, E.C = Escherichia coli, E.F = Enterococcus faecalis, K.P = Klebsiella pneumoniae, A.F = Aspergillus flavus, C.A = Candida albicans, C.N = Cryptococcus neoformans, S.P = Streptococcus pneumoniae

Table 3. Antimicrobial activity of ethanol extract of *Acalypha godseffiana* leaf on selected pathogens

Organisms	10 mg/ml	20 mg/ml	30 mg/ml	40 mg/ml	50 mg/ml	Chl(100 µg/ml)	Myz(100 µg/ml)
	Zones of inhibition (mm)						
B. S	NI	7.67±0.58^{a}	10.67±0.58^{b}	13.67±0.58^{c}	15.33±0.58^{d}	11.00±0.00^{b}	N.A
S. A	5.67±0.58^{a}	9.00±0.00^{b}	11.00±0.00^{c}	12.67±0.58^{d}	15.00±0.00^{e}	20.00±0.00^{f}	N.A
S. P	4.33±0.58^{a}	7.33±0.58^{b}	10.67±0.58^{c}	13.33±0.58^{d}	15.00±0.00^{e}	11.00±0.00^{c}	N.A
E. F	NI	6.33±0.58^{a}	9.33±0.58^{b}	12.67±0.58^{c}	14.33±0.58^{d}	14.00±0.00^{d}	N.A
E. C	NI	7.33±0.33^{a}	9.67±0.67^{b}	13.67±0.33^{c}	14.67±0.33^{c}	16.00±0.58^{d}	N.A
K. P	NI	5.33±0.58^{a}	8.33±0.58^{b}	11.33±0.58^{c}	13.67±0.58^{d}	12.00±0.00^{c}	N.A
A. F	3.67±0.58^{a}	6.33±0.58^{b}	11.67±0.58^{c}	12.00±0.00^{c}	12.00±0.00^{c}	N.A	17.33±0.58^{d}
C. A	7.67±0.58^{a}	11.33±0.58^{b}	13.67±0.58^{c}	15.67±0.58^{d}	16.00±0.00^{d}	N.A	12.00±0.00^{bc}
C. N	6.67±0.58^{a}	9.67±0.58^{b}	12.67±0.58^{c}	15.67±0.58^{d}	16.00±0.00^{d}	N.A	10.00±0.00^{b}

Values are Mean±S.E.M (mm), Values followed by different alphabet along the rows are significantly different at p≤0.05, NI = No inhibition, N.A = Not applicable, Chl = Chloramphenicol, Myz = Miconazole, B.S = Bacillus subtilis, S.A = Staphylococcus aureus, E.C = Escherichia coli, E.F = Enterococcus faecalis, K.P = Klebsiella pneumoniae, A.F = Aspergillus flavus, C.A = Candida albicans, C.N = Cryptococcus neoformans, S.P = Streptococcus pneumoniae

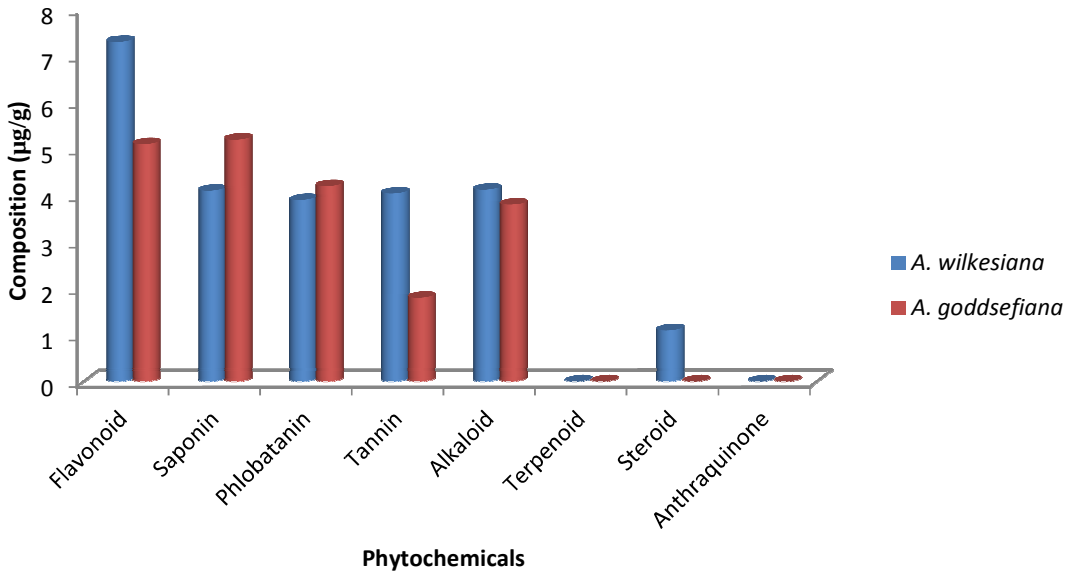


Fig. 1. Quantitative phytochemical composition of two species of *Acalypha* (µg/g of powdered sample)

Comparatively, the selected pathogens used in this study were more susceptible to the extract of *A. godseffiana* than that of *A. wilkesiana* as shown by the higher zones of inhibition on the pathogens. However, there was no significant differences ($p \leq 0.05$) between the zones of inhibition recorded for the bacteria against the extracts. However, the antifungal activities of *A. godseffiana* was significantly ($p \leq 0.05$) higher than that of A. wilkessiana. This may be due to the higher proportion of saponin in *A. godseffiana* than in *A. wilkesiana*. The antifungal activity of the plant extracts against the susceptible pathogens may be due to the presence of the identified metabolites in the plant which may be responsible for the potency of the extracts in tradomedicine [24].

4. CONCLUSION

From the foregoing, both species of *Acalypha* investigated in this work possess comparable type and quantities of phytochemicals however, *A. godseffiana* was more potent as antimicrobial agent than *A. wilkesiana* and this support their folkloric use as remedy for several disease conditions and new antimicrobial agents may be developed from them especially *A. godseffiana* which showed promising antimicrobial potency.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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