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Chemical Composition and Antimicrobial Activities of Essential Oil from the Leaves of *Acalypha wilkesiana* on Pathogenic Microorganisms

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ABSTRACT

Acalypha wilkesiana is a tropical plant used to treat a wide range of medical conditions. The leaves of *A. wilkesiana* were tested for phytochemicals with microbicide activity against a variety of microorganisms known to cause severe human infections. Antimicrobial efficacy of essential oil extracts from pulverised dried leaves of *A. wilkesiana* was examined. Phytochemical and proximate assessments of the leaves were carried out using standard techniques. The essential oil was extracted using hydro-distillation and collected using two processes. The first form was extraction for four hours straight, and the second mode involve hourly collection for four times. A total of five fractions of the essential oil were extracted. The chemical constituent of the oil was separated with Gas chromatography–mass spectrometry (GC-MS) in accordance with standard procedures. The proximate composition of leaves showed low moisture and high carbohydrate (61.39%). Tannins, flavonoids, and alkaloids were among the secondary metabolites found in the leaves. Six bioactive compounds including n-Hexadecanoic acid and 6-Benzamido-4-benzoyl-1, 2, 4-triazine-3, 5 were identified in the GC–MS analysis. The oils from *A. wilkesiana* leaves have excellent broad-spectrum antimicrobial activity at various concentrations on *Staphylococcus* species, *Bacillus coagulans*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhimurium* and *Candida albicans*. It could not, however, inhibit the growth of *Pseudomonas aeruginosa* and *Gardnerella vaginalis*. The findings justify the usage of *A. wilkesiana* leaves in ethnomedicine for possible management of skin conditions, gastrointestinal problems as well as other related microbial infections.

INTRODUCTION

Globally, plants are used primarily in health care, either in crude or refined forms (Cordell, 2009; Sofowora *et al*., 2013). They have contributed significantly to making the earth habitable for all living things, most especially man. Due to their ability to convert solar energy into metabolites, which are then turned into food and pharmaceutics for animal and human use; and have also become a valuable source of income and medications. Principally, plants contain bioactive substances such as flavonoids, alkaloids, tannins and other chemical compounds that have medicinal properties.

These plant-derived chemicals have prompted a shift in medical practices from restorative to preventative approaches, particularly as pathogens become more resistant to synthetic drugs.

Phytochemicals such as alkaloids and tannins have been extensively employed in the management of serious microbial infections. *Vernonia amygdalina* (bitter leaves), for example, has been shown in animal studies to have the ability to suppress the occurrence of diarrhea (Gudeta *et al.*, 2020) and high antibacterial properties against clinical isolates of *Enterobacter aerogenes, Escherichia coli* and *Salmonella typhi*, (Ohwofasa and Pondei, 2018*).* Several studies have also shown that essential oils derived from a variety of plants could be interesting alternatives for the treatment of infectious diseases. Essential oils are volatile and aromatic compounds which may be obtained from various plant parts, particularly leaves, flowers and seeds. Lemon grass (*Cymbopogon citratus*) essential oils have been discovered to have antibiofilm and antibacterial properties against *Candida* species, *Staphylococcus aureus*, and *Klebsiella pneumoniae* (Naik *et al*., 2018; Gao *et al*., 2020). Ghavam and colleagues reported that essential oils derived from *Salvia hydrangea* flowers and leaves are effective in treating fungal and bacterial infections. (Ghavam *et al*., 2020).

The plants of the *Acalypha* genus belong to the family Euphorbiaceae (Elkhouly *et al*., 2017) and are usually cultivated as house plants because of their attractive colours. In addition, they are traditionally known to have remarkable medicinal values and nearly every component of the plant, such as the stems, roots, and leaves, is used as a therapeutic agent to treat and control a variety of diseases owing to their broad phytochemical constituents (Seebaluck *et al.*, 2015; Asekunowo *et al.,* 2019). *Acalypha hispida* and *A. indica*, for instance, possess interesting pharmacological active constituents such as proteins, crude fats, cyanogenic glucosides, acalyphine, triacetoneamine as well as alkaloids with therapeutic potentials and as such could be used in ethnomedicine to treat bacterial infections and other diseases.

The screening of phyto-constituents of the leaves of *A. wilkesiana* (copper leaf) revealed the presence of flavonoid, carotenoids and alkaloids. These substances are thought to exert a significant role in managing the risk factors associated with cardiac infections. Likewise, the boiled decoction of the leaves has been suggested for the management of intestinal diseases, dermatitis caused by fungi including *Tinea pedis*, *Tinea corporis, Candida intetrigo* and *Pityriasis versicolar* (Ogundaini, 2005) and diabetes. Whilst most studies have focussed on the aqueous and methanolic extracts of *A. wilkesiana* leaves (Omage *et al*., 2018; Okoye and Amadi, 2019; Osibote *et al*., 2020), research on the potential benefits of its essential oil has been very limited. As a result, the current study is aimed at evaluating the antimicrobial activities of essential oil extracts from *A. wilkesiana* on pathogenic organisms and to identify potential bioactive constituents.

MATERIALS AND METHODS

Plant Materials:

Fresh *A. wilkesiana* leaves were collected from a facility within the premises of University of Lagos in Nigeria. The plant was classified as well as verified at the Department of Botany of the University (Voucher No: LUH 6497) (Osibote *et al*., 2020). The leaves were air dried before processing into rough powdery texture.

Test Microorganisms:

Eleven strains of microorganisms which were maintained in glycerol-peptone broth at 4^oC were obtained from the bacteriology laboratory of the Microbiology Department, Faculty of Science of the University. The isolates comprised *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, Methicillin Resistant *Staphylococcus aureus* ATCC 700699, *Bacillus coagulans* UL 001 and *Bacillus substilis* UL 002, *Escherichia* *coli* ATCC 35218, *Salmonella typhimurium* ATCC 13311, *Klebsiella pneumoniae* ATCC 8308, *Pseudomonas aeruginosa* ATCC 15442, *Gardnerella vaginalis* ATCC 14018 and *Candida albicans* ATCC 10231. They were grown on appropriate culture media to obtain pure culture.

Proximate and Phytochemical Composition of *Acalypha wilkesina* Leaves:

While preliminary evidence of the proximate and phytochemical findings was given in a previous article (Osibote *et al*., 2020). The total carbohydrate, fiber, fat, crude protein and moisture content in addition to the fat and ash content of *A. wilkesiana* leaves were determined in triplicate using standard methods (AOAC, 2006; Iniaghe *et al*., 2009). Besides, the nutritional contents of the leaves of three different species of *Acalypha* (*A. indica*, *A. marginata* and *A. hispida*) were also determined. The phytochemicals of *A. wilkesiana* were identified using conventional procedures (Sofowora, 2008; Osibote *et al*., 2020).

Extraction of Essential Oil:

One hundred (100) gram of the powdered leaves was mixed with 3 L of distilled water and hydro-distillation was used to recover the essential oil, which was collected in hexane (Ogunlesi *et al*., 2010). The extraction was carried out in twofold: one for four hours at a time, with the extract retrieved every four hours, and another for four hours at a time, with the extract obtained every hour. Evaporation at room temperature separated the hexane from the essential oil and any remaining water in the extract was removed using anhydrous sodium sulphate.

Essential Oil Antimicrobial Activity:

The antibacterial and antifungal activities of the essential oil from the leaves of *A. wilkesiana* was analyzed using agar well diffusion method as previously described (Ochei and Kolharkar, 2008; Osibote *et al.,* 2009). Using a colorimeter set to 540 nm, a suspension of each organism was prepared and calibrated to a concentration equivalent to 0.5 McFarland standards. 100µl the suspension was introduced into the sterile Muller Hinton agar in duplicate and spread evenly with sterile glass rod. The essential oils were poured into the wells drilled into the Mueller Hinton using an 8 mm cork borer. The inoculated plates were left standing for some minutes to allow the extracts to thoroughly dissipate. After 24 hours of incubation at 37^{o}C, the inoculated plates were examined for inhibition zones. For the bacterial organisms, imipenem (10µg) was used for quality control to determine the effectiveness of the oil against the isolates. The percentage sensitivity was calculated as follows:

$$\text{Percentage sensitivity (\%)} = \frac{\text{Diameter of zone of inhibition of extracts}}{\text{Diameter of zone of inhibition of the standard antibiotic disc}} \times 100$$

Gas chromatography- mass spectrometry (GC-MS) Analysis:

Profiling of the components of the essential oil was conducted as described previously Osibote *et al.*, 2009; Osibote *et al.*, 2020). The MS library identified the observed molecular ions (NIST MS search 2.0).

Minimum Inhibition Concentration (MIC):

Using the agar well dilution method, the minimum inhibitory concentration (MIC) of essential oil of *A. wilkesiana* was determined. Different concentrations of the essential oils were added to sterile Mueller Hintion agar plates inoculated with test organisms (Cheesbrough, 2013). The stock solution of the essential oil (extract E) was dissolved in hexane at varying concentrations ranging from 50 to 250 mg/ml. 100µl of each concentration was applied to the inoculated plates and incubated for 24 hrs. at 37^{o}C. Hexane was used as negative control. After an overnight incubation, the plates were examined for the lowest concentration that inhibited observable growth of the microorganisms, establishing the MIC.

Data Analysis:

The results of the phytochemical and proximate measurements were presented as the mean standard deviation. Microsoft Excel spreadsheet (version 2010) was used to create graphical representations of the results.

RESULTS

Phytochemical and Proximate Composition:

The basic secondary metabolites discovered were tannins, cardiac glycosides, anthraquinones, flavonoids, saponins and alkaloids. These are shown in Table 1. The results of the proximate study revealed that *A. wilkesiana* leaves possessed high carbohydrate (61.39%) and fiber (17.00%) but low moisture content (8.43%). The standard deviation for crude fiber ranges from 0.02 to 2.791 for crude fat. The percentage composition is depicted on Figure 1.

Table 1: Phytochemical Components of *Acalypha wilkesiana* leaves

Secondary Metabolites	Reagent/ Test	Outcome
Alkaloids	Dragendoff's	+ (brick red precipitate)
	Mayer's	+ (buttered colour precipitate)
	Wagner's	+ (formation of brick red precipitate)
Saponins	Frothing reaction	+ (formation of firm froth)
Anthraquinones	Chloroform/Ammonia (Borntrager's reaction)	+ (Rose pink colour)
Tannins	Ferric chloride	+ (bluish-green colouration)
Cardiac Glycoside	Keller–Killiani reaction	+ (formation of coffee-coloured ring)
Flavonoids	Magnesium turning/Concentrated Hydrogen Chloride	+ (Golden yellow colouration)

Note: +: indicates positive reaction

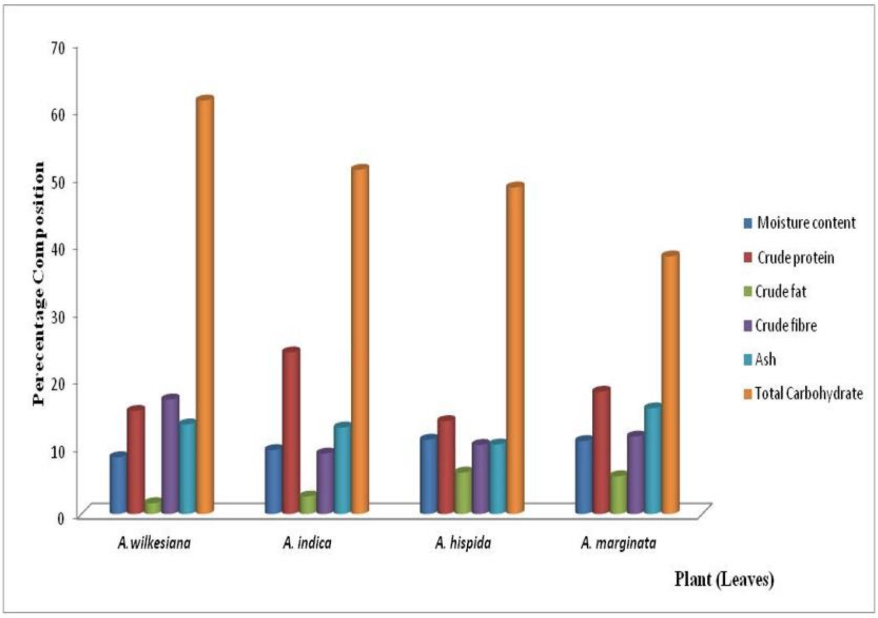

Fig.1: Proximate Contents of leaves of four *Acalypha* Species

GC-MS Analysis of The Essential Oil:

All the extracts (A-E) of the essential oil of *A. wilkesiana* leaves had sharp herbal odour and light yellow colouration. The GC/MS analysis showed the existence of six bioactive compounds, including pyrrole, and 1,3,7-Octatriene with retention time of 23.57 and 23.92 mins. respectively (Table 2). n-Hexadecanoic acid was the prominent compound.

Table 2: Bioactive constituents of essential oil obtained from *Acalypha wilkesiana* using GC-MS

Compounds	Retention Time (min)	Abundance (%)
n-Hexadecanoic acid	20.90	37.82
4-Hexen-2-one,3-methyl	20.27	8.42
1,3,7- Octatriene	23.92	0.65
2,5-Pyrrolidinedione, 1-(benzoyloxy)	25.70	0.48
Propanoic acid, anhydride	20.56	0.36
Pyrrole	23.57	0.34

Antimicrobial Susceptibility Testing:

The antimicrobial susceptibility testing of the essential oil against some bacterial and fungal organisms revealed that the plant was very effective against *S. aureus*, *S. typhimurium, B. coagulans* and methicillin resistant *S. aureus* (MRSA) (Table 3). The essential oil that was extracted for four hours stretch (extract E) was the most effective of all the extracts, more than some of the hourly extracts (A, B, C, D) (Table 3). However, it showed limited inhibitory activities against *P. aeruginosa* and *G. vaginalis*.

Table 3: Antimicrobial Activity of essential oil from *Acalypha wilkesiana* leaves

Microorganisms	Imipenem	1st hr Extract A	2nd hr Extract B	3rd hr Extract C	4th hr Extract D	4hrs stretch Extract E
S. epidermidis ATCC 12228	3+	3+	2+	2+	3+	3+
MRSA ATCC 700699	3+	2+	2+	2+	2+	3+
S. aureus ATCC 25923	2+	2+	2+	2+	2+	3+
E. coli ATCC 35218	3+	2+	2+	2+	2+	2+
S. typhimurium ATCC 13311	3+	2+	2+	2+	3+	3+
K. pneumoniae ATCC 8308	3+	2+	2+	2+	3+	3+
P. aeruginosa ATCC 15442	3+	-	-	-	-	-
G. vaginalis ATCC 14018	3+	-	-	-	-	-
B. coagulans UL 001	3+	3+	3+	3+	3+	3+
B. substilis UL 002	3+	2+	3+	2+	3+	2+
C. albicans ATCC 10231	ND	2+	3+	2+	3+	2+

+: degree of inhibition (inhibition zone measured in millimeter); 1+: 5-9mm, 2+: 10-19 mm, 3+: > 20mm3+: indicates a very good antimicrobial activity, 2+: good antimicrobial activity and 1+: fair antimicrobial activity. -ND: not detected.

Minimum Inhibition Concentrations (MICs):

The essential oil showed high antimicrobial potential against *S. aureus* compared to the control. This was followed by *S. typhimurium, B. coagulans* and MRSA. The MICs of most of the isolates were at the following concentrations: 20µg/ml (*B. coagulans; K. pneumoniae*), 40µg/ml (*S. aureus*) and 60µg/ml (MRSA; *B. subtilis*). All the essential oil (extracts A-E) were able to exhibit good inhibitory activities but extract E was the most effective since it had all the components that were present in extracts A-D (Table 4). Thus, the order of sensitivity of the essential oil extracts was E > A > B > C > D.

Table 4: Sensitivity rate the pathogenic organisms to the essential oil of *Acalypha wilkesiana*

Microorganisms	1st hr Extract A	2nd hr Extract B	3rd hr Extract C	4th hr Extract D	4hrs stretch Extract E
S. epidermidis ATCC 12228	41.6	35.4	37.5	43.8	45.8
MRSA ATCC 700699	63.3	50	56.7	40	76.7
S. aureus ATCC 25923	120	113.3	113.3	100	140
E. coli ATCC 35218	51.4	42.8	45.7	40	54.3
S. typhimurium ATCC 13311	66.7	66.7	66.7	77.8	77.8
K. pneumoniae ATCC 8308	51.6	45.2	54.8	64.5	64.5
P. aeruginosa ATCC 15442	-	-	-	-	-
G. vaginalis ATCC 14018	-	-	-	-	-
B. coagulans UL 001	72.4	65.5	68.9	68.9	96.5
B. substilis UL 002	52.9	58.8	55.8	41.2	55.8
C. albicans ATCC 10231	-	-	-	-	-

DISCUSSION

In this study, the qualitative phytochemical analysis validated our previous findings and that the leaves of *A. wilkesiana* contains a variety of phytochemicals including flavonoids, tannins, alkaloids and saponins. This was supported by the findings of other authors (Ikewuchi *et al.*, 2010). Generally, the chemical composition of plants, regardless of the parts, depends on the species, geographical region, seasonal variation, and extraction process. The phytochemicals identified in this study have been shown to have health-promoting properties (Basu *et al.*, 2007). Tannins, for example, have been shown to significantly aid in the repair of underlying tissue during wound healing (de Sousa *et al.*, 2015). Flavonoids, another significant plant constituent, have anti-inflammatory, anti-oxidative, anti-carcinogenic and anti-mutagenic effects, as well as their ability to modify essential cellular enzyme activity (Beking and Vieira, 2010). In general, antioxidants protect against radiation damage and boost the immune system.

The present investigation also revealed that the leaves of *A. wilkesiana* contain high carbohydrate (61.39%) and low moisture (8.43%). The low moisture content can be beneficial in restricting the growth of microorganisms, shelf life and quality of the leaves. This is in agreement with the results of Adeyeye and Ayejuyo (1994). In addition, we found that the leaves of *A. wilkesiana* have very high crude fiber when compared with those of *A. indica*, *A. hispida* and *A. marginata* leaves. Studies have suggested that fiber intake can help to reduce the occurrence of certain diseases such as diabetes, obesity, hypertension and gastrointestinal problems (SACN, 2008). Fiber consumption could also protect the gut by increasing faecal mass, thus diluting elevated bile concentrations associated with high fat consumption (Dillard and German, 2000). Accordingly, the importance of *A. wilkesiana* in disease management is thereby acknowledged.

Our current work supported previous studies by confirming the antimicrobial activity of *A. wilkesiana* essential oil on the pathogenic organisms. The essential oils derived from the leaves of *A. wilkesiana* have potent antifungal properties and significant inhibitory effects on *C. albicans*, *S. epidermidis*, *B. coagulans*, *S. typhimurium*, *K. pneumoniae* and *E. coli*. Significantly, the inhibitory activity was more pronounced against *S. aureus*, *S. typhimurium*, *B. coagulans* and MRSA. These organisms have been implicated in cases of skin infections, sepsis, nosocomial infections (Ayub *et al.*, 2015). Previous study of the leaves extract had indicated that the plant has significant antibacterial effects that correspond with its phytochemical properties (Akharaiyi *et al*., 2019). Also, Okoye and Amadi (2019) discovered that the aqueous extracts of *A. wilkesiana* leaves had significant inhibitory activities against some fungal species. The essential oil extracted for four hours (extract E) was the most effective in this study because it contained all of the components of the hourly extracts. Regrettably, it has minimal impact on the growth of *P. aeruginosa* and *G. vaginalis*.

The GC-MS analysis showed the existence of specific medicinal compounds that have been linked to the powerful therapeutic potential of essential oil. Compounds like pyrrole have demonstrated positive anti-malarial, antifungal, antibacterial, and anticancer properties. 2, 5 -Pyrrolidinedione, 1-(benzoyloxy) has been recognised to have antibacterial and antifungal effects. Bacteriostatic, antioxidative, and antimicrobial abilities are also possessed by 6-Benzamido-4-benzoyl-1, 2, 4-triazine-3,5 (Karrouchi *et al*., 2018). Another bioactive component of the oil is 4-Hexen-2-one, 3-methyl, which is a well-known antioxidant, anti-inflammatory, anti-tumor, and antimicrobial phytochemical that has been shown to have anti-inflammatory, lipolytic, mucolytic, anti-coagulant and stimulatory qualities. Gyawali and Kyong-Su (2012) attributed these qualities the mild electronegativity and high polarity of its ketones.

The plant also contained propanoic acid anhydride with antibacterial effect (Bertleff *et al*., 2005) and n-Hexadecanoic acid (antibacterial, anti-inflammatory, and antioxidative) (Benoit *et al*., 2009, Aparna *et al*., 2012). n-Hexadecanoic acid has a variety of medicinal benefits, including anti-inflammatory properties that make it a potential therapy for rheumatic symptoms (Aparna *et al*., 2012). Another constituents of the essential oil, 1,3,7-octatriene, has been shown to have antioxidative properties (Rezaee *et al.,* 2019).

CONCLUSION

The findings of this study indicate that the different constituents of the essential oil derived from *A. wilkesiana* have effective therapeutic activities. These properties, as well as the constituents from the oil promote its use as possible treatment option for skin conditions, salmonellosis, respiratory, and candida infections.

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