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Biological and chemical characterization of Acalypha wilkesiana leaf extracts

Olajide Ebenezer Falayi[1], Olakunle Ayodeji Fatokun[1], Adewale Elijah Fadeyi[1]
[1]Sheda Science and Technology Complex, Sheda, Abuja, Nigeria, wale.fade@gmail.com

*Correspondence: wale.fade@gmail.com

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Abstract

According to African folklore, Acalypha wikesiana is known to possess medicinal properties. A study was conducted to evaluate the presence of chemical compounds and biological properties in the non-polar and crude extracts of Acalypha wikesiana. To obtain the crude extract, the air-dried pulverized leaf of the plant was extracted with 95% ethanol and hexane. Some microbial strains associated with dermophytic diseases, including Staphylococcus aureus, Escherichia coli, Aspergillus niger, Epidermatophyton Sp, Trichophyton rubrum and Candida tropicalis were inhibited by the crude ethanol extract. No activities were observed against Methylene-resistant Staphylococcus aureus, Streptococcus pyogenes, Microsporum gypseum, Microsporum canis, Aspergillus fumigates, and Candida albicans. In the DPPH free radical scavenging assay, the crude ethanol extract exhibited acceptable robust antioxidant activity between 0.1 and 0.9 mg/mL concentration. In the following GC-MS analysis, yellow oil was obtained from the crude extract by column chromatography, which revealed the presence of some compounds, mostly terpenoids and fatty acid esters. Medicinal properties of Acalypha wikesiana leaf extracts may be attributed to these compounds.

Keywords: Acalypha wikesiana, Phytochemical, Antioxidant, DPPH, GC-MS analysis

1. Introduction

The rising demand for medicinal plants has increased in recent years, driven by the hope of discovering new, affordable pharmaceuticals (Jamshidi-Kia et al., 2017; Zahra et al., 2020). This renewed interest in herbal medicine has led to a focus on the study of botanical plants, which contain a wide range of chemicals with potential uses for human health (Ganjhu et al., 2015).

2. Literature review

Plants are rich in secondary phytochemicals, including minerals, alkaloids, flavonoids, glycosides, phenols, saponins, and terpenoids (Shakeri et al., 2012; Uttu et al., 2015). Phytotherapy plays a crucial role in modern medicine, recognized for its potential health benefits and integral to various applications, including medical, nutraceutical, pharmaceutical, and cosmetic uses (Swamy et al., 2019). A variety of phytopharmaceutical properties, including anti-inflammatory, anti-thrombogenic,

diabetic, anti-cancer, and neuroprotective properties, have been shown to work in vitro and in animals (Ezenyi et al., 2016; Gullon et al., 2017). Some of these medications are derived from plants, including aspirin, which comes from the bark of willow trees (Salix alba), and quinine, which is derived from Cinchona. The use of herbal remedies can not only cure disease but also help with wellness promotion, upkeep, and prevention (Murage et al., 2021; Lin et al., 2021). Many plants native to Nigeria have been studied for their potential therapeutic uses. *Acalypha wilkesiana,* commonly referred to as "Irish petticoat," is a visually striking plant of the Euphorbiaceae family, prized for its colorful leaves and widely cultivated in tropical and subtropical areas for its decorative appeal and belongs to the Euphorbiaceae family. It is widely planted in tropical and subtropical locations due to its high decorative value because of its strikingly coloured leaf (Kingsley & Marshal, 2014). *Acalypha wilkesiana* has been used for millennia to treat diabetes, cancer, hypertension, epilepsy, infertility, and arthritis (Mustafa, 2014) The leaves of *A. wilkesiana* are used to cure a variety of skin conditions in Northeastern Africa and the Southwest of Nigeria (Adesina et al., 2000; Madziga et al., 2010; Sharma et al., 2014). *Acalypha wilkesiana* leaf has been shown to reduce the prevalence of pathogens such as *Staphylococcus aureus*, *Staphylococcus pyogenes*, *Klebsiella aerogenes*, and *Escherichia coli* (Oluduro et al., 2011; Olude et al., 2022). The goal of this study was to use GC-MS to characterise the phytochemical, antioxidant and antibacterial characteristics of *Acalypha wikisiana* leaf extracted in hexane and ethanol.

Figure 1 : Plate 1 - Calotropis procera

3. Research methodology

3.1. Collection of *Acalypha wikesiana*

The fresh leaf of *Acalypha wikesiana* was collected from Sheda, Kwali Local Government of Abuja, and authenticated at the National Institute for Pharmaceutical Research and Development NIPRID, Abuja, with voucher number (NIPRD/H/7343). Hexane ethanol and methanol are the main solvents used. All the reagents and solvents used in this investigation were all of standard grade. Assays involving DPPH (2, 2-diphenyl-1-picrylhydrazyl) was used to measure the antioxidant activity of the extract. MHA (Mueller Hinton agar) and SDA (Sabourad dextrose agar) were used for the assay. An antimicrobial test was performed on organisms obtained from the Department of Medical Microbiology at Ahmadu Bello University Teaching Hospital in Zaria, Kaduna state, Nigeria. *Staphylococcus aureus, E. coli, Methicillin-resistant Staphylococcus aureus*, *Streptococcus pyogenes, Candida albicans, Candida tropicalis, Aspergillus nigre, Epidermatophyton sp., Microsporum gypseum, Microsporum canis, and Trichophyton rubrum* Ciprofloxacin, Fluconazole, and Fulcin were employed as the standard antibiotic agents. Hammer mill (TRP80 Rotary vacuum evaporator (BUCHI), UV spectrophotometer

(CECIL), and gas column and mass spectrometry GC-MS (Thermo-Scientific Trace GC ULTRA system) were included in this study.

3.2. Extraction of plant material

Air-dried crispy leaf samples of *Acalypha wikesiana* (100g) were ground to powder using a hammer mill, TRP80, and kept before use. The powdered leaf material was separately extracted using the maceration method for 48 hours with 2.5 liters of hexane and 2.5 liters of 95% ethanol respectively and later filtered. The resulting extracts were concentrated using a rotary evaporator at 40°C, followed by air drying to yield the resultant extracts of hexane and ethanol.

3.3. Phytochemical screening

Ethanol crude leaf extract was subjected to phytochemical screening to identify major constituents using standard methods (Trease & Evans, 2002; Singh et al., 2009).

3.4. DPPH radical scavenging assay

Using a UV-visible spectrophotometer at 517 nm, the ethanol extract's ability to neutralize free radicals against DPPH (Sigma-Aldrich) was evaluated. A slightly modified method from the one that was first outlined by (Fadeyi et al., 2022) was employed. Using the following formula, the radical scavenging activity (RSA) was determined as the percentage inhibition of DPPH discoloration:

$$\%\ Inhibition = \left\{\frac{A_b - A_a}{A_b}\right\} \times \frac{100}{1}$$

A_b is the absorption of the blank sample (without the extract) and A_a is the absorption of the extract**.**

3.5. Antimicrobial screening

Acalypha wilkesiana crude ethanol extract was utilized to screen human pathogenic microorganisms. Antimicrobial activity was evaluated using the agar well disc diffusion method (Deeni & Sadiq, 2002). Crude extract (0.1 g) was dissolved in 10 ml DMSO to yield a 10 mg/ml solution. The bacterial inoculation medium, known as Mueller-Hinton Agar (MHA), and the fungal inoculation medium, Sabouraud Dextrose Agar (SDA) plates were inoculated with 0.1 ml inoculum, then 0.1 ml crude extract was added to wells. using a sterile 6 mm corn borer. Following a 24-hour incubation period at a temperature of 37°C, the plates were subjected to examination in order to identify zones of inhibition. The determination of the lowest inhibitory concentration of the extract was conducted using the broth dilution method. A volume of 10 mL of Mueller Hinton broth was dispensed into individual test tubes. The broths were sterilized at 121°C for 15 minutes and then cooled. The concentration was determined using a McFarland 0.5 turbidity standard. The test microorganism was inoculated and incubated at a temperature of 37°C for 6 hours following the production of normal saline. Subsequently, 10 mL of the saline solution was transferred into a sterile test tube. The test microbe was diluted in normal saline until it reached the same level of turbidity as Mc-scale Farland's standard, as determined through visual comparison (Ali et al., 2017).

3.6. GC-MS analysis of extractives

Hexane oil extracts from fresh leaves were analyzed by GC-MS (7820) and identified by comparing mass spectral data to the NIST 14 Library and literature (Derwich et al., 2010; Palá-Paúl et al., 2012).

4. Results

Table 1: Phytochemical analysis of *Acalypha wilkesiana leaf extracts*

hyto-constituents	Presence/absence
Phenolics	+
Flavonoids	+
Tannins	+
Saponnins	+
Glycosides	+
Terpenoids	+
Steroids	+
Alkaloids	+
Carbohydrates	+
Resin	+

Table 2: Antioxidant activity of *Acalypha wilkesiana* ethanol leave extract

Conc.(mg/mL)	% Inhibition A.W	% Inhibition (Vit C)
0.1	73.72	60.38
0.3	62.18	72.50
0.5	44.00	77.69
0.7	32.97	77.88
0.9	16.84	81.54

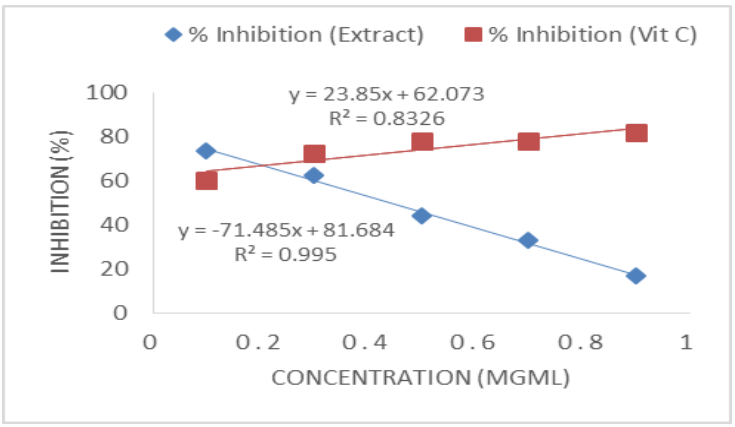

Figure 2: Antioxidant activity of *A. wilkesiana*

Table 3: Zone of inhibition of the extract on microbes

Test organisms	*A.w (mm)*	CIP (mm)	FLU (mm)	FLC (mm)	MIC (mg/mL)	MBC/MFC (mg/mL)
S. aureus	24.00	0.00	0.00	0.00	2.50	5.00
E. Coli	21.00	38.00	0.00	0.00	2.50	10.00
A. niger	21.00	0.00	0.00	29.00	2.50	10.00
E. sp	23.00	0.00	0.00	28.00	2.50	10.00
T. rubrum	25.00	0.00	0.00	27.00	2.50	5.00
C. tropicalis	27.00	0.00	32.00	0.00	1.25	5.00
M. R. St. aureus	0.00	32.00	0.00	0.00	0.00	0.00
S. pyogenes	0.00	30.00	0.00	0.00	0.00	0.00
M.gypseum	0.00	32.00	0.00	0.00	0.00	0.00
M.canis	0.00	32.00	0.00	0.00	0.00	0.00
A.fumigatus	0.00	0.00	0.00	0.00	0.00	0.00
C.albicans	0.00	0.00	34.00	0.00	0.00	0.00

Key: CIP: Ciprofloxacin, FLU = Fluconazol, FLC = Fulcin

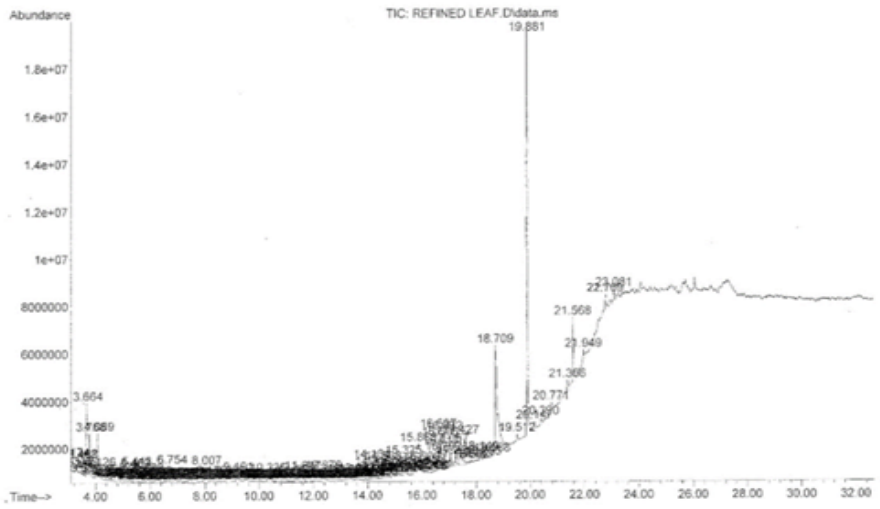

Figure 3: GCMS spectral of refined oil

Table 4: GC-MS characterization of oil extract of *A. wilkesiana* leaf

RT	% Area	Compounds
3.468	0.191	Cyclopentanol, 3-methyl-
3.670	0.146	Benzene, 1,3-dimethyl-
3.988	0.024	Cyclohexanol
4.346	0.039	Oxirane, 2-methyl-2-pentyl-
4.577	0.043	(R)-(-)-2-Methyl-2,4-pentanediol
4.710	0.060	Isothiazole, 3-methyl-
4.975	0.015	Benzene, 1-ethyl-3-methyl-
5.074	0.008	2-propenamide, 2-methyl-N-[3-[(phenylsulfonyl)amino] propyl]-
5.224	0.009	Chloromethyl 2-chloroundecanoate
5.316	0.009	1-Undecene, 5-methyl-
5.409	0.033	Mesitylene
5.894	0.113	D-Limonene
6.206	0.049	Octadecane, 1-(ethenyloxy)-
6.766	0.029	Undecane
7.014	0.021	9-Borabicyclo [3.3.1] nonane, 9-hydroxy-
7.182	0.022	Benzene, 2-butenyl-
7.615	0.011	Cyclopropane, 1-hexyl-2-propyl-, cis-
7.840	0.049	Butanedioic acid, diethyl ester
8.013	0.078	Dodecane
8.285	0.012	Pentadecanoic acid, 14-bromo-
8.672	0.014	1, E-11, Z-13-Octadecatriene
8.885	0.008	2-Piperidinone, N-[4-bromo-n-butyl]-
9.088	0.017	4-Methyl-dodec-3-en-1-ol
9.203	0.018	Tridecane
9.324	0.013	Cyclohexanol, 5-methyl-2-(1-methylethenyl)-
9.503	0.011	Naphthalene, 1,2,3,4-tetrahydro-1,4-dimethyl-
9.936	0.026	Naphthalene, 1,2,3,4-tetrahydro-1,1,6-trimethyl-
10.318	0.106	Tetradecane
10.699	0.015	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-
10.982	0.052	Dodecane, 2,6,11-trimethyl-
11.317	0.034	trans- beta –Ionone
11.507	0.048	Nonanoic acid, 9-oxo-, ethyl ester
11.756	0.049	Furan, 2,5-dibutyl-
11.860	0.074	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-
12.160	0.120	Decanoic acid, ethyl ester
12.304	0.096	5-Eicosene, (E)-

12.870	0.046	2,3-Dimethyl-8-oxo-non-2-enal
12.986	0.053	2-Cyclohexen-1-one, 4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-, [R-[R*,R*-(E)]]-
13.107	0.010	Cyclopentane, 1-butyl-2-pentyl-
13.240	0.050	Diethyl azelate
13.373	0.062	Pentadecane, 2,6,10,14-tetramethyl-
13.483	0.023	1-Octadecene
13.604	0.042	1-Hexadecanol, 2-methyl-
14.014	0.095	Tetradecanoic acid

5. Discussions

The ethanol extract of the plant contains notable chemical compounds that are responsible for its various pharmacological effects (Table 1). Flavonoids, terpenoids, tannins, alkaloids, cardiac glycosides, and carbohydrates. Terpenoids are the primary constituents of essential oils which are vital as aromatherapy (Hassanpour et al., 2020; Fongang et al., 2021). Due to their mild astringency, tannins are important secondary metabolites that have the potential to be antibacterial agents (Clinton, 2009; Voon et al., 2011; Stephane & Jules, 2020). Flavonoids, which are plant pigments, are potent antioxidants that help to prevent oxidative diseases like cardiovascular disease and certain types of cancer (Ogbuehi et al.,2014; Panche et al, 2016). Glycosides offer several medical benefits, including antioxidant, anti-inflammatory, and anti-diabetic properties, highlighting their potential in disease prevention and treatment (Tran et al., 2020). A broad class of substances recognized for their detergent and foam-forming abilities is saponins (Bezerra et al, 2018).

Free radicals, charged molecules produced by biological metabolism, could harm healthy cells Antioxidants have become linked to good health due to their capacity to mitigate some of their harmful effects (Kehrer & Klotz, 2015). In the fight against cancer, heart disease, stroke, and other immune-compromising conditions, eliminating free radicals may be helpful (Lobo et al., 2010). The linear regression coefficients of extracts and ascorbic acid in the concentration versus percent analysis were 0.995 and 0.8326, respectively. The value of the IC_{50} = 0.444. It has strong antioxidant property that is due to flavonoids and tannins (Clinton,2009), which are crucial in absorbing and neutralizing free radicals (Table 2). These phytochemicals have also been linked to lower mortality rates for a variety of different human illnesses (Panche et al., 2016; Hassanpour et al., 2011). The crude ethanol leaf extracts' bioactivities were examined against many pathogenic bacteria and fungi associated with skin and gastrointestinal disorders (Table 3). The plant extract exhibited significant antimicrobial activity against a variety of test organisms, including *Staphylococcus aureus*, *Escherichia coli, Aspergillus niger, Enterobacter sp., Trichophyton rubrum* and *Candida tropicalis* with minimum inhibitory concentrations (MICs) ranging from 1.25 to 2.50 mg/mL and minimum bactericidal/fungicidal concentrations (MBCs/MFCs) of 1.25 to 2.50 mg/mL. *S. aureus* and *E. coli* are the most common causes of disease and death in those with impaired immune systems associated with gastrointestinal disorders while *A. niger, E.sp., T. rubrum*, and *C. tropicalis* has been associated to hospital environments (Amenu, 2014; Nwachukwu *et.al.*,2016). The ethanol crude leaf extract activities agree with earlier works by (Gotep et al., 2010).

The leaf extract was relatively inactive against the test organisms such as M. R. *Staphylococcus, C. albicans*, S. *Pyogenes*, *M.gypseum*, *M.canis* and *A.fumigatus*. According to the results of the extract's phytochemical analysis, flavonoids and tannins were found. The observed bioactivity is a result of the flavonoids and tannins that constitute the plant extract. (Yu *et al.,* 2021 Alkaloids have inspired antibacterial drugs and served as crucial scaffolds for their development (Cushnie, *et al.,* 2014). The hexane leaf extract was analyzed by GC-MS, revealing unique pharmacological characteristics due to the presence of fatty acid esters and derivative. Hence the plant contain known chemicals with distinct pharmacological potencies (Onocha & Olusanya, 2021). *Acalypha wilkesiana's* leaf extracts were found to possess antioxidant and antibacterial properties, showcasing its potential as a natural antimicrobial agent, and GC-MS analysis revealed its phytochemical composition.

6. Recommendations

Assessing the biological and chemical characteristics of *Acalypha wilkesiana* leaf extracts have enhances our understanding of its therapeutic potential, hence reaveling the chemical composition of the plant and prospective pharmaceutical uses.

7. Conclusion

Among the main constituents of *Acalypha wilkesiana* oil leaf extracts are long-chain hydrocarbons, fatty acids, and their derivatives, in addition to being antibacterial and antifungal, these substances are known to have a variety of medicinal properties. This study supports the plant's historical use as a remedy for various ailments since it contains a number of phyto-constituents.

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ORCID

Olajide Ebenezer Falayi https://orcid.org/0000-0002-3463-3899
Fatokun Ayodeji Olakunle https://orcid.org/0000-0001-6581-9699
Adewale Elijah Fadeyi https://orcid.org/0000-0001-7152-7131

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