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Research Article

Phytochemical screening and Antimicrobial activity of Ethnomedicinal *Acalypha wilkesiana* leaves extracts against some clinical isolates in Dutse, Jigawa State, Nigeria

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ABSTRACT
Acalypha wilkesiana is a highly valued plant for its therapeutic potential in ethno-medicinal practices, primarily for treating gastrointestinal and skin disorders. This study aimed to investigate the phytochemical compounds and antibacterial activity of *A. wilkesiana* leaf extracts against some bacterial pathogens. Plant extraction was conducted via maceration in methanol and aqueous solvents, followed by phytochemical screening to determine the bioactive compounds using standard methods. Antibacterial activity was determined using the agar well diffusion method against the test organisms procured from the Microbiology Laboratory, Federal University Dutse, upon isolation from clinical samples in Dutse General Hospital. The preliminary phytochemicals screening observed the presence of alkaloids, flavonoids, tannins, steroids, anthraquinones, resins, and saponins in the methanolic extract. In contrast, the aqueous extract showed similar secondary metabolites except for resins, steroids, and saponins. In terms of antibacterial activity, the methanolic extract exhibited the highest antibacterial activity against the tested organisms; it produced the most significant inhibition zones of 15.10 ± 2.44 mm and 11.35± 1.60 mm against *S. aureus* and *P. aeroginosa*, respectively. However, the aqueous extract demonstrated a slightly lower antibacterial effect, yielding an inhibition zone of 7.13 ± 2.28 mm against *K. pneumoniae*. Therefore, this study underscores the potential of *A. wilkesiana* leaves, demonstrating its rich abundance of secondary metabolites and its efficacy against a broad spectrum of bacterial strains, rendering it a valuable candidate for further exploration in the development of natural antibacterial agents that could help mitigate the development of antimicrobial resistance.

Keywords: Antimicrobial; Ethno-medicine; Extraction; Maceration; Phytochemical

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INTRODUCTION

Millions of individuals in Africa and Asia, particularly in rural areas where modern healthcare services are scarce, continue to depend on medicinal plants to treat various health conditions. Traditional medicine serves as the primary source of healthcare for rural communities due to its accessibility, affordability, lower toxicity, and the transfer of knowledge across generations (Ijioma *et al*., 2021). These advantages offered by orthodox medicine have continued to play a significant role in its practices over the years. Additionally, ethno-medicine underscores the impact of medicinal plants as a vital component of healthcare systems (Jain *et al*. 2020). Medicinal plants have served as a crucial reservoir of natural bioactive compounds,

especially secondary metabolites. The phytochemicals that plants produce in response to their interactions with the environment induce the production of secondary metabolites, which confer defensive mechanisms against threats, including infectious agents (Erb & Kleibensen, 2020). The abundance of phytochemicals in medicinal plants suggests their potential for extracting bioactive compounds used in drug development and disease treatment. Their utilisation is attributed to the diversity of chemical compounds, availability, and cost-effectiveness (Kloucek *et al*., 2005; Iniaghe *et al*., 2009; Sa'id *et al*., 2024). These compounds are considered in traditional medicines and the development of medicinal drugs (Singh 2015; Kouipou & Boyom 2019). In addition to their therapeutic benefits, secondary metabolites play a crucial role in promoting human well-being and overall health, supporting various aspects of life, and contributing to a higher quality of life. The diverse benefits of these compounds in medicinal plants have generated considerable interest in using them to develop traditional medicine for treating human diseases (Kawo *et al*. 2009; Jain *et al*. 2020; Vidkjar *et al*. 2023). *Acalypha wilkesiana* is a perennial shrub from the *Euphorbiaceae*, originating from the Pacific islands but thriving in tropical regions. They are commonly referred Copper leaf, Joseph's coat, and Fire Dragon (Makoshi *et al*. 2016). A wide variety of cultivars is available globally. In Nigeria, the prevalent cultivars include *A. macarena, A. hispid, A. naginata, A. fruticosa*, and *A. racemosa*. They are popularly known in northern Nigeria as "Jiwene" (Iniaghe *et al*. 2009; Gotep *et al*. 2010). This plant possesses significant therapeutic properties and is widely used for antimicrobial treatments against gastrointestinal and dermatological infections (Akinyemi *et al*., 2005; Kabir *et al*., 2005; Erute & Oyibo, 2008). Furthermore, Gotep *et al*. (2010) reported its potential in addressing parasitic diseases, particularly malaria, as well as non-communicable conditions such as hypertension and diabetes mellitus. The plant effectiveness in treating various human ailments has sparked scientific interest in exploring the biochemical mechanisms underlying its therapeutic potential. This study aimed to investigate the bioactive phytochemical constituents and antibacterial activity of *A. wilkesiana* leaves extracts against specific bacterial pathogens.

MATERIALS AND METHODS

Source of plant material and test organisms

A. wilkesiana leaves were procured from the botanical garden, Federal University Dutse, Jigawa State, identified and authenticated at the herbarium unit, Department of Biological Science, Faculty of Science, Federal University Dutse, where they were assigned voucher number FUDHAN:006/24. According to Ehiaghe *et al*. (2013), the leaves were processed, rinsed with distilled water, and dried at ambient temperature for two weeks, followed by grinding into fine powder using an electric blender. The powder was stored in a tightly sealed container at room temperature until needed. The test organisms utilised in the study included *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,* and *Klebsiella pneumoniae.* These strains were procured from the Microbiology Laboratory, Federal University Dutse, upon isolation from clinical samples in Dutse General Hospital, subsequently followed by confirmation through conventional biochemical techniques before being stored on nutrient agar slants until use (Cheesbrough, 2002).

Extraction Protocol

To efficiently extract the desired bioactive compounds, plant extraction was conducted by maceration, as described by Murugan and Parimelazhagan (2013), with slight modifications. Briefly, approximately 20 g of the powdered sample was macerated in 100 ml of methanol and aqueous solutions at room temperature, under agitation (150 rpm) for 48 h and 18 h, respectively. The mixture was passed through double-layered muslin cloth before filtering with Whatman No. 1 filter paper. The methanolic extract was concentrated using a rotary evaporator, followed by reconstitution with DMSO. The aqueous extract was evaporated in a water bath prior to reconstitution with distilled water and then stored in an airtight polythene container at room temperature until required.

$$\text{Percentage yield (\%)} = \frac{\text{Dry weight (g)}}{\text{Extract weight (g)}} \times \frac{100}{1}$$

Phytochemical screenings

To detect bioactive compounds from *A. wilkesiana* leaves, extracts were subjected to phytochemical assays according to standard protocols by Trease and Evans (2004).

Test for Alkaloids

The procedure involved stirring 0.5 g of the extract with 3 mL of 1% aqueous HCl in a steam bath. After filtration, 1 mL of the solution was mixed with drops of Dragendoff's reagent in the first test tube, and two drops of Meyer's reagent were added to the second test tube. The appearance of an orange-red precipitate in the first test tube or a white precipitate in the second tube indicated the presence of alkaloids.

Test for Tannins

About 0.5 g of the extract was thoroughly mixed with distilled water before filtration, followed by the addition of FeCl3 solution to the resulting filtrate. The formation of a blue-black, dark-green precipitate served as an indicative marker for the presence of tannins.

Test for Resins

About 2 g of extract was dissolved in 5 ml of ethanol at the boiling point to ensure thorough mixing. The solution was then filtered using Whatman No. 1 filter paper and diluted with 4 ml of 1% aqueous HCl. The emergence of a resinous precipitate signifies the presence of resins within the extract.

Test for Steroids

About 0.1 g of the extracts were dissolved in 2 ml of chloroform before H2SO4 was carefully added to form a distinct lower layer. The presence of steroids was indicated by the appearance of a reddish-brown color at the interface between the two layers.

Test for Anthraquinones

In a dry test tube, 0.5g of the extract was combined with 5 ml of chloroform and agitated for 5 minutes. The solution was passed through a filter, and the filtrate was vigorously mixed with 100% ammonia solution. The presence of pink violet in the ammoniacal layer (bottom) indicates the presence of free anthraquinones.

Test for Flavonoids

A powdered sample (2.0g) was extracted with acetone and then subjected to a hot water bath to allow all traces of acetone to evaporate. Boiling distilled water was used to wash the extracted sample. The hot mixture was then filtered and cooled, and 5 mL of 20% NaOH was added to the filtrate to achieve an equal volume. A yellow solution confirmed the presence of flavonoids.

Test for Saponins

In the experiment, the extract (0.5 g) was added to a desiccated test tube, then water was added, and the mixture was subjected to vigorous agitation. The emergence of froth, which persists upon warming, was taken as preliminary evidence for the presence of saponins.

Test Concentrations

The extract concentrations were prepared according to Sulaiman and James (2023), with a minor adjustment. For the methanol extract, 1.0 g of the extract was dissolved in 10 ml DMSO to produce a 100 mg/ml solution, while distilled water was utilised for the aqueous extract. This solution was then subjected to serial dilutions to achieve lower concentrations of 50 mg/ml, 25 mg/ml, and 12 mg/ml. To standardize the inoculum, test organisms were cultured from a 24-hour-old culture on Mueller-Hinton agar. Each inoculum was suspended in sterile distilled water, and the turbidity was adjusted by adding 0.5 McFarland standard to achieve a density equivalent to 1.5 x 10^8 cells/mL (Cheesbrough, 2012).

Antibacterial Sensitivity Test

The antibacterial assay was conducted according to Okwori *et al*. (2007), which utilised the agar well diffusion method. Molten Mueller-Hinton agar was aseptically poured into Petri plates. Following this, 1 mL aliquots of overnight broth culture were inoculated onto the plate and gently swirled to ensure an even distribution of the test organisms. Allowed to solidify, uniform wells were created using a sterile cork borer of 6 mm diameter, and each well was filled with 0.1 mL of the various extract concentrations. A typical antibiotic, ofloxacin, served as a positive control. The plates were incubated at 37°C for 24 hours before inhibition zones' were measured to determine antibacterial activities.

Minimum Inhibitory Concentrations

The Minimum Inhibitory Concentration (MIC) was assessed using a tube dilution method according to Makhuvele *et al*. (2020). A range of extract concentrations (100, 50, 25, and 12.5 mg/ml) were prepared. About 1 mL of the extract was added to each sterile tube, followed by 5 mL of Mueller Hinton broth, and mixed thoroughly. Subsequently, 0.1 mL of a standard bacterial suspension was inoculated into each tube, and the mixture was incubated for 24 hours at 37°C. The presence or absence of turbidity was recorded. The MIC was defined as the lowest concentration that effectively inhibited the growth of the test organisms.

Statistical Analysis

The data obtained were presented as mean ± standard deviation for the diameter of inhibition in each group and analyzed using one-way analysis of variance (ANOVA) to determine whether a significant difference or otherwise existed at a 5% probability level; statistical significance was established at values ($P < 0.05$).

RESULTS

Physical characteristics of the extracts

The extraction methods employed in this study yielded varying results in terms of the physical characteristics of the extracts. Specifically, the methanol-based extraction process yielded an 8.2% extract, resulting in a dark green, viscous liquid. In contrast, the aqueous extraction method yielded a lower percentage of 5.6%, resulting in a reddish-brown extract (Table 1).

Phytochemical Screening

The qualitative phytochemical analysis revealed significant differences in the secondary metabolite profiles of the methanolic and aqueous extracts. The methanol extracts indicated the presence of

alkaloids, tannins, resins, saponins, anthraquinones, and flavonoids, whereas the ethanol extracts presented alkaloids, tannins, anthraquinones, and flavonoids (Table 2).

Antibacterial Activity

In the antimicrobial assay (Table 3), the test organisms displayed varying zones of inhibition, where methanolic extract exhibited highest activity, with inhibition zones ranging from 11.35 ± 1.60 mm (*P. aeruginosa*) to 15.10 ± 2.44 mm (*S. aureus)*. In comparison, the aqueous extracts resulted in inhibition diameters ranging from 7.13± 2.28 mm (*K. pneumoniae*) to 10.45± 2.51 mm (*S. aureus*). The standard antibiotic (Ofloxacin) demonstrated the highest inhibition zones of 24.52± 2.01 against *P. aeruginosa* and the least inhibition zones against 16.94± 1.37 *K. pneumonia*, at a 5% significant level. The inhibition zones of the extracts were compared to the standard antibiotic (control) using a one-way ANOVA. The statistical analysis indicated a significant difference ($P < 0.05$) in the effectiveness of the extracts against all tested bacteria.

Minimum Inhibitory Concentration (MIC)

Upon analyzing the Minimum Inhibitory Concentration (MIC), variable susceptibility was observed among the test organisms (Table 4). The methanol extract showed that both *K. pneumoniae* and *E. coli* exhibited an MIC of 25 mg/ml, while *S. aureus* and *P. aeruginosa* had a higher MIC of 50 mg/ml. In contrast, the aqueous extract showed reduced efficacy, with MICs of 50 mg/ml for *K. pneumoniae* and *P. aeruginosa*, whereas *S. aureus* and *E. coli* exhibited significantly elevated MICs of 100 mg/ml.

Table 1: Physical Properties of *Acalypha wilkesiana* leaves extracts

Properties	Methanol	Aqueous
Yield (%)	8.2	5.6
Colour	Dark green	Reddish brown
Odour	Sharp and alcoholic	Herbal
Texture	Thick	Thin & Watery

Table 2: Phytochemical screening of *Acalypha wilkesiana* leaves extracts

Compounds	Methanol	Aqueous
Alkaloids	+	+
Tannins	+	+
Resins	+	-
Steroids	+	-
Anthraquinones	+	+
Saponins	+	-
Flavonoids	+	+

Table 3: Antimicrobial sensitivity of *A. wilkesiana* crude extracts against some bacteria

Conc. (100 mg/ml)	Methanol	Aqueous	Ofloxacin (10 µg)
S. aureus	15.10 ± 2.44	10.45 ± 2.51	20.38 ± 1.60
P. aeruginosa	11.33 ± 1.60	08.76 ± 1.33	24.52 ± 2.01
E. coli	13.74 ± 2.33	0.00 ± 0.00	18.20 ± 1.42
K. pneumonia	14.86 ± 2.72	7.13 ± 2.28	16.94 ± 1.37

Results = Mean (mm) ± SD

Table 4: Minimum Inhibitory Concentration (MIC) of *A. wilkesiana* leaves crude extracts

Conc. (mg/ml)	Methanol				Aqueous			
	100	50	25	12.5	100	50	25	12.5
S. aureus	-	-	+	+	-	+	+	+
P. aeruginosa	-	-	+	+	-	-	+	+
E. coli	-	-	-	+	-	+	+	+
K. pneumoniae	-	-	-	+	-	-	+	+

Key: **+ =** Growth - = No growth

DISCUSSION

In the present study, methanol extraction during maceration exhibited a higher yield, which can be attributed to the effectiveness of organic solvents and a prolonged maceration period, compared to aqueous extraction, which was conducted for a short duration to mitigate microbial degradation. The physical differences between the extracts suggest variations in their chemical compositions, which were further explored in the phytochemical screening. This disparity in extraction efficiency by

the solvents indicates the influence of solvent preference on the extracted properties. A similar finding was reported by Murugan and Parimelazhagan (2013), who conducted plant macerations and observed that methanol extracts yielded the highest output compared to other solvents. Yao *et al*. (2004) demonstrated that methanol extraction is more efficient than aqueous extraction in extracting phytochemical compounds, as organic solvents are effective at dissolving and solubilizing secondary metabolites. The result of phytochemical screening demonstrated the presence of various phytochemical compounds in the methanol extract compared to the aqueous extract. This property is attributed to its higher extraction yield due to its intermediate polarity, which facilitates the dissolution of both polar and non-polar compounds. The low detection of bioactive compounds in aqueous extracts may reflect its limited capacity to solubilize specific secondary metabolites. This study is in line with the findings of Ikewuchi *et al*. (2010) and Dada *et al*. (2019), who reported numerous secondary metabolites from *A. wilkesiana* leaves extracts. However, the findings differ from those of Suleman & James (2023), who identified the presence of numerous phytochemicals in *A. wilkesiana* leaves extracts, except for alkaloids, saponins, and anthraquinones. The variation in phytochemical compounds may result from differences in climatic conditions and seasonal harvests. These findings are in agreement with the research conducted by Evans (2005), which documented a variation in the phytochemical composition of bee propolis collected from temperate and tropical climates. Climatic factors and seasonal changes significantly influence the availability of phytochemical compounds in plants (Rao and Rout 2003).

The broad-spectrum activity of the methanolic extract, particularly against *S. aureus* and *E. coli*, suggests its utility in treating infections caused by both Gram-positive and Gram-negative bacteria. This finding is consistent with previous research involving other organic solvents (Kabir *et al*. 2005; Gotep *et al*., 2010). However, Oladunmoye (2006) reported high antibacterial activity of aqueous extracts against Gram-positive bacteria. This study aligns with numerous researches have explored the antimicrobial properties of various plant species, demonstrating that these qualities are primarily due to the secondary metabolites present in the plants (Bukar *et al*., 2010, Jain *et al*., 2020; Rajkumar *et al*., 2022). The significant antibacterial activity found in the methanol extract is likely due to the substantial presence of dissolved secondary metabolites, which enhance its effectiveness. The presence of flavonoids, alkaloids, and tannins in both extracts correlates with their antibacterial effects, as these compounds are known to inhibit microbial growth through multiple mechanisms, including enzyme inhibition, metal ion chelation, and membrane disruption (Dzotam *et al*., 2016). Additionally, flavonoids destabilize bacterial cytoplasmic membranes, while tannins precipitate microbial proteins (Mariita *et al*., 2011). The absence of saponins in the aqueous extract may explain its weaker activity, as these surfactants enhance the permeability of bacterial membranes (Osbourn, 2003). These phytochemical compounds, identified through various chemical analyses, suggest a significant potential for developing natural antibiotic agents that could help mitigate the development of antimicrobial resistance.

CONCLUSION

The findings of this investigation have, therefore, established the presence of diverse bioactive constituents in *A. wilkesiana* leaves and demonstrated their inhibitory effect on the tested bacterial isolates. This underscores its significant potential in orthodox medicine to harness natural resources for pharmaceutical development, combating human diseases, and mitigating the impact of antimicrobial resistance.

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