International Journal of Science, Engineering & Environmental Technology (IJOSEET), 17(17): 22-26, 2025
ISSN 0794-9650

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Antibacterial Effect of *Acalypha wilkesiana* on Human Pathogen (*Staphylococcus Aureus*)

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Abstract: ***Acalypha species are traditionally used in the treatment and management of diverse ailments ranging from parasitic diseases to non-parasitic diseases. This study focuses on the antimicrobial effect of the various extracts of Acalypha wilkesiana on Staphylococcus aureus. The antimicrobial screening method was carried out using agar well diffusion method and nutrient broth media and the solvents for the extraction ethanol, ethyl acetate, petroleum ether and methanol. The percentage yields of the extracts in ethanol, ethyl acetate, petroleum ether and methanol were 86.7%, 86%, 83.6% and 78.3% respectively. Ethanolic extraction produced better activity than ethyl acetate, methanol and petroleum ether. Results of this study shows good antibacterial activity of the plant extracts against Staphylococcus aureus and Methicillin Resistant Staphylococcus aureus (MRSA) which are gram-positive organisms. The study showed that Acalypha wilkesiana has significant and comparable antibacterial activity against the pathogens tested (Gram positive bacteria) with the ethanolic extract containing bioactive phytochemical and antimicrobial activity at higher concentration. Hence, this plant has the potential of being developed as a typical agent that can be used to treat asymptomatic MRSA carriers.***

Keywords: Acalypha wilkesiana, Antibacterial activity, Pathogen, Solvent extraction

1. Introduction

Most of the World's population use extracts from plants, for their primary health care. The struggle between man and illness as well as drugs resistance has led to the return to nature in the form of medical plant (Geoffrey et al., 2009). Different phytochemical compounds and enzymes are well reserved in plants. Researchers (Patil et al. 2009; Painuli et al., 2011; Kaitibi et al., 2023) has confirmed the presence of alkaloids, tannins, volatile oils, flavonoids, saponins, tannins, phenoantioxidantides, etc. which have all been assessed for their anti-oxidant, anti-mutagenic, anti-carcinogenic and other biological effects. Over twenty-five percent of people in the world depend on traditional medicinal plants as drugs for curing various diseases and ailments (Painuli et al., 2011). The results generated from the search for antimicrobial agents from plants like garlic, ginger, thyme, tea leaves have been a growing interest in the last few decades (Kunle et al., 2012). The regular use of antimicrobials for treatment therapy or prophylaxis promotes the development of resistant. Through antimicrobial-driven selection and the exchange of genetic resistance elements, multi-drug resistant strains of bacteria emerge. Antimicrobial sensitive microorganism that are part of the endogenous flora are suppressed, while the resistant strains survive. Many strains of pneumococci, staphylococci, enterococci, and tuberculosis are currently resistant to most or all antimicrobials which were once effective (John and Herin, 2011) With the constant challenge of discovering new drugs to replace existing ones due to antimicrobial resistance, preliminary studies on their efficacy are needed to verify claims of clinical efficacy in humans.

Acalypha wilkesiena (Red acalypha), belongs to the family *Euphorbiaceae*. Due to its reddish colour it is commonly referred to as red acalypha (Ogundaini, 2005). Oladunmoye et al. (2006), acknowledged that the genus Acalypha comprises about 570 species, a numerous proportion of which are weeds while the others are ornamentals. There are a quite reasonable number of cultivars worldwide, the macrophylla, hofammani, godseffiana, macefeena, hispida marginata and racemose are peculiar cultivars within Nigeria (Many cultivars are available with different leaf forms and colors) (Ogundaini, 2005). *Acalypha wilkesiana*, being a popular medicinal plant has been used by herbal doctors to treat *P. versicolor* (PV), a common superficial mycosis that is associated with cosmetic disfigurement and reduced quality of life. The leaves have been used for headache, swelling, cold and wound dressing (Ikewuchi et al., 2011). Chopped pieces of the dried stem and root in past studies were steeped in alcohol and used for stomach ache and as worm expellant in man in the Delta region

of Nigeria (Onocha and Olusanya, 2010). The leaves of this plant were recorded to be eaten as vegetables in the management of hypertension, in Southern Nigeria (Iwu, 1993; Ikewuchi et al., 2011; Kaitibi et al., 2023).

Extracts of the leaves of *Acalypha wilkesiana* (Macrophylla) have shown to possess a wide range of antibacterial and antifungal activity (Oladunmoye, 2006; Adeshina *et al.*, 2010). Oyelami *et al* (2003) evaluated the efficacy and safety of *Acalypha wilkesiana* ointment in superficial fungal skin diseases. Their formulation produced total inhibition of the growth of *Tinea pedis*, *Pityriasis versicolor* and *Candida intetrigo*. A comparative antimicrobial study on two varieties of *Acalypha wilkesiana* (Macrophylla and Hoffmanni) showed that it possessed a broad spectrum of activity on both bacteria and fungi (Oladunmoye, 2006). In a study by Jekayinfa *et al* (1997), the aqueous extract of *Acalypha wilkesiana* (Macrophylla) showed significant antibacterial and antifungal properties *in vitro* and was found to be reasonably useful in the treatment of eczema. The *in vitro* antihelminthic activity of plant extracts of *A wilkesiana* against *Fasciola gigantica*, *Taenia solium* and *Pheritimap asthuma* has also been documented (Onocha and Olusanya, 2010). They are traditionally used in the treatment and/or management of diverse ailments such as diabetes, jaundice, hypertension, fever, liver inflammation, schistosomiasis, dysentery, respiratory problems including bronchitis, asthma and pneumonia as well as skin conditions such as scabies, eczema and mycoses, (Ikewuchi et al., 2011; Seebaluck et al., 2015). It is frequently used in traditional medicine, exclusively or as a major constituent of many herbal preparations for the management or treatment of hypertension. The preliminary phytochemical screening of the leaves of *Acalypha wilkesiana* revealed the presence of alkaloids, carotenoids, flavonoids (catechins and flavones), saponins, tannins, anthraquinones and glycosides, all of which have potential health promoting effects, at least under some circumstances (Oladunmoye, 2006; Basu et al., 2007). However, a comparative evaluation of the antimicrobial activities and phytochemical screening of two varieties of AW showed that the inhibitory effect against microorganisms differ among the varieties of this plant (Oladunmoye, 2006).

Staphylococcus aureus is a major bacterial human pathogen that causes a wide variety of clinical manifestations. It is said to possess a battery of virulent factors (Kim et al., 2016). They are found on the skin and mucous membranes, (Boucher et al., 2008) and it is estimated that up to half of all adults are colonized and are the major reservoir for these organisms. Approximately 15% of the population persistently carry *S. aureus* in the anterior nares and can be transmitted person-to-person by direct contact or by fomites (Tong et al., 2015). Some populations tend to have higher rates of S. aureus colonization (up to 80%), such as health care workers, persons who use needles on a regular basis (i.e., diabetics and intravenous (IV) drug users), hospitalized patients, and immunocompromised individuals. *S. aureus* are the causative agents of multiple human infections, including bacteremia, infective endocarditis, skin and soft tissue infections (e.g., impetigo, folliculitis, furuncles, carbuncles, cellulitis, scalded skin syndrome, and others), osteomyelitis, septic arthritis, prosthetic device infections, pulmonary infections (e.g., pneumonia and empyema), gastroenteritis, meningitis, toxic shock syndrome, and urinary tract infections. Treatment remains challenging to manage due to the emergence of multidrug resistant strains such as MRSA (Methicillin-Resistant Staphylococcus aureus) (Boucher et al., 2008). This study was aimed to determine and compare the antibacterial effect of different extracts of *Acalypha wilkesiana* on *Staphylococcus aureus* and resistant strain MRSA (Methicillin-Resistant Staphylococcus aureus) at different concentrations.

2. Methodology

Fresh sample of *Acalypha wilkesiana* leaves were collected (within Ede, Osun State) and identified by a botanist in the department and was there after taken to the laboratory for further preparations. The leaves were detached from the main plant, rinsed with running laboratory tap water, sliced into 1mm and dried at a temperature between 40-45^{0}C. Once dried it was grinded to powder, weighed and divided into four equal parts for extraction. Ethanol, Methanol, Petroleum ether and ethyl acetate (sigma product) was used as solvents. 200g was extracted with absolute ethanol (sigma product) and other fractions of solvents per time and concentrated via rotary evaporator.

The nutrient broth was used for sub culturing the test organisms (which was sub-cultured from laboratory) to obtain an 18hour culture, and the Mueller Hilton agar was used for the antimicrobial assay using well diffusion method. Mueller Hilton Agar. The required gram of the agar was weighed (according to manufacturer's prescription; 38g of MHA in 1000ml of distilled water) using a weighing balance. The weighed media was transferred into an already sterilized conical flask to which appropriately measured distilled water was added. A magnetic stirrer was put into the conical flask containing the prepared media for the purpose of uniformity and complete dissolution of the media. After uniformity was achieved, the well covered flask was sterilized by autoclaving at 121^{o}C for 15minutes (Haruna et al., 2013). The sterilized media was allowed to cool; after which it was poured into labeled petri dishes.

The antimicrobial screening method carried out was the agar well diffusion method. The extracted sample for the antimicrobial analyses was brought out. Prior to this, the sub cultured 18 hours test organisms were spread on the labeled and separated nutrient filled Petri plates in duplicates appropriately using cotton swabs. Holes were made on solidified nutrient plates containing the test organisms using a 6mm cork borer which was repeatedly flamed and sterilized in ethanol after each hole was made. The negative controls, DMSO (dimethyl sulfoxide) were dispensed into their appropriate holes. The extracted samples were tested at 100%, 75%, 50% and 25% concentrations. This procedure was carried out for the sample with four solvents (Ethanol, Methanol, Petroleum ether and ethyl acetate extracts). The inoculated plates were incubated at 38^{o}C for 24hours. The results of the clear zone inhibition in mm were measured and recorded. The 100% concentration of the extract constituted the undiluted form, 0.1ml of the sample was carefully dispensed into the appropriate holes using a syringe, avoiding spillage and flooding the plate with the sample. The various concentrations (100%, 75%, 50% and 25%) were all repeated appropriately as stated above. After 18hrs-24hrs the clear zone of inhibition were recorded and measured in mm diameter.

3. Discussion of Results

Table 1: Percentage yield of extraction from 200g of A. wilkesiana using different solvent

Extract	Yield (%)
Ethanol	86.7
Ethyl Acetate	86
Petroleum Ether	83.6
Methanol	78.3

Table 1 shows the ethanolic extraction of sample yielded 173.4g from 200g. The percentage of the yield was 86.7%. The methanol extraction of sample yielded 156.6g from 200g. The percentage of the yield was 78.3. The petroleum ether 40^{o}C-60^{o}C extraction of sample yielded 167.2g from 200g. The percentage of the yield was 83.6. The ethyl acetate extraction of sample yielded 173.2g from 200g. The percentage of the yield was 86%. The percentage yields of the extracts in ethanol, ethyl acetate, petroleum ether and methanol were 86.7%, 86%, 83.6% and 78.3% respectively were good yields based on Vogel's textbook of Practical Organic Chemistry (Furniss et al.,2022).

Table 2: The mean of Zone of inhibition (mm) on the Antimicrobial activity of the various solvent extract of *A. wilkesiana*

Organisms	Concentration	Ethanolic extract	Methanolic extract	Petroleum ether	Ethyl acetate
Staphylococcus aureus	25%	13	05	0 0	15
	50%	14	10	0 0	17
	75%	15	15	0 0	20
	100%	17	15	0 0	23

MRSA	25%	15	05	0 0	0 0
	50%	16	07	0 0	0 0
	75%	17	08	0 0	0 0
	100%	18	10	0 0	0 0

From the antimicrobial screening, the plant extracts have some antibacterial potential as demonstrated in Table 2. All the leaf extracts except petroleum ether extract had some degree of antibacterial activity against the test organisms even at low concentration, hence they possess inhibitory properties. The antibacterial activity exhibited by ethanolic and methanolic extracts was comparable as they all showed considerable activity at all extracts concentrations. Katibi et al., (2023) reported similar results in which 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 the insolubility of the active ingredients of *A. wilkesiana* in petroleum ether solvent. The findings of the study agree with the earlier studies of Oluduro et al., (2011); Akpomie and Olorungbon, (2011); and Odeja et. Al., 2016. Ethyl acetate extract had the highest inhibition zone for *S. aureus* but had no effect on MSRA which also corresponds with Haruna et al., (2013) discoveries.

On the contrary, Gotep et al. (2010) and Akpomie and Olorungbon (2011) recorded higher values of growth inhibition in *S. aureus* and other organisms (*K. aerogenes and S. typhi*) as against the lower values of inhibition observed in this present study with the test organism *S. aureus* and MSRA. The variation observed may be due to environmental factors which may include climatic conditions, geographical locations, extraction techniques and solvents.

There is an observation of color changes in the culture using ethanol extract of the plant, the color changes from green to deep brown color while methanol extract color changes to a slightly brown color but there is no any appearance of color changes in ethyl acetate and petroleum ether extract because the color still remain greenish brown this could be related to the polarity of the solvent used in the study. However, the ethanolic extraction produced better activity than methanol. These variations may be due to the difference in its polarity as well as dielectric constant, which play a vital role in the solubility of phytochemical compounds in respective solvents. Therefore, this result confirms the effect of solvent system on the extract yield that consequently confirms the richness of this plant in polar substances. The effectiveness of the ethanolic extract against *Staphylococcus aureus* was similar when compared to the activity of the Mupirocin cream which is usually used to treat infections caused by *Staphylococcus aureus*. The ethanol extract which was the most effective solvent in this study is commonly used in traditional medicine. De Silva et al. (2017) investigated and confirmed aqueous and methanolic extracts at 100mg/ml concentration and Oyedeji and Olajide (2015) confirmed aqueous and ethanolic extracts at 100mg/ml concentration were effective on the inhibition of selected bacterial strains causing wound infections, including *S aureus.* Comparing the results of these studies, we can say that the studies observed significant antibacterial activity of *Acalypha wilkesiana* (Red Acalypha) extracts against *S. aureus*. However, the methods used to evaluate the antibacterial activity differed. De Silva et al. (2017) and the current work used the agar well diffusion method, which involves pouring agar plates with bacterial cultures and creating wells where the plant extract is added but differed slightly in the use of aqueous solution. In contrast, Oyedeji and Olajide (2015) used the disc diffusion method, which involves placing paper discs soaked in plant extracts on agar plates inoculated with bacterial cultures. Both methods have been widely used to evaluate the antibacterial activity of plant extracts, but they may yield slightly different results due to variations in the concentration and diffusion of plant extracts. Nevertheless, the empirical evidence from these studies supports the potential use of *Acalypha wilkesiana* (Red Acalypha) extracts as a natural source of antibacterial agents against *S. aureus*.

4. Conclusion and Recommendation

The study showed that *Acalypha wilkesiana* has significant and comparable antibacterial activity against the pathogens tested (Gram positive bacteria) with the ethanolic extract containing bioactive phytochemical and antimicrobial activity at higher concentration. This observation has substantiated the logic behind the use in folk medicine and open up more conviction in harnessing the plant as an antimicrobial agent

The study however recommends that *Acalypha wilkesiana* be used as cheap and readily available sources of skin medication and therapy for gastrointestinal disorders in the country but at appropriate dosage recommended by a specialist. More research on the use of this plant in treating multidrug resistant infections should be considered.

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