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Evaluation of the Antibacterial Activities of Methanol and Aqueous Leaf Extract of *Chromolaena odorata* Against Selected Bacterial Pathogens

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ABSTRACT
The choice of extraction solvents is key in the development of a drug candidate from plants, and methanol and water are some of the widely used choices among researchers. Therefore, this study aims to evaluate the antibacterial potential of their respective extracts in an effort to validate a solvent for extracting *C. odorata* leaves in antimicrobial studies. Freshly harvested leaves of *Chromolaena odorata* were dried at room temperature and then ground to a fine powder. Exactly 500 g of the powder was subjected to extraction in water and methanol, and the resulting filtrate was concentrated with the aid of a rotary evaporator and subsequently stored at 4°C. Media preparation and antibacterial screening were performed using standard procedures. Results obtained from the study showed that the zones of inhibition recorded following exposure of *S. aureus, E. coli, P. aeruginosa, Salmonella sp,* and *Bacillus sp* to 400 mg/ml of methanol leaf extract of *Chromolaena odorata* were not significantly ($p>0.05$) different from those recorded following exposure of the isolates to 80 mg/ml of a standard antibiotic (gentamicin). On the contrary, zones of inhibition observed after exposing isolates to the aqueous extract of *C. odorata* were significantly ($p<0.05$) lower than those reported for 80 mg/ml of gentamicin. This study unveiled that the methanol leaf extract of *C. odorata* was more potent against the aforementioned microorganisms than its aqueous counterpart.

Keywords: Microorganisms, Gentamicin, Antimicrobial, *Chromolaena odorata*

INTRODUCTION
The use of plants for treating human ailments dates back to prehistoric times (Atanasov *et al.*, 2021). Over the centuries, medicinal plants have remained integral to healthcare, serving as the foundation for numerous commercialized therapeutic products, including antibiotics (Atanasov *et al.*, 2021). In contemporary times, the quest for plant-based medications has intensified, reflecting their growing acceptance among the global populace (Veeresham, 2019). This rising interest is evident in their extensive application in health management across both developed and developing countries (Jamshidi-Kia *et al.*, 2018).

The World Health Organization reinforces this widespread acceptability by reporting that approximately 80% of the world's population depends on medicinal plant preparations for primary healthcare needs (World Health Organization, 2024). Medicinal plants are particularly valued not only for their efficacy in treating a wide range of diseases but also for their relatively low incidence of adverse effects, a feature that significantly enhances their appeal (Jamshidi-Kia *et al.*, 2018). Their therapeutic potential is largely attributed to the diverse bioactive compounds they possess, which serve as the biochemical basis for their medicinal properties (Atanasov *et al.*, 2021).

The term "bioactive" is a conjunction of two words: "bio," derived from Greek, meaning life, and "activus," derived from Latin, meaning dynamic or full of energy. Phytochemicals with significant biological activities are termed plant bioactive compounds. Although produced by plants primarily for survival functions such as defense, these compounds indisputably account for the known pharmacological or toxicological effects associated with various plant parts (Wink, 2018).

A critical step in accessing these bioactive constituents is extraction, and the significance of extraction solvents in drug development cannot be overemphasized. Solvents play an indispensable role in the isolation and purification of active pharmaceutical ingredients (APIs) from biological sources. The overall quality of products emerging from an extraction procedure is greatly influenced by solvent choice, as it determines the purity, safety, and efficacy of the isolated drug compound (Azmir *et al.*, 2019).

Traditionally, water has been the most commonly used extraction solvent, especially among local communities who rely on medicinal plants for treating ailments. Scientific investigations have also demonstrated the effectiveness of water-based extracts in delivering therapeutic benefits. However, the true extent of their efficacy can only be established when compared with extracts obtained using organic solvents such as methanol, which are widely believed to yield a richer spectrum of phytochemicals, including flavonoids and steroids (Zhang *et al.*, 2018; Azmir *et al.*, 2019). This comparison is essential, as solvent polarity directly influences the types and concentrations of compounds extracted.

Chromolaena odorata, commonly known as Christmas bush, is an herb belonging to the *Asteraceae* (sunflower) family. Water-based extracts derived from various parts of *C. odorata* have been traditionally used to treat infections caused by pathogenic microorganisms, with notable success (Omonije *et al.*, 2019). Despite its extensive ethnomedicinal applications, there remains a paucity of scientific data comparing the efficacy of *C. odorata* extracts obtained using water and other reputable solvents, thereby underscoring the need for comprehensive solvent-based evaluations.

MATERIALS AND METHODS

Collection and Proessing of *Chromolaena odorata* leaf

Fresh leaves of *Chromolaena odorata* were obtained from the wild in Samaru, Zaria, Kaduna State. The leaves were then taken to the herbarium unit of the Department of Biological Sciences, Ahmadu Bello University, for authentication. After authentication, the leaves were washed thoroughly with clean tap water. Subsequently, they were spread

on a clean flat surface and dried at room temperature in the laboratory. The dried leaves were ground thoroughly, and the resulting powder was sieved to obtain a fine powder.

Extraction of *Chromolaena odorata* Exactly 500 g of powdered *C. odorata* leaf was macerated in 1000 ml of methanol and allowed to stand for 72 hours with intermittent shaking (Akinmoladum *et al.*, 2007). For the aqueous extract, another 500 g of the powdered sample was suspended in 1000 ml of distilled water and left to stand for 24 hours with intermittent stirring. Each mixture was first sieved through muslin cloth and then filtered using Whatman No. 1 filter paper. The filtrates were concentrated using a rotary evaporator and stored at 4°C. The percentage yield of each crude extract was calculated as described by Lovet and Douye (2013).

$$yeild(g/mg) = \frac{W1 \times 100}{W2}$$

W2 depicts the weight of the plant material as powder, while W1 represents the extract residue weight after the solvent has been eliminated.

Antibacterial Analysis

Culture Media Preparation and Antibacteria Screening

The antibacterial activity of the methanol and aqueous leaf extracts of *C. odorata* was evaluated against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella* spp., and *Bacillus* spp. Zones of inhibition were measured in millimeters (mm) using a ruler, and all measurements were performed in triplicate. Gentamicin served as the positive control, while bacteria treated only with the respective solvents constituted the negative control. The antibacterial activities of both the extracts and the standard antibiotic were assessed in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2021).

STATISTICAL ANALYSIS

Data generated from this study was analyzed with the aid of SPSS version and expressed as mean ± standard deviation of three determinations. Mean zone inhibition across bacterial species was compared using analysis of variance (ANOVA). $P < 0.05$ was considered statistically significant.

RESULTS

The zone of inhibition of the methanol leaf extract of *Chromolaena odorata* shown in Table 1 indicates that the zones of inhibition recorded following exposure of *S. aureus*, *E. coli*, *P. aeruginosa*, *Salmonella spp.*, and *Bacillus spp*. to 400 mg/mL methanol leaf extract of *C. odorata* were not significantly ($p>0.05$) different from those recorded following exposure to gentamicin (80 mg/mL), but were significantly ($p<0.05$) higher than those recorded following exposure of isolates to 200 mg/ml, which were not significantly ($p>0.05$) different from those observed following exposure to 100 mg/mL. The zones of inhibition at 100 mg/ml were significantly ($p<0.05$) higher than those reported after exposing isolates to 50 mg/mL, which were also significantly ($p<0.05$) higher than those reported for 25 mg/mL.

Table 1: Zone of inhibition of Methanol Leaf extract of *Chromolaena odorata* (Christmas bush)

	Zone of inhibition (mm)				
Treatment (mg/mL)	*S. aureus*	*E.coli*	*P. aeruginosa*	*Salmonella Sp*	*Bacillus Sp*
400 mg/mL COMLE	26.49±0.01^{d}	26.98±0.29^{c}	26.67±0.28^{c}	26.90±0.37^{c}	26.80±0.27^{c}
200 mg/ mL COMLE	25.60±0.29^{c}	25.60±0.30^{b}	25.40±0.31^{b}	25.00±0.36^{b}	25.60±0.36^{b}
100 mg/ mL COMLE	25.01±0.05^{c}	25.00±0.43^{b}	25.02±0.33^{b}	25.03±0.38^{b}	25.00±0.38^{b}
50 mg/ mL COMLE	24.20±0.18^{b}	24.01±0.26^{a}	24.30±0.43^{a}	24.30±0.19^{a}	24.30±0.41^{a}
25 mg/ mL COMLE	23.08±0.27^{a}	23.70±0.75^{a}	23.09±0.39^{a}	23.90±0.26^{a}	23.09±0.43^{a}
80 mg/mL Gentamicin	26.03±0.31^{d}	27.01±0.21^{c}	26.31±0.33^{c}	26.40±0.33^{c}	26.90±0.51^{c}

Results are expressed as mean ± standard deviation. Values with the same superscript in a row are not significantly ($p<0.05$) different

COMLE = *Chromolaena odorata* Leaf Extract

The zone of inhibition of the aqueous leaf extract of *Chromolaena odorata* shown in Table 2 indicates that the zones of inhibition recorded following exposure of *S. aureus*, *E. coli*, *P. aeruginosa*, *Salmonella spp.*, and *Bacillus spp.* to 400 and 200 mg/ml were not significantly different ($p<0.05$), but were significantly lower ($p>0.05$) than those recorded after exposing isolates to gentamicin (80 mg/ml). However, exposing isolates to 100, 50, and 25 mg/ml did not elicit inhibition.

Table 2: Zone of Inhibition of Aqueous Leaf extract of *Chromolaena odorata* (Christmas bush)

	Zone of inhibition (mm)				
Treatment (mg/mL)	*S. aureus*	*E.coli*	*P. aeruginosa*	*Salmonella Sp*	*Bacillus Sp*
400 mg/ mL COALE	15.50±0.01^{b}	15.00±0.31^{b}	15.27±0.23^{b}	16.00±0.37^{b}	15.80±0.21^{b}
200 mg/ mL COALE	14.98±0.92^{b}	15.30±0.32^{b}	14.81±0.38^{b}	15.01±0.37^{b}	15.10±0.37^{b}
100 mg/ mL COALE	0.01±0.05^{a}	0.00±0.43^{a}	0.02±0.33^{a}	0.03±0.38^{a}	0.00±0.38^{a}
50 mg/ mL COALE	0.00±0.12^{a}	0.00±0.01^{a}	0.00±0.13^{a}	0.00±0.11^{a}	0.00±0.41^{a}
25 mg/ mL COALE	0.00±0.17^{a}	0.00±0.75^{a}	0.00±0.02^{a}	0.00±0.06^{a}	0.00±0.03^{a}

80	mg/mL	22.03±0.31^{c}	22.60±0.21^{c}	22.31±0.33^{c}	22.40±0.33^{c}	22.90±0.51^{c}
Gentamicin						

Results are expressed as mean ± standard deviation. Values with the same superscript in a row are not significantly ($p<0.05$) different

COALE = *Chromolaena odorata* Aqueous Leaf Extract

DISCUSSION

The area on a culture medium where an antimicrobial agent suppresses bacterial growth is called a zone of inhibition. It is instrumental in determining the effectiveness of antimicrobial substances, aiding clinical treatment and drug discovery. It is an important measurement in laboratory pre-diagnosis, and the final output is instrumental in providing effective treatment against bacterial infections (Balouri *et al*., 2016). The methanol leaf extract of *Chronolaena odorata* was effective against the test bacteria (*S. aureus*, *E. coli*, *P. aeruginosa*, *Salmonella sp, Bacillus sp*) in a dose-dependent manner.

This was contrary to the observations made on the aqueous extract of the leaf of *Chronolaena odorata*. The reportedly impressive antibacterial activity of the methanol leaf extract of *C. odorata* could be attributed to flavonoids, which have been scientifically proven to exhibit antibacterial activity (Odutayo *et al*., 2017). This is consistent with the finding of Phetburom *et al* (2025), which demonstrated the antibacterial activity of ethanol leaf extract of *Chronolaena odorata* against some bacterial strains associated with the skin. The outcome of our study is in tandem with the findings made by Omeke *et al*. (2019), which showed that isolates of *P. aeruginosa* were susceptible to the methanol crude extract of *C. odorata* leaf at high concentrations. The same study also revealed that the antibacterial activity of *C. odorata* methanol leaf crude extract was potentiated with low concentrations of standard antibiotics. The poor antibacterial activity observed in the aqueous leaf extract of *C. odorata* could be attributed to the limited solubility of active non-polar compounds in water, while methanol has the potential to extract a broad spectrum of compounds owing to its ability to extract both polar and semi-polar compounds.

CONCLUSION

The study evaluated the antibacterial activity of the methanol leaf extract of *C. odorata* to assess potency relative to the conventionally used aqueous extract. The results revealed that the methanol leaf extract was more effective than its aqueous counterpart.

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CONFLICTS OF INTREST

The authors declare no conflicts of interest

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