Sokoto Journal of Medical Laboratory Science 2024; 9(2): 98 - 105

SJMLS - 9(2) - 013

Phytochemical and Antibacterial Studies of the Aerial Parts of *Euphorbia convolvuloides* Extracts

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https://dx.doi.org/10.4314/sokjmls.v9i2.13

Abstract

The growing resistance of anti-microbial agents to current antibiotics gives emphasis to the urgency of seeking new antimicrobial alternatives, especially from natural sources. The aim of this study was to evaluate the phytochemical and antibacterial efficacy of n-hexane, dichloromethane, ethyl acetate, n-butanol, methanol and water fractions derived from aerial parts of *Euphorbia convolvuloides* with a view to identify the most active fraction from the plant and to determine the reliability of the use of the plant in ethno-medicine. Phytochemical screening was conducted using standard procedures and the antibacterial property of the plant extract was assessed against the test isolates using agar well diffusion and broth dilution methods. The results of the phytochemical analysis of the extracts revealed the presence of carbohydrates, saponins, phenols, flavonoids, tannins, alkaloids, anthraquinones, diterpenoids, steroids and cardiac glycosides. The n-butanol fraction exhibited good activity against *E. coli* (30 mg/cm^3) and methanol against *S. aureus* (22 mg/cm^3), *E. coli* (21 mg/cm^3), *S. typhi* (19 mg/cm^3), and P. *aeruginosa* (17 mg/cm^3) while ofloxacin (5µg) was used as standard. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the n-hexane, n-butanol and methanol fraction were 3.13-100 mg/cm^3 and 12.50-100 mg/cm^3 respectively against the tested organisms. Methanol fraction possessed the most significant antibacterial activity among the fractions tested against the pathogens.

Keywords: *Euphorbia convolvuloides*, Antibacterial, Extraction, Phytochemical, micro-organisms.

Introduction

The approaching "post-antibiotic era" presents urgent challenges that must be addressed by developing new therapeutic approaches to enhance the treatment of bacterial infections. In a society undergoing rapid demographic changes, antibiotic resistance and untreatable bacterial infections hold significant importance (Sen Karaman *et al.,* 2020).

The increasing resistance of bacteria to antibiotics has become a pressing concern, leading to hundreds of thousands of deaths annually. The escalating number of bacteria resistant to commonly used antibiotics poses a particularly serious threat. In recognition of the widespread rise in multi-drug resistant strains globally, the World Health Organization (WHO) designated this phenomenon as a major global health threat in 2014 (Urban-Chmiel *et al.,* 2022).

The use of herbal medicines has seen a great upsurge globally where over 80% of the world's population depend on traditional medicines for primary healthcare according to the World Health Organization (Tuo *et al.,* 2015). In developing countries, traditional medicinal practice forms the backbone of rural health care especially in Africa, supporting an estimated 80–90% of the population, a scenario largely influenced by cultural beliefs (Mensah *et al.,* 2019). In Nigeria, the use of medicinal plants in the form of traditional medicine is also very common. Due to economic factors, coupled with some cultural preferences, traditional medicine has gained appreciable popularity (Clement *et al.,* 2020). Traditional and ethical medicinal practice provides information on the therapeutic effects of plants, and these have resulted in some notable drug discoveries (Zhang *et al*., 2020). Recently, the development of 'crude drugs', which are largely whole plants or parts of plants which have medicinal properties, are being used by pharmacists and pharmacologists for treating several ailments. Though such plants may qualify as medicine, it is however essential to scientifically validate phytochemical make up of such plants using different techniques (Daniel, *2021).*

Plants offer substantial advantages, such as deterring other organisms, combatting diseases, and aiding in the healing of wounds, although not directly contributing to steady growth or reproduction, secondary metabolites serve as survival strategies for plants, and these strategies have beneficial implications for humans. Secondary metabolites, exemplified by their contribution to antibiotics, food additives, and pesticides, constitute a valuable resource (Wink, 2015). It is estimated that over 50,000 plant species are utilized in traditional and alternative medicine (Sadiq *et al.,* 2018). Despite the documentation of 20,000 plant species with medicinal significance, only 10% of the world's biodiversity has been evaluated for medicinal purposes. Consequently, the exploration of new medications derived from natural sources is poised to become more prevalent (Antonelli *et al.,* 2020).

The *Euphorbiaceae* family, comprising around 300 genera and 8000 species, stands out as one of the most widely recognized flowering plant families globally, especially in tropical and temperate regions (Awaad *et al.,* 2017). However, previous studies have primarily focused on the biological properties of the *Euphorbiaceae* family (Fais *et al.,* 2021). The greater diversity of species implies a rich supply of raw materials for both biotechnology and the development of medicinal compounds (Cavazos, 2021). According to Abubakar (2009), *Euphorbia hirta* harbors potent antibacterial agents; its phytochemical screening revealed the presence of tannins, saponins, phenolics, flavonoids, cardiac glycosides, anthraquinones, and alkaloids in the raw extracts which is attributed to the existence of these bioactive components present in *Euphorbia hirta.*

According to Jain *et al.* (2017), certain Nigerian herbal practitioners apply a mixture of powdered *E. convolvuloides* and palm oil to rashes caused by smallpox and chickenpox, this remedy is believed to expedite the drying process of the rashes, while in Ghana, the plant is consumed both orally and through enemas as a laxative and for managing urethral discharges but in Ivory Coast, a collyrium made from *E. convolvuloides* is utilized for addressing eye issues. The plant extract is employed for treating asthma and bronchial inflammation. Additionally, adhering to the Signatures theory, latex is applied to women's breasts with the belief that it enhances lactation. Oluranti *et al.* (2012) documented the utilization of *E. convolvuloides* for treating peptic ulcer disease in Sokoto State, Northwestern Nigeria. *E. convolvuloides* remains relatively unexplored, with limited information available in the current literature regarding both its morphological features and biological activities (Mahomoodally *et al.,* 2020). The aim of the study was to investigate the potential antimicrobial properties of natural compounds derived from *E. convolvuloides*.

Materials and Methods

Sample Collection and Identification

The sample of *E. convolvuloides* was collected from its native habitat in Dukai village, located in the Silame Local Government Area of Sokoto State, Nigeria. The identification process was conducted at the herbarium unit of Pharmacognosy and Ethnopharmacy, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto where a voucher specimen (PCG/UDUS/EUPH/0013) was issued.

Sample Preparation

To ease extraction, the dried sample obtained after shade-drying the aerial parts of the plant was pulverized with a clean mechanical grinder; and the powdered sample was stored in an airtight container until required for analysis.

Extraction Procedure

The pulverized plant material (1.5 kg) was subjected to cold maceration as described by Umar and Muhammad (2015) with 20 L of methanol for 72 hours after which the extract was concentrated using a rotary evaporator set at 40 °C to obtain the crude extract. The crude methanol extract was mobilized with silica gel and loaded into a column containing silica gel and subsequently partitioned into different fractions using organic solvents in increasing order of polarity including n-hexane, dichloromethane (DCM), ethyl acetate, n-butanol, methanol, and finally washed with water to obtain the different fractions. The percentage yield was then calculated using the relationship:

$$\%\, yield = \frac{Extract\ weight}{sample\ weight}\ x\ 100 \quad ....(1)$$

Phytochemical Screening

The fractions of *E. convolvuloides* aerial parts (n-hexane, dichloromethane (DCM), n-butanol, and methanol) were analyzed through standard procedures for phytochemical screening to determine the phytoconstituents within the extracts as reported by Silva *et al.* (1998), Shaikh and Patil (2020), and Uba *et al.* (2021).

Test Organisms

The Enteropathogenic bacteria used in this study were obtained from the Department of Pharmaceutical Microbiology Laboratory, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto. Nigeria. The organisms include *Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Staphylococcus aureus,* and *Pseudomonas aeruginosa* and they were maintained on nutrient agar slant in a refrigerator at 4°C.

Anti-bacterial Screening

The antimicrobial activity of the extract was done using the agar well diffusion method described by (Gashe and Zeleke, 2017). One (1 cm^3) of each standardized inoculum was flooded over the surface of a well-dried agar plate, and the excess fluid was discarded into a jar containing a disinfectant solution. The inoculated plates were then allowed to dry for 15 minutes. The cork borer mark 8mm in diameter was used to bore four holes on each plate separately. The wells were filled with 0.1cm^3 of the solution of the extracts (2 mg) at varying concentrations. Ofloxacin 5ug was used as a positive control. After introducing the extract, the plates were allowed to stand for 1hour for pre-diffusion time. The plates were then incubated for 24 hours at 37°C. The presence of a zone of inhibition around the hole was observed for each plate. Using a ruler in millimeter marking, the diameter of the zones of inhibition was measured and recorded (Jumare *et al.,* 2022).

Determination of the Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of *E. convulvoloids* fractions against the pathogens was determined using the broth dilution method, following the procedure described by Ali *et al.* (2019). The plant fractions were serially diluted into various concentrations ranging from 100, 50, 25, 12.50, 6.25, 3.13, 1.56, 0.78, and 0.39 mg/cm^3 in nutrient broth. Next, 0.1 cm^3 of broth culture of the test organism (1.5 x 10^8 CFU/cm^3) was added to each of the test tubes containing the fractions. The tubes were then incubated at 37°C for 24 hours, after which the plates of the medium were observed for colony growth.

Minimum Bactericidal Concentration (MBC)

The MBC was conducted to ascertain whether the test bacteria were destroyed or if only growth was hindered. Mueller Hinton agar broth was prepared and sterilized at 121°C for 15 minutes, and then transferred into sterile petri dishes to cool and solidify. The contents of the MIC in the serial dilution were then sub-cultured into the prepared medium and incubated at 37°C for 24 hours. After incubation, the plates were observed for colony growth; the MBC was determined as the plate with the lowest concentration of the compound in the serial dilution without any colony growth was noted (Ali *et al.,* 2019).

Results and Discussion

The percentage yield of the different solvents used for extraction is shown in Table 1. The findings revealed that methanol had the highest percentage yield of 6.8%, whereas dichloromethane yielded only 0.5%. The superior ability of methanol to permeate the cellular membrane and extract intracellular components from the plant material was evident (Adham, 2015). This could be as a result of abundance of polar compounds in plants (Dhawan *et al.,* 2017).

Percentage Yield

Table 1 : Percentage yield of *E. convolvuloids* aerial parts fractions

Solvent	Weight (g)	Yield (%)
n-Hexane	7.48	1.50
Dichloromethane	11.55	0.50
n-Butanol	52.89	3.50
Methanol	102.2	6.80

Phytochemical screening

The qualitative phytochemical screenings of the fractions of *E. convolvuloids* aerial parts using four different solvents viz. n-hexane, DCM, n-butanol, and methanol as presented in Table 2. The results revealed that the methanol and n-butanol fractions yielded positive results for nine out of ten phytochemical compounds, specifically carbohydrates, saponins, phenols, flavonoids, tannins, alkaloids, anthraquinones, steroids, and cardiac glycosides. These findings closely correspond to those reported by Anyasor *et al.* (2014), with the exception of diterpenoids which was absent. The n-hexane fraction showed the presence of six out of ten phytochemical compounds tested thus; carbohydrates, phenols, flavonoids, tannins, steroids, and cardiac glycosides while DCM fraction demonstrates positive results for four out of ten compounds tested, that are carbohydrates, diterpenoids, steroids, and cardiac glycosides. Only DCM revealed the presence of diterpenoids. These findings are consistent with prior research on *Euphorbia* species, which are known to have more than 80 phytoconstituents (Sinan *et al.,* 2020).

Table 2 : Phytochemical Analysis of *E. convolvuloids* aerial parts fractions

Constituents	Test	n-Hexane	DCM	n-Butanol	Methanol
Carbohydrates	Molich	++	++	++	++
Saponins	Froth	-	-	+++	+
Phenols	Ferric chloride	+	-	+++	++
Flavonoids	Alkaline reagent	+	-	+++	+++
Tannins	Lead acetate	++	-	+++	+++
Alkaloids	Mayer	-	-	+	++
Anthraquinones	Bontrager	-	-	+	++
Diterpenoids	Copper acetate	-	+	-	-
Steroids	Salkowki	+++	+++	++	+++
Cardiac glycosides	Killer-killiani	+++	+	++	+++

+++ = significantly present; ++ = moderately present; + = trace amount; - = not detected

Antibacterial Studies

The occurrence of life-threatening diseases caused by resistant microorganisms has risen globally, raising the prospect of a future era where common infections may become fatal due to antibiotic resistance (Abdullahi *et al.,* 2017). The results of antibacterial activity of the fractions of *E. convolvuloides* aerial parts are presented in Table 3. The findings from the antibacterial investigations demonstrated that the various fractions of the extract from the aerial parts of *E. convolvuloides* displayed good antibacterial properties against the pathogens under study. The susceptibility testing revealed inhibition zones ranging from 9 to 30 mm across all organisms tested (Table 3). Notably, the n-butanol fraction demonstrated the highest sensitivity, showing a 30 mm inhibition zone at 100 mg/cm³ against *E. coli*, while the methanol fraction exhibited the lowest sensitivity, with a 9 mm inhibition zone at 40 mg/cm³ against *P. aeruginosa.* It is important to highlight that the dichloromethane fraction demonstrated no inhibition against any of the tested organisms, and the n-hexane extract exhibited no inhibition against *E. coli* and *P. aeruginosa.* The MIC range of 3.13–100 µg/cm^3 and MBC range of

12.50–100 μg/cm^3 suggest that the fractions exhibit moderate antibacterial activity against susceptible organisms, given that fractions with MICs lower than 100 μg/cm^3 are considered to possess potent antibacterial properties. The antimicrobial properties of active phytochemical constituents of *E. convolvuloides* aerial parts are present in a solvent of high polarity. The fractionation process capacity to selectively concentrate an active compound in certain fractions due to the different solubilities of phytochemicals could reveal why the methanol fraction of the extract displayed better activity compared to the other three fractions.

Table 3: Zones of inhibition of *E. convolvuloides* aerial parts fractions against the test organisms

		Zone of inhibition (mm) (Concentrations in mg/cm^3)				
Test Organisms	**Fractions**	**100**	**80**	**60**	**40**	**Ofloxacin 5μg**
Escherichia coli	HF	-	-	-	-	31
	DF	-	-	-	-	31
	BF	30	25	25	21	31
	MF	21	19	16	15	31
Salmonella typhi	HF	15	14	12	12	30
	DF	-	-	-	-	30
	BF	18	17	16	15	30
	MF	19	16	15	14	30
Staphylococcus aureus	HF	15	13	12	10	33
	DF	-	-	-	-	33
	BF	15	13	13	10	33
	MF	22	20	16	16	33
Pseudomonas aeruginosa	HF	-	-	-	-	20
	DF	-	-	-	-	20
	BF	-	-	-	-	20
	MF	17	14	12	9	20

Key: HF= n -hexane fraction, E F= Ethyl acetate f raction, D F= Dichloromethane fraction, BF= Butanol fraction, MF= Methanol f raction, mm= millimeter, μg= micro - gram.

Table 4: MIC of *E. convolvuloides* aerial parts fractions against the test organisms

Sub-fractions	**Test Organisms**	**Concentration (μg/cm^3)** 100.00	**50.00**	**25.00**	**12.50**	**6.25**	**3.13**	**1.57**	**0.70**	**0.35**	**G**	**S**
HF	*S. typhi*	-	-	+	+	+	+	+	+	+	+	-
	S. aureus	-	+	+	+	+	+	+	+	+	+	-
BF	*E. coli*	-	-	-	-	+	+	+	+	+	+	-
	S. typhi	-	-	-	+	+	+	+	+	+	+	-
	S. aureus	-	-	-	+	+	+	+	+	+	+	-
MF	*E. coli*	-	-	-	-	+	+	+	+	+	+	-
	S. typhi	-	-	-	-	+	+	+	+	+	+	-
	S. aureus	-	-	-	-	+	+	+	+	+	+	-
	P. aeruginosa	-	-	-	-	-	-	+	+	+	+	-

Key: + = Turbidity (growth), - = no turbidity (no growth) , HF= n -hexane f raction, DF= Dichloromethane fraction, BF= n-butanol fraction, MF= Methanol fraction, G = Growth control, S = Sterility control.

Table 5: MBC of *E. convolvuloides* aerial parts fractions against the test organisms

Sub-Fractions	Test Organisms	Concentration (µg/Cm^3) 100.00	50.00	25.00	12.50	6.25	3.13	1.57	0.70	0.35	G	S
HF	*S. typhi*	+	+	+	+	+	+	+	+	+	+	-
	S. aureus	+	+	+	+	+	+	+	+	+	+	-
BF	*E. coli*	+	+	+	+	+	+	+	+	+	+	-
	S. typhi	+	+	+	+	+	+	+	+	+	+	-
	S. aureus	+	+	+	+	+	+	+	+	+	+	-
MF	*E. coli*	+	+	+	+	+	+	+	+	+	+	-
	S. typhi	+	+	+	+	+	+	+	+	+	+	-
	S. aureus	-	-	-	-	+	+	+	+	+	+	-
	P. aeruginosa	-	-	-	-	+	+	+	+	+	+	-

Key: + = Turbidity (growth), - = no turbidity (no growth), HF= n -hexane fraction, D F= Dichloromethane fraction, BF= n-butanol fraction, MF= Methanol fraction, G = Growth control, S = Sterility control.

Conclusion

In conclusion, the study's findings indicate that the aerial parts of *E. convolvuloides* contains bioactive constituents with antimicrobial properties, supporting its traditional medicinal application in treating bacterial infections such as diarrhoea, and dysentery. Further investigation involving isolation and characterization of the active compounds is currently ongoing.

Declaration of competing interest

The authors affirm that they do not have any competing interests.

Acknowledgements

The authors acknowledged Mrs Hadiza Danjumma for providing the plant materials, Dr. Nasir Ibrahim for the bench space at Natural Product Chemistry Research Laboratory, Department of Pharmaceutical and Medicinal Chemistry, and Department of Pharmaceutical Microbiology Usmanu Danfodiyo University Sokoto, Nigeria.

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Citation: Hadiza Adamu Kwazo, Umar Zaki Faruq, Lawal Gusau Hassan, Masud Enaji Sadiq, Mustapha Salihu, Abdullahi Bako Rabah. Phytochemical and Antibacterial Studies of the Aerial Parts of *Euphorbia convolvuloides* Extracts. ***Sokoto Journal of Medical Laboratory Science*; 9(2): 98 – 105**. https://dx.doi.org/10.4314/sokjmls.v9i2.13