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Full Length Research Paper

Phytochemical screening and antimicrobial activities of *Euphorbia balsamifera* leaves, stems and root against some pathogenic microorganisms

A.S. Kamba[1]* and L. G. Hassan[2]

[1]Department of Chemistry, Institute for Quran and General Studies, Sokoto, Nigeria.

[2]Department of Pure and Applied Chemistry, Usmanu Danfodiyo University, Sokoto, Nigeria.

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Plants and plant-based products are the bases of many of the modern pharmaceuticals we use today for various ailments. The objective of the study is to determine the bioactive chemical constituents and to evaluate extracts of *Euphorbia balsamifera* leaves, stem and root for *in vitro* antimicrobial activities by seagar dilution method. Phytochemical analysis of the crude extracts of the plant parts revealed the presence of tannins, saponins, steroid, terpenoid, flavonoids, cardic glycosides and balsam (gum). The extract of *E. balsamifera* leaves, stem and roots were mostly effective against the *Salmonella typhimurium, Pseudomonas aeroginosa, Klebsiella spp., Escherichia coli and* Candida *albicans.* Thin layer chromatography (TLC) revealed four, three and two spots for ethanol, petroleum ether and chloroform extracts respectively using ethyl acetate: hexane solvent mixture. The minimum inhibitory concentrations (MIC) of the crude extracts were determined for the various organisms which ranged between 5.0 and 6.0 mg/ml while the minimum bactericidal concentration (MBC) ranged between 4.5 and 6.0 mg/ml. *E. balsamifera* could be a potential source of antimicrobial agents.

Key words: *Euphorbia balsamifera,* phytochemical screening, plant extracts and zone of inhibitions.

INTRODUCTION

Medicinal plants are of great value to mankind and the society in general. The medicinal value of these plants can be observed from the chemical agents they possess which may alter certain physiologic actions in the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (Hill, 1952). Many of these indigenous medicinal plants are used as spices and food plants. They are also sometimes added to foods meant for pregnant and nursing mothers for medicinal purposes (Okwu, 2001).

Knowledge of the chemical constituents of plants is very important, not only for the discovery of drugs and other therapeutics agents, but also in disclosing new sources of such economic materials as tannins, oils, gums, precursors for the synthesis of complex chemical substances (Mojab et al., 2003).

In Nigeria, almost all plants are associated with some medicinal value. The application of medicinal plants especially in traditional medicine is currently well acknowledged and established as a viable profession (Kafaru, 1994). Extraction of bioactive compounds from medicinal plants permits the demonstration of their physiological activity; it also facilitates pharmacology studies leading to the synthesis of pure and potent compounds with decreased toxicity (Manna and Abalaka, 2000). Furthermore, the active components of herbal remedies have the advantage of being combined with many other substances that appear to be inactive. However, these complementary components give the plant as a whole a safety and efficiency much superior to that of its isolated and pure active components (Shariff, 2001).

*Corresponding author. E-mail: shafiuabdullahi@yahoo.com. Tel: +234 07032302272.

Objective

The objective of the study is to determine the phytochemical active constituents and antimicrobial activities of the *Euphorbia balsamifera* parts (leaves stem and roots) used in various disease states.

MATERIALS AND METHODS

Collections and identification

Fresh leaves, roots and stem of *E. balsemifera* were collected from Gagi village in Kware local Government, Sokoto state, Nigeria during the month of May, 2007. The plant was identified at the Taxonomy unit of the biological sciences, Usmanu University, Sokoto Nigeria.

Processing of plant samples

The fresh samples were properly washed with tap water, rinsed with sterile water, dried in the sun and then pulverized with an electric blender.

Extraction of plant samples

The ethanol extract of the active ingredient of the leaves, stem and root were carried out using methods previously described (Okogun, 2000; Mbata and Saikia, 2008). A fifty gram (50 g) sample of dried plant material was extracted with 200 ml of solvent (in the ratio of 9:1 ml ethanol: water respectively). The sample was completely submerged in the solvent and then covered with aluminium foil. Extraction was allowed to proceed for 48 h. The extract was decanted and the solvent removed by evaporation at room temperature (28 + 30°C) to obtain the extract. The air dried extract was stored for 48 h in sterile universal bottles at room temperature. The sterility of the extract was tested before use.

Antibacterial test

The antibacterial tests of the plant samples were tested using the agar-gel diffusion inhibition test described by (Opara and Anasa, 1993; Mbata and Saikia, 2008) . About 0.2 ml of a 24 h broth culture containing 1 × 106 cells/ml of organism was ascetically introduced and evenly spread using bent sterile glass rod on the surface of gelled sterile Mueller-Hinton agar plates. Three wells of about 6.0 mm diameter were ascetically punched on each agar plate using a sterile cork borer, allowing at least 30 mm between adjacent wells and between peripheral wells and the edge of the Petri dish. Fixed volumes (0.1 ml) of the extract were then introduced into the wells in the plates. A control well was in the center with 0.01 ml of the extracting solvent. The plates were allowed on the bench for 40 min for pre-diffusion of the extract to occur then incubated at 37°C for 24 h (Esimone et al., 1998). The resulting zones of inhibition were measured using a ruler calibrated in millimeters. The average of the three readings was taken to be the zone of inhibition of the bacterial isolate in question at that particular concentration. (Abayomi, 1982; Mbata and Saikia, 2008)

Maximum inhibitory concentration (MIC)

The MIC of the plant extracts was determined on solid medium (Nutrient agar) using method of Siddiqui and Ali (1997). Standardized suspensions of the test organism was inoculated into a series of sterile tubes of nutrient broth containing two-fold dilutions of leaf extracts and incubated at 37°C for 24 h. The MICs were read as the least concentration that inhibited the growth of the test organisms.

Minimum bactericidal concentration (MBC)

The MBCs were determined by first selecting tubes that showed no growth during MIC determination; a loopful from each tube was subcultured onto extract free agar plates, incubated for another 24 h at 37°C. The minimum bactericidal concentration was considered as the lowest concentration that could not produce a single bacterial colony (Mbata and Saikia, 2008).

Thin layer chromatography (TLC)

Thin layer chromatography (TLC) was used to separate the plant extracts into different spots on the chromotoplate. The chromatograms developed on the microscope slide, were dried and observed visually for the various leaves components. The developing solvents used were ethyl acetate:Hexane (1:1), mixture. All the corresponding spots were again subjected to antibacterial activity.

Phytochemical screening of crude extracts

The phytochemical components of the medicinal plants were screened using the methods (Harbone, 1984; Trease and Evans, 1989). The components analysed for, were saponins, steroid, cardiac glycosides, anthraquinones, tannins, flavonoids, alkaloid, terpenoids and balsam (gum).

Test for terpenoids (Salkowski test)

To 0.5 g of each the extract was added 2 ml of chloroform. 3 ml of concentrated sulphuric acid (H_2SO_4) was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids.

Test for anthraquinones

About 0.5 g of the extract was boiled with 10 ml of sulphuric acid (H_2SO_4) and filtered using Whatman filter paper No.1.The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipetted into another test tube and 1 ml of 10% of dilute ammonia was added. The resulting solution was observed for colour changes.

Test for flavonoids

Two methods were used to test for flavonoids. First, 10% of dilute ammonia (5 ml) was added to a portion of an aqueous filtrate of the extract. Concentrated sulphuric acid (1 ml) was added. A yellow colouration that disappears on standing indicates the presence of flavonoids. Second, a few drops of 1% aluminium solution were added to a portion of the filtrate. A yellow colouration indicates the presence of flavonoids.

Test for saponins

To 0.5 g of extract was added 5 ml of distilled water in a test tube.

The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Test for tannins

About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added and observed for brownish-green or a blue-black colouration.

Test for alkaloids

About 0.5 g of extract was diluted in 10 ml of 1% aqueous hydrochloric acid, boiled and filtered. 2 ml of dilute ammonia was added to 5 ml of the filtrate. 5 ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Draggendorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Draggendorff's reagent) was observed indicating the presence of alkaloids.

Test for cardiac glycosides (Keller-Killiani test)

To 0.5 g of extract diluted in 5 ml of water was added 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout the layer.

RESULTS

The results of phytochemical screening and microbial activities of the plants parts under study showed the presence of secondary metabolites and other activities demonstrated in the Tables (1 - 5). Antibacterial activity of the leaves, stem and root extracts of *E. balsamifera* on pathogenic microorganisms.

DISCUSSION

The phytochemical screening of the plants studied showed that the leaves, stems and root were rich in most of the secondary metabolites analyzed using different solvents as shown in Tables (1a to 1c). These chemical constituents were reported to show medicinal activity as well as exhibiting physiological activity (Sofowora, 1993). Some of these plants metabolite were found to be absent in some of the plant parts analyzed. It should be noted that compounds demonstrating steroidal activity are of importance and interest in pharmacy due to their relationship with sex hormones (Okwu, 2001). It has been reported that several phenolic compounds like tannins present in the cells of plants are potent inhibitors of many hydrolytic enzymes such as proteolytic macerating enzymes used by plant pathogens. Other compounds like saponins also have antifungal properties (Aboaba and Efuwape, 2001). Many plants contain non-toxic glycosides that can get hydrolyzed to release phenolics that are toxic to microbial pathogens (Aboaba and Efuwape, 2001). Therefore the principle active compounds detected here may be responsible for the antimicrobial activity of the tested organisms.

The results in Table 3 indicate that the minimum inhibitory concentration (MIC) of the leaves, stems and roots extracts of the plant ranged between 5.5 and 6.0 mg/ml. The effect of the plant extract on the MIC for the test microorganisms is in line with the report that microorganisms varied widely in the degree of their susceptibility (Aboaba and Efuwape, 2001). Antibiotic drugs used in the study are penicillin (10 µg/disc) and streptomycin (10 µg/disc) serving as control.

An antimicrobial agent with high activities against an organism yields a low MIC while a low activity against an organism has a high MIC that is, the roots extracts has 5.0 MIC, which is lower than the leaves (5.8) and stems (6.0) respectively. This indicates that roots of the *E. balsamifera* has high activity against the tested organisms when compared with the leaves and stems extracts. The minimum bactericidal concentration (MBC) of the leaves extract of the plant ranged between 4.5 and 6.0 mg/ml (Table 3). The MIC and MBC is normally used to evaluate the efficacy of the agents such as antiseptics, disinfectants and indeed chemotherapeutic agents under standard conditions also support the sensitivity test results. In order for an antibiotic to be effective an MIC or MBC must be able to be achieved at the site of the infection.

The pharmacological absorption and distribution of the antibiotic will influence the dose, route and frequency of administration of the antibiotic in order to achieve an effective dose at the site of infection.

The potent activity of extracts *Euphorbia balsamifera* warrants further investigation and rapid development to add to the list of currently available antimicrobial agents. Certainly, this could decrease cost, improve efficacy, and decrease mobidity and mortality.

Tables (4a to 4c) showed the results of weight of solute extracted from 50g of powdered leaves and percentage yield of the plant crude extract using different solvents. The results indicate that ethanolic extract has the higher percentage yield with 4.03 g representing 8.06% in leaves followed by root with 4.0 g having 8.0% and the least in the stems with 3.03 g representing 6.06%. The percentage yield of various solvent used is significantly lower then the crude water extract that has 5.56 g representing 27.8%, this is because, it does not contain any organic solvent. i.e the crude water extract has the highest percentage yield in all the four solvents used during the analysis. Therefore the overall yields in percentages are as follows: leaves (45.48%), roots (48.45%) and roots (37.56%). Our results indicate that

Table 1a. Phytochemical analysis of leaves extract using various solvents.

Active principle	Chloroform	Ethanolic extract	Petroleum ether	Crude water extract
Steroids	+	+	-	+
Terpenoids	-	_	+	_
Tanin	+	-	_	+
Flavonoids	+	+	+	+
Alkaloids	_	+	+	+
Saponins	+	+	-	
Cardiac- glycosides	-	-	+	+
Anthraquinone	+	+	+	+
Volatile oil	_	+	+	_
Balsams	+	+	-	-

Table 1b. Phytochemical analysis of stem extracts using various solvents.

Active principle	Chloroform	Ethanolic extract	Petroleum ether	Crude water extract
Steroids	+	+	-	+
Terpenoids	-	_	+	+
Tanin	+	+	+	+
Flavonoids	+	+	+	+
Alkaloids	-	+	-	+
Saponins	-	+	+	_
Cardiac-glycosides	+	_	+	+
Anthraquinone	+	+	+	+
Volatile oil	_	+	_	_
Balsams	+	+	+	+

Table 1c. Phytochemical analysis of roots extracts using various solvents.

Active principle	Cholrofom	Ethanolic extract	Petroleum ether	Crude water extract
Steroids	+	+	-	+
Terpenoids	-	+	+	+
Tanin	+	+	+	+
Flavonoids	+	+	+	_
Alkaloids	+	+	+	+
Saponins	+	+	-	+
Cardiac glycosides	-	-	+	+
Anthraquinone	-	+	+	-
Volatile oil	+	_	_	+
Balsams	+	+	+	+

ethanol appears to be a better solvent for extraction when compared with petroleum ether and chloroform. Water was the best of all three solvents used.

The results of thin layer chromatography TLC in Tables (5a to 5c) using ethyl acetate:Hexane solvent mixture which revealed four spots for ethanol, two spots for petroleum ether and three spots for chloroform extractions in the leaves experiments Also, the roots demonstrated four spots for the ethanol, two spots for petroleum ether and three spots for chloroform. The stem experiments demonstrated four spots ethanol, two spot for petroleum ether and chloroform extraction respectively.

Antibiotics provide the main basis for the therapy of bacterial infections. However, the high genetic variability of bacteria enables them to rapidly evade the action of antibiotics by developing antibiotic resistance. Thus, there has been a continuing search for newer and more

Table 2a. Antimicrobial activities of leaves extracts.

S/No.	Bacterial strains used	Zone of Inhibition in mm with penicillin and streptomycin vs various concentration of extracts					
		Penicillin (10 µg/disc)	Streptomycin (10 µg/disc)	50 mg/ml	100 mg/ml	200 mg/ml	300 mg/ml
1.	*Salmonella typhimurium*	19.80 ± 0.81	18.70 ± 0.35	08.60 ± 0.72	11.80 ± 0.66	12.40 ± 0.58	16.87 ± 0.44
2.	*Pseudomonas aeroginosa*	15.30 ± 0.33	9.90 ± 0.47	07.20 ± 0.48	10.88 ± 0.39	13.96 ± 0.36	17.10 ± 0.54
3.	*Klebsiella sp*	16.10 ± 0.25	11.60 ± 0.71	09.10 ± 0.51	12.64 ± 0.42	13.96 ± 0.33	15.80 ± 0.53
4.	*Escherichia coli*	11.70 ± 0.60	16.10 ± 0.25	07.40 ± 0.54	08.72 ± 0.46	11.08 ± 0.45	14.08 ± 0.71
5.	*Staphylococcus aureus*	23.80 ± 0.25	21.40 ± 0.35	08.60 ± 0.61	11.68 ± 0.33	13.37 ± 0.50	16.87 ± 0.58
6.	*Candida albicans*	19.10 ± 0.50	21.80 ± 0.45	08.10 ± 0.11	11.90 ± 0.55	14.70 ± 0.60	16.20 ± 0.65

Table 2b. Antimicrobial activities of stems extract.

S/No.	Bacterial strains used	Zone of Inhibition in mm with penicillin and streptomycin vs various concentration of extracts					
		Penicillin (10 µg/disc)	Streptomycin (10 µg/disc)	50 mg/ml	100 mg/ml	200 mg/ml	300 mg/ml
1.	*Salmonella typhimurium*	19.80 ± 0.81	18.70 ± 0.35	7 ± 0.23	12.64 ± 0.42	12.40 ± 0.58	16.87 ± 0.44
2.	*Pseudomonas aeroginosa*	15.30 ± 0.33	9.90 ± 0.47	7.20 ± 0.48	09.58 ± 0.39	12.06 ± 0.32	17.10 ± 0.54
3.	*Klebsiella sp*	16.10 ± 0.25	11.60 ± 0.71	7.40 ± 0.54	0.4 ± 0.22	13.96 ± 0.33	14.80 ± 0.35
4.	*Escherichia coli*	11.70 ± 0.60	16.10 ± 0.25	7.40 ± 0.54	08.72 ± 0.46	11.08 ± 0.45	14.02 ± 0.71
5.	*Staphylococcus areus*	23.80 ± 0.25	21.40 ± 0.35	8.60 ± 0.61	11.78 ± 0.45	13.23 ± 0.60	15.87 ± 0.57
6.	*Candida albicans*	19.10 ± 0.50	21.80 ± 0.45	8.10 ± 0.11	11.90 ± 0.55	14.70 ± 0.60	16.20 ± 0.65

Table 2c. Antimicrobial activities of roots extract.

S/No.	Bacterial strains used	Zone of Inhibition in mm with penicillin and streptomycin Vs various concentration of extracts					
		Penicillin (10 µg/disc)	Streptomycin (10 µg/disc)	50 mg/ml	100 mg/ml	200 mg/ml	300 mg/ml
1.	*Salmonella typhimurium*	19.80 ± 0.81	18.70 ± 0.35	7 ± 0.65	12.64 ± 0.42	12.40 ± 0.58	16.21 ± 0.44
2.	*Pseudomonas aeroginosa*	15.30 ± 0.33	9.90 ± 0.47	6.20 ± 0.48	09.58 ± 0.39	12.06 ± 0.32	16.10 ± 0.54
3.	*Klebsiella sp*	16.10 ± 0.25	11.60 ± 0.71	8.40 ± 0.54	0.6 ± 0.22	14.96 ± 0.33	14.80 ± 0.35
4.	*Escherichia coli*	11.70 ± 0.60	16.10 ± 0.25	6.40 ± 0.23	07.72 ± 0.46	12.08 ± 0.45	13.02 ± 0.78
5.	*Staphylococcus aureus*	23.80 ± 0.25	21.40 ± 0.35	7.60 ± 0.51	12.78 ± 0.45	13.23 ± 0.60	16.80 ± 0.56
6.	*Candida albicans*	19.10 ± 0.50	21.80 ± 0.45	8.43 ± 0.10	11.30 ± 0.55	14.80 ± 0.10	17.20 ± 0.25

Table 3. Minimum bactericidal concentration (MBC) and Minimum inhibitory concentration (MIC) of extracts of *E. balsamifera*-leaves, stems and roots.

Plants extracts	MIC (mg/ml)	MBC (mg/ml)
Leaves	5.8	5.0
Stems	6.0	4.5
Roots	5.0	6.0

Table 4a. Percent yield of aqueous and organic solvent extracts of *Euphorbia balsamifera leaves.*

Fractions	Weight of the powdered sampled (g)	Weight of the sample extract (g)	Percent yield (%)
Ethanol	50	4.03	8.06
Petroleum ether	50	2.56	5.12
Chloroform	50	2.25	4.50
Crude water extract	20	5.56	27.8

$$\text{Percentage yield} = \frac{\text{Weight of the sample extract obtained (g)}}{\text{Weight of the powdered sampled used (g)}} \times 100$$

Overall yield = 8.06 + 5.12 + 4.50 + 27.8 = 45.48%

Table 4b. Percent yield of aqueous and organic solvent extracts of *Euphorbia balsamifera* stems.

Fractions	Weight of the powdered sampled (g)	Weight of the sample extract (g)	Percent yield (%)
Ethanol	50	3.03	6.06
Petroleum ether	50	2.02	4.52
Chloroform	50	2.25	4.20
Crude water extract	20	4.56	22.8

$$\text{Percentage yield} = \frac{\text{Weight of the sample extract obtained (g)}}{\text{Weight of the powdered sampled used (g)}} \times 100$$

Overall yield= 6.06 + 4.02 + 4.50 + 22.8 = 37.56%

Table 4c. Percentage yield of aqueous and organic solvent extracts of *Euphorbia balsamifera* roots

Fractions	Weight of the powdered sampled (g)	Weight of the sample extract (g)	Percent yield (%)
Ethanol	50	4.00	8.00
Petroleum ether	50	2.56	4.50
Chloroform	50	3.46	6.92
Crude water extract	20	4.86	24.03

$$\text{Percentage yield} = \frac{\text{Weight of the sample extract obtained (g)}}{\text{Weight of the powdered sampled used (g)}} \times 100$$

Overall yield = 8.00 + 4.50 + 6.92 + 27.8 = 43.72%

potent antibiotics (Heisig, 2001; www.uni-mysore.ac.in). Report of infectious diseases, overcoming antibiotic resistance is the major issue of the WHO for the next millennium. (www.who.int, 2000). Hence the last decade has witnessed an increase in the investigation of plants materials as a source of human disease

Table 5a. TLC Result of ethanol, petroleum ether, and chloroform leaves extracts.

Extract	Solvent system	Number of component	Distance of spot (cm)	Solvent front (cm)	R_f value
Ethanol	Ethyl acetate:	4	6.5	7.0	0.93
	Hexane (1:1)		5.3	7.0	0.76
			3.2	7.0	0.46
			1.5	7.0	0.21
Petroleum ether	Ethyl acetate:	2	6.5	7.0	0.93
	Hexane (1:1)		4.0	7.0	0.57
Chloroform	Ethyl acetate:		6.5	7.0	0.93
	Hexane (1:1)	3	5.4	7.0	0.77
			1.7	7.0	0.24

Table 5b. TLC Result of ethanol, petroleum ether, and chloroform stems extracts.

Extract	Solvent system	Number of component	Distance of spot (cm)	Solvent front (cm)	R_f value
Ethanol	Ethyl acetate:	4	6.6	7.0	0.94
	Hexane (1:1)		5.3	7.0	0.76
			3.2	7.0	0.45
			1.5	7.0	0.21
Petroleum ether	Ethyl acetate:				
	Hexane (1:1)	2	5.5	7.0	0.78
			4.0	7.0	0.57
Chloroform	Ethyl acetate:	2	6.5	7.0	0.93
	Hexane (1:1)		5.4	7.0	0.77

Table 5c. TLC Result of ethanol, petroleum ether, and chloroform roots extracts.

Extract	Solvent system	Number of component	Distance of spot(cm)	Solvent front(cm)	R_f value
Ethanol	Ethyl acetate:	4	5.3,	7.0	0.76
	Hexane		5.3	7.0	0.76
	(1:1)		3.2	7.0	0.46
			1.5	7.0	0.21
Petroleum ether	Ethyl acetate:				
	Hexane	2	6.5	7.0	0.93
	(1:1)		3.2	7.0	0.45
Chloroform	Ethyl acetate:				0.93
	Hexane	3	6.5	7.0	0.485
	(1:1)		3.4	7.0	
			1.7	7.0	0.24

Conclusion

This study has provided some preliminary evidence of antibacterial interaction between the various solvent extracts of plants parts and the observed antibacterial effects on the isolates associated with the presence of alkaloids, tannins, flavonoids, saponins, steroid, terpenoid, cardic glycoside and balsam, which have been

shown to possess antibacterial properties. Therefore the *E. balsamifera* leaves, stems and roots extracts can be used to discover bioactive natural products that may serve as lead for the development of new pharmaceutical agents to address unmet therapeutic needs. Such screening of various natural organic compounds and identification of active agents is the need of the hour. The plants studied here can be seen as a potential source of useful drugs. There is need for further studies on the plants parts in order to isolate, identify, characterize and elucidate the structure of antimicrobial bioactive compounds.

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