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Euphorbia Graminea Jacq. (Euphorbiaceae): A Comparative Antimicrobial Evaluations of Stem and Root Extracts

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ABSTRACT: Antimicrobial resistance is a global issue. Euphorbia plants are used locally to treat microbial infections. This study examined the antimicrobial potential of *Euphorbia graminea* stem and root extracts. The stems and roots extracts of *E .graminea* were extracted using 80% methanol and tested for antimicrobial activity at concentrations between 4.69-300mg/mL against non-clinical isolates (*S. aureus*, *E. coli*, *P. aeruginosa*, *C. albican*, *A. niger*). The active roots extract was fractionated using vacuum liquid chromatographic fractionations (VLC) and the resulting fractions bulked and tested against the organisms at 6.25-100mg/mL. The MIC of extracts and vlc bulked fractions were tested at 0.39-6.25 mg/mL. The root extract recorded higher antimicrobial activities over the stem extract especially against *S. aureus* and *E.coli*, hence was fractionated. Among the vlc sub-fractions of the roots extract, fractions A (2) recorded no activity against the test organisms while fractions C (9-10) recorded 7.50 and 3.50 mm against *S. aureus* and *E. coli* only at the maximum concentration of 100mg/mL. However, fractions B (3-8) conspicuously gave zones of inhibitions far higher than the other fractions. This study has shown that the roots extract of *E. graminea* has higher antimicrobial activities more than the stems, further justifying the ethno-botanical potentials of the plant in treating skin infections.

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For a long period of time, plants have often been a useful source of natural products and medicines (Atanasov, 2021). Many plants today, however, have recently developed new natural products that can make a major difference in the quality of human health (Newman and Cragg, 2020). Most developed countries including the United States of America, use conventional medicine that has ingredients derived from plants, hence it would be prudent to examine plants in order to fully understand their properties, safety and security and performance (Miller *et al*, 2022). It has been reported that the amount of antibiotic resistance has not decreased in spite of production of all of the newer antibiotics created over the last three decades (Larsson, *et al*, 2022). Due to the rise in microbe resistance, it is unclear whether any more medications will be useful in the next few years (Dadgostar, 2019). According to Aslam *et al*, (2018), the antibiotic resistance epidemic will persist for a long time in humans and animals, thus, the development of an alternative medication is required. As one of the most diverse genus, Euphorbia has more than 2100 species, which can be found all over the world. Of the thirty Euphorbia species in West Africa,

only 21 are well-known in Nigeria. Although other researchers claim to have seen the plant in Nigeria as far back as the late 1990s, *Euphorbia graminea* was not recognized until the mid-2000s (Aigbokhan *et al*, 2007). Our previous research (Ikpefan *et al*, 2020) on the extract and fractions of *E.graminea* leaves revealed significant antimicrobial activity. This study aimed to investigate the antimicrobial activities of the extract and fractions of other morphological parts of the plant (stem and roots).

MATERIALS AND METHODS

Collection and processing of plant materials: The stems and roots of *E.graminea* were collected in Benin City, Edo State, Nigeria at Ekosodin and were named and properly named by Dr. Henry Akinnibosun of the University of Benin's Department of Plant Biology and Biotechnology, Faculty of Life Sciences. The herbarium number (FHI 109024) was obtained after it was authenticated at the Forest Research Institute of Nigeria (F.R.I.N). The plant parts (stems and roots) were washed, rinsed with distilled water and finally dried in shade and subsequently in an oven (Teltech Lo001) at 40 ^{o}C. The dried samples were then grinded into coarse powder form with an electric machine.

Extraction of powdered plant samples: A total of 950 g of each powdered plant materials were extracted with 80 % methanol using a cold maceration technique for a period of 72hrs. Using a rotary evaporator, the filtrates were reduced to a slurry under vacuum at 40 ^{o}C. The yield of the extracts were recorded and were then stored in small sample bottles which were kept in a refrigerator at 5 ^{o}C.

Phytochemical screening: This was done using previously mentioned methods on the extract and vlc sub-fractions of the active root extract of *E.graminea* (Ukwubile *et al*, 2019; UKwubile *et al*, 2017).

Collection and processing of isolates: The experimental organisms (*Staphyloccocus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albican*) were human-pathogenic microorganisms from cultures obtained from the Pharmaceutical Microbiology LAB, Delta State University**.**

The bacterial and fungal isolates were stocked on nutrient agar and were subsequently sub-cultured for purification at 40 ^{o}C prior to biochemical identification. The identities of these organisms were identified using standard biochemical test technique previously described (Balouiri *et al*, 2016). The inoculum preparation method used was based on the method of (Anie *et al*, 2022).

Phytochemical screening of extracts: This was done using previously reported methods (Ukwubile *et al*, 2020).

Antimicrobial evaluations of the stem and roots extracts of E.graminea: Agar plates were streaked with bacterial and fungal suspensions (108 cfu / mL) according to the Jimoh *et al*., (2020) procedure. We inoculated wells (6 mm in diameter) with 200 l each of the extract and partitioned fraction working concentrations (4.69-300 mg/mL) and vlc fraction (12.5-200 mL). The experiment had three (3) replications. These included Ciprofloxacin (200 mg/100 mL) and Clotrimazole (20 mg/mL).

Vacuum liquid chromatographic Fractionation of the active roots extract of E. graminea: This was carried out adopting established procedure (Ikpefan *et al*, 2020). By this method, a total of 30 g of the methanol extract was loaded on a silica gel G (30-70 µm) in a Sintered Glass (No.4) attached to a Buchner flask connected to a vacuum pump. The eluting solvents were 300mL of C_6H_{14} (100%), C_6H_{14}-$CHCl_2$ (1:1; 1:3), $CHCl_2$ (100%), $CHCl_2$-$CH_3COOC_2H_5$(3:1,1:1,1:3), $CH_3COOC_2H_5$(100%), $CH_3COOC_2H_5$–CH_3OH (1:1) and CH_3OH(100%) . Analytical thin layer chromatographic analyses of crude extract were carried out on a pre-coated aluminum plate of Silica gel GF_{254} using C_6H_{14}- $CH_3COOC_2H_5$ (4:1). After development, the plates were sprayed with concentrated H_2SO_4 and subsequently heated for 5min at 110 ^{o}C. The colored spots were noted and their R_f values were recorded. Based on the TLC profile, fractions 3-8, 9-10 were bulked. Fraction 2 was left alone. The bulked-up fractions were subject to phytochemical screening as well as antimicrobial assay against the selected organisms.

Determination of Minimum Inhibitory Concentration (MIC) of extracts and vlc fractions: The (MIC) of the extracts and vlc fractions against the isolates (mg/mL) was carried out adopting method with a moderate change in the concentrations used (Mostafa *et al*, 2018).

Statistical analysis: The Graphpad software was used and all experimental data were presented as Mean ± Standard Error of Mean of the results taken. The level of statistical significance was done using one-way anova.

RESULTS AND DISCUSSION

Yields of the Plant Extracts: The powdered plant samples (950 g) of *E. graminea* yielded 38.65 and 49.26 g of of the stem and root extracts corresponding to 4.07 and 5.19 % respectively. The ten vlc fractions

of the active root extract yielded three sub-fractions (2, 3-8, 9-10) which weighed 0.52, 6.78 and 12.60 g respectively.

Preliminary Phytochemical Screening: Preliminary phytochemical examination of the extract of the stems, roots and corresponding fractions of *E. graminea* disclosed varying levels of phytochemical groups in the test samples (Table 1).

Sensitivity test: extracts and fractions of E. graminea: The antimicrobial activity of the extract recorded a marginal activities compared to the control drugs used. The root extract exhibited higher activities more than the stem extract. For example, at 75 mg/mL, the root extract recorded 7.3, 4.0, 2.5 and 2.0 mm zones of inhibitions against *S. aureus, E. coli, C. albican* (Table 1). However, at the same concentration against the stem extract, only *S. aureus* and *E. coli* were susceptible as 4.5 and 2.0 mm zones of inhibitions were recorded (Table 2). The activity of the root extract was observed to further enhance upon fractionation via vlc. Among the vlc sub-fractions of the root, fractions 3-8 (B) gave a higher antimicrobial activity than any other fractions. The fractions like the extracts were more susceptible to *S.aureus* and *E.coli* with increased in concentrations. The active fraction "B"recorded 9.46 and 4.81 mm zones of inhibitions against *S.aureus* and *E.coli* at 25 mg/mL. The activity was later increased to 12.25 and 7.66 mm as well as 16.32 and 12.18 mm at 50 and 100 mg/mL respectively (Table 3).

Table 1: Phytochemical screening of the extracts and fractions of *E. graminea*

Phytochemical groups	**Extracts**		**VLC sub-fractions of the root extract**		
	Stem	Root	2 (A)	3-8 (C)	9-10(D)
Alkaloid	-	+	-	+	+
Anthraquinones	-	+	-	+	-
Cardiac glycoside	+	+	-	+	+
Flavonoids	+	+	-	+	-
Saponins	+	+	-	++	-
Steroids	+	+	+	+	-
Tannin	+	++	-	++	+
Terpenes	+	++	++	++	-

(–): absent; (+): low presence; (++): medium presence; (+++): high presence

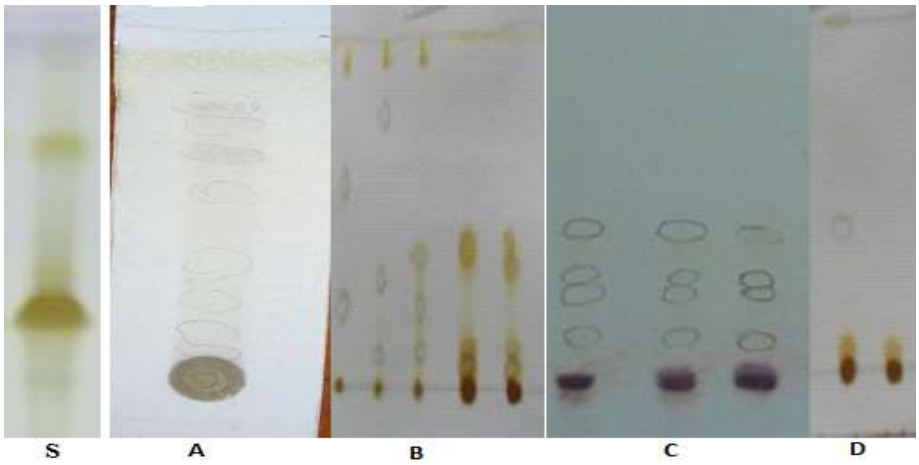

Fig 1: Chromatogram of the stem extract (S) root extract of *Euphorbia graminea* (A) and its vlc chromatographic products (B, C, D)

MIC Results of extracts and fractions of E.graminea: The result of the MIC of extracts and fraction against the selected organisms. Among the extracts, while the stem extract recorded no MIC, the roots extract recorded MIC at the maximum concentrations of 6.25mg/mL against *S. aureus. Also*, for the root fractions, fraction B (3-8) recorded MIC at 0.79 and 1.57 mg/mL against *S. aureus* and *E. coli* with the other fractions showing no MIC (Table 4)

Antimicrobial agents are useful tools for controlling pathogenic microbes by interfering with the growth or metabolism of microorganisms in a way that is harmful to them (Kebede *et al*, 2021). Microbial diseases are known to pose a serious threat to human health due to a lack of vaccines and limited chemotherapy (Mostafa *et al*, 2016). The organisms used in the tests are known to cause enteric infections in humans (Sonibare *et al*, 2016). The ability of a plant material to inhibit the growth of bacterial and fungi organisms can be used to determine its antimicrobial activity (Vaou *et al*, 2021).

Table 2: Antimicrobial **activity of the roots and stems extract of *E. graminea***

Treatment	Conc. (mg/mL)	Zones of inhibitions (mm)				
Root extract*		*S. aureus*	*E. coli*	*P. aeroginosa*	*C. albican*	*Aspergilus niger*
	300	11.60±0.50	8.15±0.15	7.50±0.03	6.00±0.03	-
	150	9.50±0.50	6.55±0.50	5.65±0.01	4.32±0.00	-
	75	7.30±0.50	4.05±0.62	2.51±0.11	2.00±0.10	-
	37.5	5.50±0.01	2.57±0.25	-	-	-
	18.75	3.50±0.01	-	-	-	-
	9.38	-	-	-	-	-
	4.69	-	-	-	-	-
Stem extract*	300	8.50±0.50	6.00±0.50	1.600±0.03	-	-
	150	5.50±0.50	4.50±0.30	-	-	-
	75	4.50±0.50	3.00±0.01	-	-	-
	37.5	2.00±0.01	-	-	-	-
	18.75	-	-	-	-	-
	9.38	-	-	-	-	-
	4.69	-	-	-	-	-
Ciprofloxacin[a]	5µg/mL	39.22±1.31	36.72±1.95	33.52±2.10		
Clotrimazole[b]	10 µg/mL				24.50±1.26	21.26±1.55

*Values are Mean ±SEM of three replicates (n= 3). a=antibacterial control drug, b= antifungal control drug, - = inactive. Values of * were significantly different from control at p<0.05*

Table 3: Antimicrobial activity of the vacuum liquid fractions of root extract of *Euphorbia graminea*

VLC Bulked fractions	Conc. (mg/mL)	Zones of inhibitions				
		S. aureus	*E. coli*	*P. aeroginosa*	*C. albican*	*A. niger*
2*	100	-	-	-	-	-
	50	-	-	-	-	-
	25	-	-	-	-	-
	12.5	-	-	-	-	-
	6.25	-	-	-	-	-
3-8	100	16.32±1.57	12.18±0.50	8.22±1.50	5.02±1.27	-
	50	12.35±0.98	7.66±1.28	3.11±1.28	-	-
	25	9.46±1.68	4.81±1.34	-	-	-
	12.5	5.67±1.52	-	-	-	-
	6.25	3.92±0.05	-	-	-	-
9-10*	100	7.50±1.50	3.50±0.50	-	-	-
	50	-	-	-	-	-
	25	-	-	-	-	-
	12.5	-	-	-	-	-
	6.25	-	-	-	-	-
Ciprofloxacin (Antibacteria[a]	5 µg/mL	28.61 ±1.44	23.09±1.32	25.01±1.10		
Clotrimazole[b] (Anti-fungal)	10 µg/mL				15.50 ±0.91	21.5±1.44

*Values are Mean ±SEM of three replicates (n= 3). Values of * were significantly different from control at p<0.05. - = no inhibition*

Previous investigations of *E.graminea* leaves extract showed potential antimicrobial efficacy against similar organisms (Ikpefan *et al*, 2020). This informed the choice of the roots and stems of this plant for this study. The findings of this research indicated that the methanol extracts of *E. graminea* roots recorded higher antimicrobial activity than the stems extract as evidenced by the different concentrations at which their zones of inhibition were observed. The reason for this could be as a result of the time of collection of the different parts of the plant as there could be a greater availability of metabolites in the plant's roots than the stem. This was also reflected in the results of the phytochemical screening, which demonstrated that the root extracts contained a greater number of metabolites than the stem extracts did. This distribution could also explain why the roots extract showed higher activity than the stem extract. The works of Rajeh *et al* (2010) recorded higher an antibacterial and fungi activity of the roots over the stem extract of *Euphoribia hirta* at which is in line with our work. All crude plant extracts had some physiologically active chemicals, and these compounds could be present in extremely high concentrations. The higher antimicrobial activity recorded by the root extract over stem extract which necessitated its fractionation could be as a result of higher concentrations of certain plant groups of metabolites.

Table 4: Minimum inhibitory concentration (MIC) of extracts and fractions of *E.graminea*

Extract	Conc.	Zones of inhibitions				
		S. aureus	*E. coli*	*P. aeroginosa*	*C.albican*	*Aspergilus niger*
Stem	6.25	+	+	+	+	+
	3.13	+	+	+	+	+
	1.57	+	+	+	+	+
	0.79	+	+	+	+	+
	0.39	+	+	+	+	+
Roots	6.25	-	+	+	+	+
	3.13	+	+	+	+	+
	1.57	+	+	+	+	+
	0.79	+	+	+	+	+
	0.39	+	+	+	+	+
	VLC bulked fractions of roots extract					
2	6.25	+	+	+	+	+
	3.13	+	+	+	+	+
	1.57	+	+	+	+	+
	0.79	+	+	+	+	+
	0.39	+	+	+	+	+
3-8	6.25	-	-	+	+	+
	3.13	-	-	+	+	+
	1.57	-	-	+	+	+
	0.79	-	+	+	+	+
	0.39	+	+	+	+	+
9-10	6.25	+	+	+	+	+
	3.13	+	+	+	+	+
	1.57	+	+	+	+	+
	0.79	+	+	+	+	+
	0.39	+	+	+	+	+
Ciprofloxacin	5 µg/mL	-	-	-	-	
Clotrimazole	10 µg/mL				-	-

Key: - = +: growth; -: No growth

Antimicrobial resistance in organisms is something that can be fought with the use of phytochemicals like flavonoids, tannins, alkaloids, terpenoids, etc (Khare *et al*, 2021). According to Coker *et al*, the antimicrobial properties of many plant and their compounds have been documented. For example, alkaloids have been documented to exhibit potent antibacterial activity against Gram-positive bacteria (Gurrapu and Mamidala, 2017; Mabhiza *et al*. 2016). Terpenoids commonly found in many plants have demonstrated antibacterial activity against bacteria (Ludwiczuk *et al*., 2016). The antimicrobial activities of the extracts were relatively weak compared to the control drugs which this is consistent with our previous work (Ikpefan *et al*, 2020). The higher activity recorded by the root extract over stem necessitated its fractionation. Despite the presence of several phytochemicals observed during the phytochemical screening, the roots extract still recorded minimal antimicrobial activity compare to the control drugs. However, upon fractionation, the activity of the roots extract was further enhanced. For example, at 50 mg/mL, vlc fraction B (3-8) recorded 12.6, 7.66 and 3.11 mm zones of inhibitions against *S.aureus*, *E.coli* and *P.aeroginosa* respectively while fractions A (2) and C (9-8) were inactive at similar concentrations. This further attest to the fact that the constituents of the roots which are responsible for the activities are concentrated more in the bulked-fractions B (3-8). The MIC which serve as a reference for the cure for most infections was determined for the extracts and fractions. This further provides evidence of the effectiveness of fraction B especially against *S. aureu.* The lower MIC range for *S. aureus* and *E. coli* recorded by the fraction B suggests the presence of a potential antimicrobial agent making it possible to reduce the growth of micro-organisms, thus, it may be used as an antimicrobial agent. This is because at this concentration there was no visible growth. However, the growth of the other organisms at the same concentration were not inhibited. The MIC showed that the bulked fraction B (3-8) produced more inhibitory effect on the test organisms as compared to the extracts. This may be as a result of the diffusion rate of the bioactive components of the fractions. This research has provided additional information on the antimicrobial studies of *E. graminea*.

*Conclusion***:** The study has further shown that apart from the leaves earlier reported, other parts of plant *E.graminea* has antimicrobial activities. However, unlike the stem, the roots of *E. graminea* demonstrated a higher antimicrobial potential which resides more in the vlc sub-fraction B. Further investigation on the

active fraction for a possible isolation of the active principles which could serves as antimicrobial agent for the development of drugs, needs to be carried out in future studies.

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