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Phytochemical Screening, Cytotoxicity, Antioxidant and Antimicrobial Activities of Stem and Leave Extracts of Euphorbia Heterophylla.

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Abstract

Phytochemical screening carried out on the stem and leave extracts of *Euphorbia heterophylla* (Euphorbieacea) confirmed the presence of Carbohydrates, gycosides, reducing sugar, saponins, taninns, phlobatanins, cardiac glycosides, steroids, triterpenes, and flavonoid. cytotoxicity test using brine shrimp lethality assay gave the LC_{50} value ($\mu g/cm^3$) are 20.67, 25.07, 158.56,and 176.55 for n-Hexane fraction, Ethyl Acetate fraction, Butanol fraction and Aqueous fraction of the stem while 23.50, 30.20, 164.10 and 179.80 for n-hexane fraction, Ethyl Acetate fraction, Butanol fraction and Aqueous fraction of the leave extracts. The results of antioxidant properties of the stem and leaves extracts showed that the extracts exhibited strong activity as a radical scavenger in the experiment using 2, 2-diphenyl-1-picryhydrazyl radical (DPPH) indicating that the plant has strong ability to

donate hydrogen when compared with the standard butyrate hydroxyl anisole (BHA). The antimicrobial activity of the extracts was carried out against *Staphylococcus aureus, E.coli , Pseudomonas aeruginosa, Streptococcus pneumonia,* and *Candida albicans,* the results showed moderate to low activity for the test organisms.

Keywords: Phytochemical screening, Antimicrobial activity, Antioxidant activity, *Euphorbia heterophylla.*

1. Introduction

Plants or herbs have been found to have medicinal and therapeutic importance in the prevention, treatment or cure of diseases and ailment. This knowledge has been passed down from one generation to another either verbally or in writing (Sofowora, 2008).The universal role of plants in the treatment of diseases is exemplified by their employment in all major systems of medicine (Okeniyi *et al*., 2007). *Euphorbia heterophylla* is a toxic plant which belongs to the family of *Euphorbiaceae*. It is commonly called Mexican fire plant, milk weed and Spurge weed in English. The toxicity of the plant, especially the root and latex is recognized in Africa. The latex is acrid and the toxic principle is neither alkaloid nor glycoside but probably a resin which can prove fatal (Hartwell *et al*., 1969). Despite the toxicity hazard of this plant, it has various medicinal properties which include its use in the reatment of gonorrheal, respiratory tract infection, malaria, eczema, asthma and wart cure.

The report by Omale and Emmanuel, (2010) shows that the Ethanol extract and water free extract of *Euphorbia heterophylla* leave contain some wound healing properties. (Falodun and Agbakwuru, 2004) reported the isolation of a flovonoid, quercetin from crude extract of the leaves of this plant. The leave is known to posse's antibacterial activity (Falodun *et al* 2003). Toxicity is documented in most of the genus Euphorbia with individual sensitive to latex. This study investigated the antioxidant, cytotoxicity, and antimicrobial activity of stem and leave extracts of *Euphorbia heterophylla* plant.

2. Materials and Methods

Plant Collection and Identification; The stem and the leave of *Euphorbia heterophylla.* Was collected from Nigerian defence academy Afaka area Kadunna, the sample was identified by Mr Yahaya Abdullahi of Herbarium section, Biological Science Department, Nigerian Defence Academy. The collected leave and stem were cleaned, air dried and pulverized.

Extraction; A portion (200 g) each of the pulverized plant material were separately extracted using soxhlet apparatus with (500 cm^3) methanol each as solvent . The extracts was collected and concentrated with the aid of a rotary evaporator at 40^oC to obtain the crude extracts which was weighed and kept at ambient temperature.

Fractionation:

A portion (21g) of crude extract was dissolved in 250 cm^3 distilled water in a separatory funnel and250 cm^3 of n-hexane were added, shaken and allow to stand over night for the two layers to

separate. The two layers were drain separately and the upper n-hexane layer was evaporated using rotary evaporator, The same procedure was repeated using - Ethyl acetate (250 cm^3) and Butanol (250 cm^3) to get Hexane, Ethyl acetate and Butanol fraction. (Aliyu *et al* 2008).

Phytochemecal Screening; The crude extracts above was used to test for the presence of the following secondary metabolites: alkaloids, flavonoids, steroids, saponins, phenols, tannins, glycosides, reducing sugars, anthrquinones, carbohydrates, resin, cardiac glycosides, and tannins, using standard methods described by Okeniyi *et al*., (2007) and sofowora (2008).

Brine Shrimp Cytotoxicity Test; screening of the extracts against Brine shrimp larvae was carried out according to Falope *et al* (1993) and Oloyede *et al* (2010). In this assay, a drop of dimethyl sulphoxide (DMSO) was added to test and control vials to enhance the solubility of the test materials.

Antioxidant Activity

In order to investigate the antioxidant properties of the extracts, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay were employed.

Scavenging effect on DPPH; 0.5M of the free radical source 2.2-diphenyl-l-picry hydrazyl radical (DPPH) solution in methanol was prepared and 3$cm^3$of this solution was mixed with 2cm^3 of the extract solution at varying concentration(1.0 mg/ml, 0.5mg/ml. and 0.25 mg/ cm^3) **(**Cow-chin *et al*, 1994, Mellors and Tappel, 1996, and Lugasi *et al*, 1999**)** The decrease in absorption at 517 nm of DPPH was measured after 10 minutes of incubation. The actual decrease in absorption was measured against that of control and the percentage inhibition was calculated. The same experiment was carried out using Butyrate Hydroxyl Anisole (BHA), Vitamin C and a-Tocopherol, a known antioxidant which were all used as the standard. All tests and analysis was carried out in triplicates and the results obtained were averaged. The activity was determined as a function of their % inhibition which was calculated using the formula;

$\%RSA \text{ or } \% \text{ inhibition} = \{(A_{DPPH} - A_S)/A_{DPPH}\} \times 100$

Where A_S =Absorbance of the solution A_{DPPH} = Absorbance of the DPPH solution (Hatano *et al*, 1988).

Antimicrobial Assay

The antimicrobial assay of each extracts was evaluated using method described by Egwaikhide *et al*., 2008 and Okeniyi *et al*., 2000.

Table 1. Result of Phytochemical screening of Stem and Leaves of *Euphorbia heterophylla*

2° metabolites	Crude extract of stem	Crude extract of leaves
Alkaloids	_	_
Carbohydrates	+	+
Glycosides	+	+
Free anthraquinone	_	_

Combined anthraquinone	–	–
Reducing sugar	+	+
Saponins	+	+
Tannins	+	+
Cardiac glycosides	+	+
Steroids	+	+
Triterpenes	+	+
Flavonoids	+	+
Phlobatannins	+	+

Where: + = present - = negative

Table 2. Result of Brine –shrimp lethality test of Euphorbia heterophylla stem and leave Extracts.

Sample	Plant part	LC_{50} µg/cm^3
n-Hexane fraction	Stem	159
n-Hexane fraction	Leaves	165
Ethyl Acetate fraction	Stem	25
Ethyl Acetate fraction	Leaves	30
Butanol fraction	Leaves	21
Butanol fraction	Stem	23
Aqueous fraction	Stem	247
Aqueous fraction	Leaves	280

Table 3. % Inhibition of DPPH –Free –Radical Scavenging Activity of Stem and Leave Extracts

Conc. mg/cm^3	hexane stem %	Ethyl acetate stem %	Butanol stem %	Hexane leave%	Ethyl acetate leave%	Butanol leave %	AA %	BHA %	α-Tocopherol %
0.25	65	73	79	55	80	45	44	91	10
0.5	84	78	81	65	94	65	65	93	12
1.0	90	83	88	89	98	94	68	94	12

KEY: BHA = Butylated Hydroxyl Anisole. AA= Ascorbic Acid

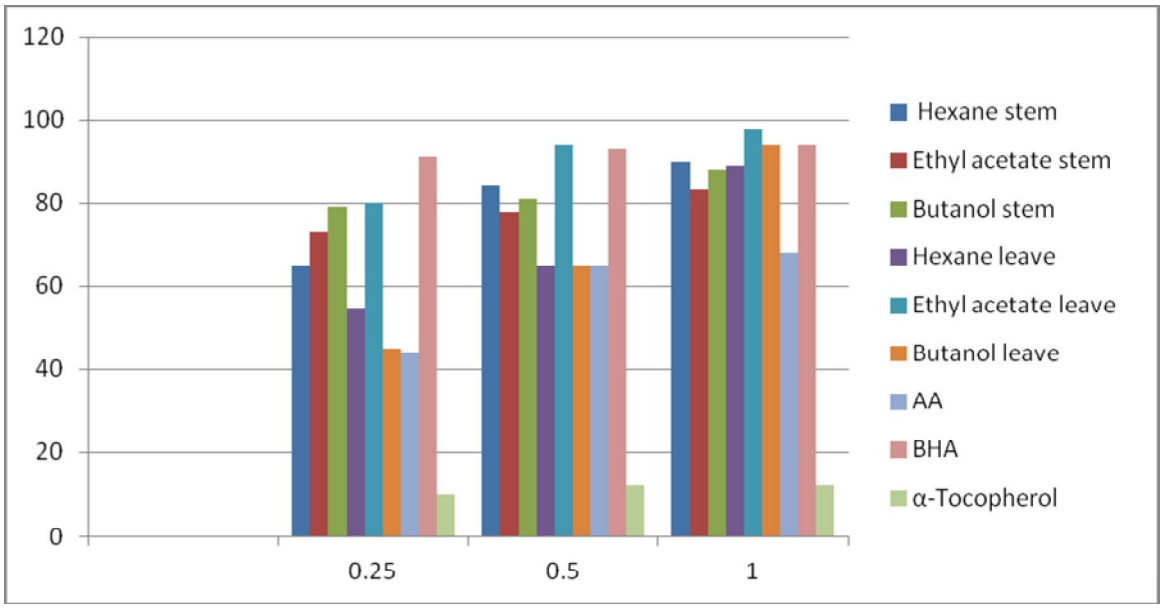

Figure 1. percent inhibition scavenging activity of DPPH of the stem and Leave extracts of *Euphorbia heterophylla*

Table 4. Result of Antimicrobial test of *Euphorbia heterophylla* stem and leave Extracts

Plant part	Extracts	Concentration (mg/cm^3)	S.a	E.coli	P.sa	Strep	C.a
Stem	Hexane	0.25	11	16	11	10	13
		0.5	14	20	14	16	15
		1.0	16	25	18	24	19
		Control	NI	NI	NI	NI	NI
	Ethyl acetate	0.25	15	16	14	13	14
		0.5	24	23	21	18	19
		1.0	30	29	30	24	28
		Control	NI	NI	NI	NI	NI
	Butanol	0.25	15	12	10	16	15
		0.5	17	21	20	21	22
		1.0	27	31	28	25	31
		Control	NI	NI	NI	NI	NI
Leave	Hexane	0.25	8	9	11	10	10
		0.5	11	12	14	16	14
		1.0	14	18	18	19	17
		Control	NI	NI	NI	NI	NI
	Ethyl acetate	0.25	17	18	15	14	13
		0.5	23	26	23	23	19
		1.0	27	30	31	25	27
		Control	NI	NI	NI	NI	NI
	Butanol	0.25	16	14	10	16	15
		0.5	19	20	20	21	22
		1.0	26	32	27	24	31
		Control	NI	NI	NI	NI	NI

KEY: S.a=staphylococcus aureus, E.coli=Escherichia coli, Ps.a=pseudomonas aeruginosa, strep=streptococcus pneumonia, and C.a=Candida albicana

3. Result and Discussion

The result of phytochemical screen presented in Table 1 showed the presence of saponinns, reducing sugar , Glycosides, Triterpenes, flavonoids, Cardiac Glycoside, Carbohydrate, steroids , tannins and phlobatannins.in the stem and leave of *Euphorbia heterophlla* with exception of alkaloids and anthraquinone in both sample. The presence of this phytocompounds in the methanol extract have been reported to be responsible for the anti inflammatory and anti microbial properties displayed by many medicinal plants (Taylor *et al*,1996) Falodun *et al* 2006 : Salihu and Garba, 2008. The result of brine shrimp lethality test (BST) (Table 2) are in agreement with those of phytochemical where the majority of the phytocompound appeared to be present in the crude extract of the plant (Table 1&2). The activities exhibit by crude extracts of stem and leave from highly polar solvent extracts to non-polar solvent extracts (Butanol, Ethyl acetate and n-Hexane). The highest, stem extract (Butanol) and leave (Butanol) (BST LC_{50} 21 µg/cm^3 and 23 µg/cm^3) to the lowest stem (n-Hexane) and leave (n-Hexane) (BST LC_{50} 159 µg/cm^3 and 164 µg/cm^3) respectively. However the aqueous extracts of stem and leave (BST LC_{50} > 1000 µg/cm^3) was in active. In this anti microbial assay, zone diameter of inhibition of 21- 25mm and 26-35mm correspond to moderate and maximum activities respectively (Aminabhavi *et al*., 1984). The result obtained (Table 4) show that most of the extract indicated high presence of phytocompound as well as high activities against the shrimp larvae also showed high activities against the test organisms. For instance stem extract(Butanol) and leave (Butanol) (BST LC_{50} 21 µg/cm^3 and 23 µg/cm^3) showed high activity against all the test microbes with maximum zone inhibition diameter of 31 & 32 mm against *E.coli*, and *C. albicans* (see Table 4). Similarly stem extract (Ethyl acetate) and leave extract (ethyl acetate l) (BST LC_{50} 25 µg/cm^3 & 30 µg/cm^3) inhibited the growth of all the test microbes , (except. *Streptomycin*) with maximum inhibition diameter of 30 & 31mm against *Pseudomonas aeruginosa*. However, low activities were generally recorded in the n-hexane extracts which is conformity with low activity in BST (see Table 2-3). stem extract (n- Hexane) (BST LC_{50} 159µg/cm^3) and leave extract (n-Hexane) (164µg/cm^3) exhibited low activities against all test microbes(Table 3).The moderate anti microbial activities recorded in different solvent extracts of stem and leave of *E. heterophlla* against *E.coli*, *Candida albican*, *Streptomycin*, *staphylococcus aureus* and *Pseudomonas aeruinosa* suggest that this plant s may be potential source of ingredients that may be employed in the treatment of typhoid, malaria, boil, respiratory tract and other diseases cause by the test organisms. Antioxidant activity of all extracts as measured by ability to scavenge (DPPH) free radicals was compared with the standard s Ascorbic acids, Butylated Hydroxyl Anisole (BHA) and α- Tocopherol. It was observed that Ethyl acetate of leave extracts had higher activity than the n-Hexane and Butanol extracts of the leave. At concentration of 1.0mg/cm^3 .The scavenging activity of ethyl acetate extract reached 97%. n-Hexane and Butatnol reached 89% and 93% respectively. The DPPH radicals scavenging ability of all the extracts were closer to that of BHA and higher than Ascorbic acid and α-Tocopherol(see fig 1). The ethyl acetate of both stem and leave scavenging ability was found to be higher than BHA 94% at 1.0mg/cm^3 the study show that the extracts have the proton donating ability and could serve as free –radical inhibitor s..

4. Conclusion

The maximum antimicrobial activities exhibited by the ethyl acetate and Butanol extract of stem and Leave of *E. heteophylla* against *E. coli*, and *Candida albicans* respectively suggest that their extracts may be used for the treatment of typhoid, malaria, boil, respiratory tract and other diseases cause by the test organisms. The higher antioxidant activities exhibited by the extracts indicate that the extracts have the proton donating ability and could serve as free –radical inhibitor. This shows that the plant could be useful as antitumor, anticancer and as antimicrobial agent.

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