Bima Journal of Science and Technology, Vol. 8(1B) Apr, 2024 ISSN: 2536-6041

DOI: 10.56892/bima.v8i1.623

Fractionation and Characterization of Natural Antibacterial Compounds from E*uphorbia hirta Linn* (Asthma Plant)

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ABSTRACT

Euphorbia hirta Linn (asthma plant) has for long been utilized as an herbal medicine globally in remedying and curing many an infection and disease in humans. Different parts of *Euphorbia hirta* for instance roots, leaves, saps, stems, flowers have been employed in ethnomedicine as it possesses a broad range of biological and pharmacological activities. The objective of this research study was to fractionate and characterize the natural antibacterial compounds from *Euphorbia hirta* plant. In the course of work, three different extracts (methanol, n-hexane and aqueous) of the plant were subjected to qualitative phytochemical analysis and the result signified the presence of metabolites. Crude extracts of the plant were tested against *Escherichia coli, Klebsilla pneumoniae, Staphylococcus aureus* by the cup plate agar diffusion assay method to explore the anti-microbial activity for its therapeutic applications. The plant exhibited an extensive antibiotic activity against the tested bacteria, calculated by measuring diameters of the zone with no microbial growth. The structure of compounds obtained from sample fractions through Column Chromatography were established using a combination of FT-IR and GC-MS. The following functional groups were detected; C-H vibration stretching at 2950cm^{-1} and 2974cm^{-1}, CH_2 bend at 1424cm^{-1}, 1452cm^{-1} and 1454cm^{-1}, C-H (stretch aliphatic) at 2917cm^{-1} and 2889cm^{-1}, C-O (esters) at 1296cm^{-1}, 1274cm^{-1} and 1275cm^{-1}, C=O (carboxylic) at 1703cm^{-1}, 1654cm^{-1} and 1714cm^{-1}.The peaks obtained from GC-MS compared with corresponding library hits showed that the major compounds were vinyl laurate, monoethylhexyl phthalate and lauric acid triglyceride. From the results of this study, *Euphorbia hirta* plant can be a potential source of useful antibiotic drug.

Key words: *Euphorbia hirta*, fractionation, phytochemical screening, bacteria, FT-IR, GCMS.

INTRODUCTION

From the time immemorial, plants have been employed in the globe by diverse societies in treating communicable diseases and ailments principally to keep stable health. Scores of drugs that are made use of by consumers for medical and therapeutical functions are obtained from plants. Plants can be referred to as the primary stream of medicine since they can produce bulky bioactive compounds for pharmacological activity. The compounds are utilized by plants to safeguard attack from herbivorous insects and other are derivatives of growth and development processes in plants. Many a number of these compounds possess biochemical properties which are valuable to the well-being of humans. (Buli *et al*., 2015).The account of herbal medicine therapy is directly associated with conventional medicine and is known as botanical medicine or phyto-medicine (Bent,

DOI: 10.56892/bima.v8i1.623

2008). It dates back to several years that the use of plant parts like leaves, bark, seeds, roots, juice or flowers for the treatment of various infections and ailments. (Falodun, 2010). In rural parts of under-developed countries, the dependence of medicinal herbs as primary health care is incredibly prominent and people at grass root especially in Nigeria are not excepted. The application of herbal medicine derived from plants especially for prophylactic and curing of ailments and communicable diseases has greatly enhanced the health of people (Nascimento and Locatellic, 2000). Presently in many developing countries, manufactured drugs are not only costly and insufficient for the management and combating of diseases but are also often contaminated with consequential side effects (Shariff, 2001).

Euphorbia hirta (Figure 1) is a popular medicinal plant with wide distribution in the Africa, Australia, and Asia. It is native to Central America.(Ghosh *et al*, 2019).

Figure 1: *Euphorbia hirta* Linn

Euphorbia hirta is popularly known as "*Irawo- Ile"* by the Yoruba tribe, South-Western Nigeria. It is slender-stemmed and typically spring upright up to 40cm high, although at times seen creeping over the ground. It has a furry stem with numerous branches from the base to the top. The leaves are opposite, elliptical, oblong-lanceolate, with a faintly toothed margin and darker on the upper surface. The flowers are tiny, numerous and crowded together in dense cymes (dense clusters in upper axils) about 1 cm in diameter. The stem and leaves produce a white or milky sap when cut. It is frequently seen occupying open waste spaces, banks of watercourses, grasslands, road sides, and pathways (Sandeep *et al*.,2009). All the parts of *Euphorbia hirta* are used in folk medicine. The plant is known for its anti-bacteria, anti-malaria, anti-inflammatory, galactogenic, anti-asthmatic, anti-diarrhea, anti-oxidant, anti-infertility, anti-amoebic and anti-fungal properties as well as therapeutic effect on urine output and electrolytes (Kumar *et al*., 2010).

In Nigeria, the plant's extracts or exudates are employed as ear drops, boils treatment, sore and facilitation of wound healing (Igoli *et al*., 2005). *Euphorbia hirta* is known for an analgesic to suppress chronic headache, colic, toothache, rheumatism and pains during pregnancy. It is used as an antidote and pain relief of scorpion stings and snakebites. The use of its latex to facilitate removal of thorns from the skin is common (Singh *et al*.,2005). The increase in antibiotic resistance, cost and inaccessibility to some orthodox modern antibiotics is however making local herb to gain more popularity (Doughari *et al*., 2008). In addition to the numerous applications of this plant, this work was set out to fractionate, characterize and determine the antimicrobial activity of *Euphorbia hirta* against some pathogenic bacteria.

Bima Journal of Science and Technology, Vol. 8(1B) Apr, 2024 ISSN: 2536-6041

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Taxonomic classification of *E. hirta*: (Ghosh *et al*, 2018: Al-Snafi, 2017: Asha *et al*., 2014).

Table 1: Taxonomic classification of *Euphorbia hirta*

Kingdom	***Plantae***	**Superorder**	***Rosanae***
Subkingdom	*Viridaeplantae*	Order	*Malpighiales*
Infrakingdom	*Straptophyta*	Family	*Euphorbiaceae*
Division	*Tracheophyta*	Genus	*Euphorbia*
Subdivision	*Spermatophytina*	Species	*Euphorbia hirta*
Infradivision	*Angiosperms*	Botanical Name	*Euphorbia hirta Linn*
Class	*Magnoliopsida*	Common Name	Asthma plant, milk weed, Cats hair, hairy spurge or snake weed

MATERIALS AND METHODS

Collection and identification of plant sample

The fresh plant of *Euphorbia hirta* was collected around the Faculty of Health Sciences of the University of Ilorin, Kwara State, Nigeria in September of 2014. Taxonomical Identification of the plant was confirmed at Botany Department, Faculty of Physical Sciences, University of Ilorin, Nigeria where voucher sample with specimen number UIH001/848 dated 22-10-2014 was deposited for future reference.

Extraction Procedure

The extracts of the plant were achieved according to an earlier technique (Leonard *et al*., 2013). The plant of *Euphorbia hirta* was air-dried at ambient temperature, pulverized to obtain 350g of powder. The pulverized form of the plant sample was extracted in succession with 600ml n-hexane and methanol at ambient temperature for seven days each. Each extract was decanted, filtered and concentrated to dryness using rotary evaporator (Buchi, R-200 Switzerland) to obtain the crude n-hexane and methanol extracts respectively. For aqueous extraction, about 150g of the pulverized form of *Euphorbia hirta* were macerated in 1.0 Litre of hot water and allowed to stand at room temperature for 24 hours. The hot water extract was then filtered through muslin cloth on a plug of glass wool in a glass column. The resulting hot water extract was concentrated and evaporated to dryness using rotary evaporator at an optimum temperature of between 40 and 45^{o}C to avoid denaturation of the active ingredients. The concentrated extracts were all stored in the refrigerator at 0^{o}C until further analysis.

Phytochemical Screening of Crude Extracts

Phytochemical involves chemical properties of plants or plant-derived products. Phytotherapy is a way of taking care and prevention of numerous diseases with the use of the bioactive compounds which are secondary metabolites of medicinal plants (Ghosh *et al*, 2018; Ghosh *et al*, 2019; Mohammad *et al*., 2017). The crude extracts of *Euphorbia hirta* were subjected to qualitative phytochemical analysis for the presence of some metabolites such as terpenoids, alkaloids, flavonoids, tannins, saponins, phenolic compounds, phytosterols, glycosides, carbohydrates, proteins and amino acids using standard procedures (Sofowara, 1993: Harbone, 1998; Trease and Evans 2002).

Test Organisms

The bacterial strains (*Escherichia coli, Klebsilla pneumoniae,* and *Staphylococcus aureus*) were obtained from the Microbiology Laboratory, Department of Microbiology, University of Ilorin, Nigeria.

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Culture Media and Inoculums Preparation

The nutrients agar media were used for the culturing of bacterial strains. Aseptically inoculation of loops of bacteria culture in a conical flask containing 30ml of nutrient broth were incubated at 37°C for 24hours in order to obtain active strain. The pure cultures on the nutrient agar plates were used as the inoculums. The nutrient broths were poured into Petri-plate for test culture. The content of the plates were allowed to solidify, then 0.25ml of test strains were inoculated in the agar plates separately and wells approximately 4 mm in diameter and 2.0 mm deep were bored on the surfaces of the agar medium using a sterile cork borer

Antimicrobial Susceptibility Assay

Crude methanol, n-hexane and aqueous extracts obtained from the plant sample were analyzed for their antibiotic activity by agar well diffusion method (Esimone *et al*., 1998). The solution prepared for each of the extracts of*Euphorbia hirta* contained different concentration (100mg/ml, 75mg/ml, 50mg/ml), and thesewere introduced into the well and plates were incubated at 37°C for 24hours. The growth of bacteria was established by evaluating the zone of inhibition diameter (clear zone around each well) in millimeter (mm) (Ogbulie *et al*., 2007). For each bacteria strain, negative controls were maintained as DMSO solvent and the antibiotic Ciprofloxacin (50mg/ml) was used as a positive control for comparison.

RESULTS AND DISCUSSION

Plants play a crucial role in traditional system of medicine. Studies on medicinal plants are gaining consensus in recent years in most part of the world. In the present study, three different extracts (methanol, n-hexane and aqueous) of *Euphorbia hirta* were subjected to percentage yields, qualitative phytochemical analysis, fractionation and characterization of natural antibacterial.

The percentage yields of extracts and the phytochemical constituents of the plants are shown in table 2 and 3. respectively.

Table 2: Percentage yield of the crude extracts of *Euphorbia hirta*

Extraction solvent	Raw plants powder (g)	Extracted plant powder (g)	Percentage yield (%)
Methanol	100	12.0	12.0
N-hexane	100	6.9	6.9
Aqueous (Distilled Water)	100	19.5	19.5

Table 3: Phytochemical screening of extract of E*uphorbia hirta*

S/N	Phytochemicals	Test performed	Methanol	N-hexane	Aqueous
1	Terpenoids	Salkowski	+	+	+
2	Alkaloids	Mayer	+	-	+
3	Flavonoids	Alkaline reagent	+	+	+
4	Saponins	Froth	+	-	+
5	Tannins	Gelatin	-	+	+
6	Glycosides	Modified Borntrager	+	+	+
7	Phenolic compounds	Ferric chloride	+	+	+
8	Carbohydrates	Benedict	-	-	-
9	Proteins and Amino acids	Xanthoproteic Test:	+	-	+
10	Phytosterols	LibermannBurchard	+	+	+

Keys: + = Present, - = Absent.

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The highest yield of plant extract was found when extraction was done with aqueous (19.5%), followed by methanol (12%) and the lowest for n-hexane (6.9%). This is most probably occasioned by differences in the solvents polarity.The percentage yield is directly proportional to polarity of the solvent used i.e. aqueous > methanol > n-hexane. These yields were far higher than what was obtained for the aqueous, methanol and n-hexane extraction of the same plant with the corresponding yield of 3.9% , 1.8% and 1.3% respectively (El-Mahmood 2009). Yield of 5.29% ethanol extract, 4.72% chloroform extract and 1.84% n-hexane extract of *Euphorbia hirta* leaves while 3.26% ethanol extract, 42.54% chloroform extract and 1.63% n-hexane extract of *Euphorbia hirta* flowers have been reported by (Waseem *et al*., 2017).Factors like the age of the plant, time of extraction, temperature, nature of solvent, solvent concentration, and polarity significantly affect extraction yield of bioactive materials (Ncube *et al*., 2008). Thus in this study, distilled water with the yield of 19.50% was found to be the best solvent for the extraction of this plant material.

The phytochemical screening showed the presence of contains terpenoids, alkaloids, flavonoids, tannins, saponins, phenolic compounds, phytosterols, glycosides, proteins and amino acids. These compounds have potential application against human pathogens, including those that cause enteric infections (El-Mahmood *et al*., 2008). A number of work has connected the presence of these bioactive compounds to the antimicrobial properties of crude plant extracts (Owolabi *et al*., 2007) ; Ogbulie *et al*., 2007; Sahm and Washington, 1990; Adesokan *et al*., 2007; Oyeleke *et al*., 2008). The presence of alkaloids is particularly exciting, because many of them are used as analgesic, antimalaria, and stimulant (Duke and Ayens, 1985). The presence of saponins glycosides and flavonoids are known to hinder lump development and serve also to guard against gastrointestinal infections. These are of pharmacognostic importance and give credence to the use of the plant in ethno medicine. Herbs that have tannins as their components are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery thus exhibiting antibacterial activity (Akinpelu and Onakoya 2006). Tannins are widely used in traditional medicine in treating wounds and to arrest bleeding (Nguyi, 1988). Some of these bioactive compounds which are synthesized as secondary metabolites as the plant grows also serve to protect the plant against microbial attacks and predation by animals (El-Mahmood *et al*., 2008). Phytochemical study of methanol and water extract of *Euphorbia hirta* leaf indicated the presence of reducing sugar, saponins, phenols, flavonoids and xanthoprotein.

The results of the antibacterial screening of the different concentrations of the extract on the test isolates are shown in Tables 4.

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Table 4: Antibacterial activity of *Euphorbia hirta* plant extracts in different solvents.

Extracts	Conc. of Extracts (mg/ml)	Diameter of Zone of inhibition of bacteria (mm)		
		Escherichia coli	*Klebsilla pneumoniae*	*Staphylococcus aureus*
Methanol	100	11	9	8.3
	75	8	7	5
	50	6	NI	NI
N-hexane	100	9	6.	7.0
	75	6	5.2	4.8
	50	4	NI	NI
Aqueous	100	13	12.0	9.6
	75	8	9.4	6.8
	50	5.6	5.5	4.3
Ciprofloxacin	50	23	21	20
DMSO solvent		NI	NI	NI

Ciprofloxacin and DMSO were used as control.
NI = no inhibition

Diameter of Inhibition zone (mm) of different concentration (100mg/ml, 75mg/ml, 50mg/ml).

The diameters of the zone ofinhibition for *Euphorbia hirta* plant of methanol, n-hexane andwater extract against tested bacteria were measured in millimeters.All test organisms were inhibited by up to20% concentration and the efficiency was a direct function of concentration. At 100 mg/ml, aqueous, methanol, and n-hexane extracts showed the maximum zone of inhibition against*Escherichia coli* as 13mm, 11mm and 9mm respectively. At 100 mg/ml, aqueous, methanol, and n-hexane extracts showed the maximum zone of inhibition against*Klebsilla pneumoniae* as 12mm, 9mm and 6mm respectively. At 100 mg/ml, aqueous, methanol, and n-hexane extracts showed the maximum zone of inhibition against*Staphylococcus aureus*as 9mm, 8mm and 7mm respectively. At 50 mg/ml n-hexane extracts showed no inhibition against*Klebsilla pneumoniae* and *Staphylococcus aureus*as 9mm, 8mm and 7mm respectively. This indicates that aqueous extract showed the broadest spectrum of activity against all the tested organisms. All the extracts in most cases exhibited appreciable spectra of activities and were more effective on the test microorganisms and results showed that increased concentration of extract increased the zone of their growth inhibition. All tested bacterial had no inhibition on DMSO solvent. The growth of all the bacteria were inhibited though to varying levels, thus establishing the use of the plant in treating enteric infections. Most of the organisms used in this study were the causative agents responsible for diarrhea, asthma, bronchitis, eczema and dysentery. This result supported the earlier report that *Euphorbia hirta* can be used in the control of diarrhea, dysentery and many a bacteria associated with enteric infections (Kokwaro, 1993).

Bima Journal of Science and Technology, Vol. 8(1B) Apr, 2024 ISSN: 2536-6041

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Figure 2: Zone of Inhibition (mm)

Column chromatographic separation of the methanol crude extracts

About 5.6g of concentrated methanol extract was subjected to column chromatography on silica gel and eluted with solvents in increasing order of polarity using only n-hexane, n-hexane : methanol (2;1), n-hexane: methanol (1;2), and only methanol. 13 fractions of about 50ml each were collected. The fractions were examined for their TLC profile and fractions with similar constituents were pulled together and concentrated via evaporation. Fractions obtained were coded EH_1, EH_2 and EH_3.

Spectroscopic analysis

The partially purified fractions were characterized using data from Gas Chromatography-Mass Spectroscopy (GC-MS) and Fourier Transform Infrared (FT-IR) Spectroscopy.

Tables 5 shows the results of GC-MS analysis of the fractionates of *Euphorbia hirta* coded EH_1, EH_2 and EH_3 respectively.

Table 5:GCMS Analysis of *Euphorbia hirta* (EH_1, EH_2 and EH_3)

SN/Fraction	Name of the proposed compounds	$R_{.Time}$	Area %	Major fragments, *m/z*)
EH_1				
1	Monoethylhexyl phthalate	40. 161	96.55	55, 70, 83, 95, 104, 132, 149, 167, 183
EH_2				
1	Vinyl laurate	37.264	13.90	41, 43, 57, 71, 85, 98, 112, 127, 158, 171, 183
2	Lauric acid triglyceride	37.451	33.40	41, 43, 57, 71, 85, 98, 112, 127, 158, 171, 183,201.
EH_3				
1	Lauric acid triglyceride	24.576	39.50	41, 43, 57, 71, 85, 98, 112, 127,140, 155

Tables 6 shows the results of FT-IR analysis of the fractionates of *Euphorbia hirta* coded EH_1, EH_2 and EH_3 respectively.

Table 6: Characteristics FTIR data of the fractionated compounds.

S/N	Sample code	C-H Stretch (cm^{-1})	CH_2 Bend (cm^{-1})	C-O Ester (cm^{-1})	C-H (stretch aliphatic) (cm^{-1})	C=O Carboxylic (cm^{-1})
1	EH_1	2950.06	1424.50	1296.39	2917. 14	1703. 20
2	EH_2	2974.23	1452.40	1274.95	2835.36	1714.92
3	EH_3	2974.23	1454.92	1274.95	2889.37	1714.92

DOI: 10.56892/bima.v8i1.623

The FT-IR data also supported the observation recorded with GC-MS result. Two peaks were obtained from GC-MS of EH_1. The comparison of these peaks with the corresponding library hits showed that the major compound was Monoethylhexyl phthalate. The FT-IR data for EH_1 obtained indicated the C-H vibration stretching at 2950cm^{-1}, CH_2 bend at 1424cm^{-1}, C-H (aliphatic) at 2917cm^{-1}, C-O (esters) at 1296cm^{-1} and C=O (carboxylic) at 1703cm^{-1} respectively. Thus the IR data agreed with the functional groups present in the compound suggested by the GC-MS. Regarding EH_2, five peaks were obtained from GC-MS. The comparison of the various peaks with the GC-MS library showed that the major compounds were Vinyl laurate and Lauric acid triglyceride. The C-H vibration stretching appeared at 2974cm^{-1}, CH_2 bend at 1452cm^{-1}, C-H (stretch aliphatic) at 2835cm^{-1}, C-O (esters) at 1274cm^{-1}and C=O (carboxylic) at 1714cm^{-1}. Similarly, nine peaks which corresponds to nine compounds were obtained from GC-MS results. When compared with machine library showed that the major compound was Lauric acid triglyceride. The FTIR data obtained for EH_3 showed the C-H stretch vibration frequency at 2974cm^{-1}, CH_2 bend occurred at 1454cm^{-1}, C-H (stretch aliphatic) at 2889cm^{-1}, C-O (esters) at 1274.95 cm^{-1}and carboxylic C=O at 1654cm^{-1}. The compounds Vinyl laurate and Lauric acid triglyceride are both fatty acid compounds present in several medicinal plants (Williams and Fleming, 1995) and had been shown to possess antimicrobial properties (Ajoku et al., 2015; Bodoprost and Rosemeyer, 2007). The proposed structures of the compounds obtained from *Euphorbia hirta* is shown in Figure 7(a, b and c) below.

CONCLUSION

In the aspect of drugs innovation and design, plants have proved to be a rich source of restorative compounds. Over the last three decades, many a new drug have been discovered, essentially due to continuous researches executed by pharmaceutical industry and academia. From this study, *Euphorbia hirta* plantcan be seen as a potential stream of valuable phytochemical and antimicrobial activity.. It was found that extracts of the plant contain enormous quantity of terpenoids, alkaloids, flavonoids, tannins, saponins, phenolic compounds, phytosterols, glycosides, proteins and amino acids. Although *Euphorbia hirta* was found to contain some bioactive compounds with bio-efficacy and bioactivity within moderate limit under the fractionation work, further work will give emphasis to the isolation and characterization of active principles. This could be done by employing enhanced modern techniques such as HP-LC, NMR and the likes in identification of molecular level to improve drug formulation of this plant which will definitely give adequate promising results.

Acknowledgement

The authors would like to express our hearty gratitude to the God Almighty whose way of life has been a continuous guidance for us. The lead author specially addressed his sincere appreciation to his resourceful team on this work for their kind support and necessary help to make the original research paper.

Conflict of Interests

The authors have not declared any conflict of interests.

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