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EXTRACTION, FRACTIONATION AND *IN VITRO* PHYTOCHEMICAL INVESTIGATION OF CRUDE LEAF EXTRACT OF ETHYL ACETATE AND FRACTIONS OF *EUPHORBIA HETEROPHYLLA*

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Abstract

Euphorbia heterophylla's leaf is widely used in indigenous medicinal practices in Kano State, Nigeria and other parts of the world, because of its tremendous medicinal properties which include purgative, treatment of gonorrheal, respiratory tract infection, malaria, Eczema, Asthma and wart cure. The present study was designed to use standard procedures to extract, fractionate and investigate (*in vitro*) the phytochemical ingredients of ethyl acetate extract and fractions of *Euphorbia heterophylla's* leaf. Extraction of the plant's leaf was done using ethyl acetate as a solvent to obtain the crude ethyl acetate extract (CEE), while fractionation of the crude was done using petroleum ether, chloroform and ethyl acetate to afford petroleum ether fraction (Eh_1), chloroform fraction (Eh_2) and ethyl acetate fraction (Eh_3). The *in vitro* phytochemical test to investigate the phytochemical content of the crude extract and fractions was carried out via standard protocols, which revealed the presence of carbohydrates, flavonoids, saponins, steroids and tanins. These phytochemical ingredients justifies some aspects of indigenous mental acceptance on the *Euphorbia heterophylla's* leaf and validate its popular usage in traditional medicinal practices in Kano State, Nigeria and other part of the world for the treatment of infectious diseases that need clinical attention.

Keywords: Extraction, Fractionation, phytochemical screening and *Euphorbia heterophylla.*

Introduction

Medicinal plants have biologically active compounds that are used for treating various human diseases and also play important roles in curing (Arsule and Sable, 2017). Due to the presence of biologically active secondary metabolic constituents, they showed antifungal, antibacterial, antidiabetic and anti-inflammation activities (Waseem *et al*., 2017). This knowledge has been passed down from one generation to another either verbally or in writing (Sofowara, 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).

The world Health Organization supports the use of traditional medicines, provided they are proven to be efficacious and safe (WHO, 1995). In the developing countries, vast number of people live in extreme poverty and some are suffering and dying for the demand of clean and safe water, medicine and have no alternative for primary health care. Therefore, the need to use medicinal plants as alternatives to orthodox medicines in the provision of primary health

care cannot be over-emphasized (WHO, 1995). More so, herbal medicines have received much attention as sources of lead compounds since they are considered as time tested and relatively safe for both human use and environment friendly (Fazly-Bazzaz *et al*., 2005). They are also cheap, easily available and affordable. Therefore, there is need to look inwards to search for herbal medicinal plants with the aim of validating the ethnomedicinal use, with subsequent isolation and characterization of biologically active compounds which will be added to the potential lists of drugs in clinical usage (Falodun *et al*., 2008).

Euphorbia heterophylla linn is a medicinal plant belonging to the *Euphorbiaceae* family. It is an erect, annual weed growing to about 3 ft high and locally abundant in Nigeria and East Africa (Jaiyesimi and Abo, 2010; Okeniyi *et al*., 2012). It is commonly known in Nigeria as Nonon-kurchiya (Hausa), Egele (Igbo) and Adimeru (Yoruba). It is referred to as Mexican fire plant, milk weed and Spurge weed in English (Okeniyi *et al*., 2012). In East Africa, *E. heterophylla* is used for the treatment of gonorrhea, accelerate wound healing and as purgative (Erden *et al*., 1999), as a lactogenic agent (Dokosi, 1998) as a cure for migraine and warts (Falodun *et al.*, 2003; Falodun and Agbakwuru, 2004), while the latex of the plant is used as fish poison and insecticide (Rodriguez *et al*., 1976). Previous biological studies have reported the antibacterial activity of the leaf of *E. heterophylla* (Falodun *et al.*, 2003; Okoli *et al*., 2009), its anti-inflammatory activity (Falodun *et al*., 2006), as well as its wound healing potentials (Omale and Emmanuel, 2010).

The present study was designed to use standard protocols to extract, fractionate and investigate (*in vitro*) the phytochemical ingredients of the crude ethyl acetate extract and fractions of the leaf of *Euphorbia heterophylla linn*.

Materials and Methods

Plant collection and identification

Fresh leaf sample of *Euphorbia heterophylla* was collected from the plant in its natural habitat, at Old Campus, Bayero University Kano, Kano state, Nigeria. It was identified and authenticated at the Herbarium unit, Department of Plant Biology, Bayero University Kano, with Herbarium Accession Number BUKHAN 0055.

Sample preparation and Extraction Process

The sample was washed with cleaned and running tap water, followed by distilled water, air-dried under shade at ambient temperature for three (3) weeks. It was ground mechanically into fine powder, weighed, packaged in an airtight container, labelled (SEh-Leaf) and kept away from any form of contaminant and was later subjected to extraction using ethyl acetate (Kumar, 2015).

Preparation of Solvent Extract

The powdered sample (800 g) was soaked in ethyl acetate (2.0 L) with frequent agitation at regular interval for 15 days. After which the extract was separated from the debris by filtration. The debris was again soaked in another fresh volume of ethyl acetate (1.5 L) for another 15 days. The extract was again separated from the debris by filtration using and then concentrated under vacuum at 40°C by the use of rotary eva porator (R110). The crude ethyl acetate extract was then allowed to dry at room temperature until a constant weight is obtained (Shovon *et al*., 2016).

Fractionation process of the Crude Extract

The crude ethyl acetate extract (CEE) was fractionated with petroleum ether (400 mL) in part (50 mL), chloroform (350 mL) in part (50 mL) and ethyl acetate (150 mL) in part (50 mL) to incur fractions of petroleum ether, chloroform and ethyl acetate and were coded EhF_1, EhF_2

and EhF_3 respectively. Each fraction obtained was concentrated at room temperature, weighted and stored in a glass beaker (100 mL) for further analysis (Abdulrahman and Adamu, 2019).

In vitro Phytochemical Investigation

Qualitative phytochemical investigation to detect the presence of alkaloids, anthraquinones, carbohydrates, flavonoids, saponins, steroids, tannins and terpenoids were carried out according to standard protocols adopted from Trease and Evans (1989), Harborne (1998) and Gurav *et al*. (2014).

Results and Discussions

Physical properties of crude extract and Fractionation

The ethyl acetate crude extract (CEE) of the leaves of *Euphorbia heterophylla* is obtained as a dark green and sticky/gummy weighing 22.40 g in mass with a percentage yield of 2.80%. The physical properties of the three (3) fractions obtained through polarity gradient fractionation of 20 g by mass of the crude can be seen in the table one (1) below.

Table 1: Physical appearance of fraction of *Euphorbia heterophylla's* extract

Fraction	Texture	Colour	Weight(g)	%Yield
Eh_1	Sticky	Dark green	10.20	51.00
Eh_2	Sticky	Dark green	5.10	24.50
Eh_3	Non-sticky	Light green	2.80	14.00

Eh_1= petroleum ether fraction, Eh_2= chloroform fraction and Eh_3= Ethyl acetate fraction

As can be seen from the table one (1) above, petroleum ether fraction presented highest yield (51.00%), followed by chloroform fraction (24.50%), while the ethyl acetate fraction is the one that presented the least yield (14.00%). The promising yield of petroleum ether fraction suggested that, the leaf extract contained much of non-polar phytochemicals than polar ones

Phytochemical Ingredients

The phytochemical test results for the detection of secondary metabolites imbedded in the crude ethyl acetate extract and those of fractions are shown in the table (2) below.

Table 2: Phytochemical test of *Euphobia heterophyta's* leaf

Phytochemicals	CEE	Eh_1	Eh_2	Eh_3
Alkaloid	-	-	-	-
Anthraquinone	-	-	-	-
Carbohydrates	+	+	+	+
Flavonoids	+	+	+	+
Saponins	+	-	-	+
Steroids	+	+	+	+
Tannins	+	-	-	+

+ = present and - = absent

CEE= crude ethyl acetate extract Eh_1= petroleum ether fraction, Eh_2= chloroform fraction and Eh_3= Ethyl acetate fraction

In the present study, when we observe keenly and move across the table (2), we can see that, the ethyl acetate crude extract, contained five out of the six (6) phytochemicals

(carbohydrate, flavonoid, saponin, steroid and tannin) tested, but showed negative test for alkaloids (Falodun *et al*., 2006; Okeniyi *et al.*, 2012), and anthraquinone, whereas, only the fraction of ethyl acetate (Eh_3) contained Saponin and tannin which conformed with some previous reports by Edeoga *et al*. (2005) and Rahilla *et al*. (1994). So also, Ponnambalam *et al*. (2019), carried out the phytochemical analysis of *Euphobia heteropylla* and uncovered the presence of various active components like saponinns, triterpenes, flavonoids, carbohydrate, quinones, steroids and tannins.

Conclusion

Apparently, the results of the extraction and fractionation revealed that, the leaf of *Euphobia heteropylla* contained much of non-polar phytochemicals than polar ones. This can be seen in the percentage yield (51.00%) presented by a fraction obtained using the least polar solvent (Petroleum ether) during fractionation.

Phytochemical test to detect the secondary metabolites imbedded in the crude ethyl acetate extract and those of fractions is a very significant way to establish that, the leaf of *Euphobia heteropylla* may be used as potent drugs. The plant species is selected for our present study, because it is a well-known medicine for the treatment of gonorrheal, respiratory tract infection, malaria, Eczema, Asthma (as it has a special reputation for causing bronchial relaxation), wart cure, purgative action and commonly found and easily available at Old Campus of Bayero University and other parts of Kano State, Nigeria.

The evidences at hand clearly present that the leaf of *Euphobia heteropylla* studied here can be seen as a potential source of useful phytochemicals like carbohydrates, flavonoids, saponins steroids and tannins. If they are found non-toxic and biologically active, may be used as important sources of antimicrobial agents of natural origin.

Further research is recommended to focus on antimicrobial and antioxidant activities, cytotoxicity studies, isolation and characterization of phytochemical ingredient(s) responsible for bio-efficacy and bioactivity.

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Competing Interests

Authors have declared that no competing interests exist.

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