WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Phytochemistry and Antioxidant Studies of the Hexane Fraction of *Euphorbia Heterophylla* Leaves

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A B S T R A C T

This analysis was carried out to identify the phytochemicals and antioxidants present in the leaves of Euphorbia Heterophylla. Leaves of Euphorbia heterophylla has being specifically used for treatment of Gastro Intestinal disorders (diarrhea, dysentery, intestinal parasitosis etc.), bronchial and respiratory diseases (asthma, bronchitis, hay fever etc.) and in conjunctivitis. The Extracts have been found to depict anticancer pharmacological activities and active plant materials or secondary metabolites such as alkaloids, flavonoids, terpenes, saponins, steroids and inert or active materials were separated with n- hexane solvent through extraction.

Retention factor of the constituents using hexane acetate ratio of 7:3, 8:2, 9:1 was calculated and a comparison made. Thin layer chromatography was done, and a comparison made hexane ethyl acetate ratio of 7:3 separates more than the hexane ethyl acetate ratio of 9:1, clearly showing the constituents when viewed in UV in the Phytochemicals, the result for qualitative phytochemical analyses in tables indicated that saponin, flavonoids, alkaloid, glycosides and steroids were found to be present in the plant extracts.

Keywords: Phytochemicals, Euphorbia Heterophylla, Thin layer chromatography, 2,2-diphenhydramine-1-picrylhydrazyl, Hexane fraction

1.INTRODUCTION.

Euphorbia heterophylla L., commonly termed as fireplant, is traditionally beneficial in a series of folk medicine practices. Claims on the medicinal potential of *Euphorbia heterophylla* leaves, especially for the Hexane Fraction, have been subjected to various assertions(Anarado C, 2020). Euphorbia is among the largest plant genera containing more than several hundreds species in which some of them are medicinal plants. *Euphorbia heterophylla* or fireplant has been used among popular medicine as a folk against different diseases(Harborbe JB, 1998). Though the pharmacological potential of some *Euphorbia* species has been investigated in different investigations, knowledge about Hexane Fraction from *Euphorbia heterophylla* leaves is scanty. Phytochemical investigations *of Euphorbia* spp. based on the standard procedures have indicated that they contain among other constituents, alkaloids, flavonoids, and terpenoids. For instance, secondary metabolites such as what this study aims to cover are often associated with diverse therapeutic activities of ranging from anti-inflammatory to anticancer effects. However, the detailed analysis of the Hexane Fraction's phytochemical composition in *Euphorbia heterophylla*is notably lacking in existing literature. There are a lot of Literatures on the involvement of phytochemicals which include alkaloids, flavonoids, and terpenoids as some of the possible contributors to the therapeutic potentials of extracts from plant sources. Some of the phytochemical composition responsible for medicinal properties of the Hexane Fraction of *Euphorbia heterophylla* leaves is unique knowledge in determining how its therapeutic properties could be fully explored medicinally(Azwandi, N,N, 2015) and oxidative stress-related diseases are highly linked with the effect of antioxidants; overall, such evaluation could indicate what this fraction can offer for reducing disease risks. From this background, we shall attempt to bridge this knowledge gap by exploring the Hexane Fraction of *Euphorbia heterophylla* leaves. This paper aims to investigate potential antibacterial, anti-fungal and anti-inflammatory activities providing evidence for traditional uses. This paper involves the extraction of the bioactive compounds from the plant using the cold maceration method, fractionation using a

separating funnel to separate the pure compound from the aqueous layer using hexane, thin layer chromatography to determine the purity of the isolated compounds from the plant extract to ensure their quality for further analysis and applications, the phytochemicals present in the plant.

Euphorbia heterophylla is an ornamental plant, and it can be cultivable for its succulent. The leaves are two large and overlying long leaves originating from the base of the plant. It bore shiny, green leaves with pointed ends. The plant *Euphorbia heterophylla* is native to arid lands that cross Central America, South America and Africa. King of the Mauritania Region by 30BC named a local African plant *Euphorbia* II as a consideration to Euphorbus, a renowned medical doctor who discovered medicinal properties of *Euphorbia* resinifera. Linnaeus then adopted the name *Euphorbia* to describe the whole genus. *Heterophylla* derives from Greek; heteros = different and phyllon = leaf, after the variation in the form of leaves this species displays (Kissman and Groth, 1993). The specific aim of this study is to investigate the phytochemical and antioxidant properties of the leaves of *euphorbia heterophylla.* The specific objectives include:

●To carry out methanol extraction of the leaves of *euphorbia heterophylla* using cold maceration method

●To obtain a hexane fraction of the methanol extract.

●To determine the phytochemical present in the methanol extract present in the hexane fraction of leaves of *euphorbia heterophylla*

●To compare the phytochemicals present in the methanol extract and leaves of *euphorbia heterophylla*

●To determine the best solvent system to be used.

●To evaluate the antioxidant activity of the hexane fraction of DPPH assay.

Figure 1: Full view *of Euphorbia Heterophylla* leaves

Figure 2: Side view of *Euphorbia Heterophylla* leaves

IDENTITY PREFERRED SCIENTIFIC NAME: *Euphorbia heterophylla L. (1753),* PREFERRED COMMON NAME: wild poinsettia, OTHER SCIENTIFIC NAMES: Euphorbia geniculata Ort. (1797), Euphorbia prunifoliaJacq.Euphorbia taiwaniana, Euphorbia zonospermaMüll, Poinsettia geniculata (Ort.)Klotzsch&Garcke, Poinsettia heterophylla (L.)Klotzsch&Garcke

LOCAL COMMON NAMES: Argentina: lecherón, Brazil: adeus-brasilcafé-do-diaboleiteiramata-brasil, Italy: poinsettia d'America.

●TAXONOMIC TREE

Domain:-Eukaryota
Kingdom:- Plantae

Phylum:- Spermatophyta

Subphylum:- Angiospermae

Class:- Dicotyledonae

Order:- Euphorbiales

Family:- Euphorbiaceae

Genus:-Euphorbia
Species:-Euphorbia heterophylla

Mature Size:- 30-100 cm tall

Benefits Ornamental, medicinal

1.1 EXTRACTION OF PLANTS

Plant extraction is described as a procedure through which active plant materials or secondary metabolites such as alkaloids, flavournoids, terpenes, saponins and steroids, in the manner of their realization or inactiveness are separated by use an appropriate solvent. Commonly used methods in the extraction of medicinal plants

●**MACERATION.** This is the technique for extraction wherein drug material either leaves or stem bark or root bark in coarsely powdered form are put inside the container; then the menstruum is poured on top until completely covered the drug material. The container is then closed and kept for at least three days. The content is periodically stirred and if placed in the bottle, should be time to time shaken to its bottom to extract everything out of it. After extraction, at the end of it, mac gets separated from micelle by filtration or decantation. Thereafter, evaporation of the menstruum within an oven or on top of water bath is done in order to remove the micelle. This method is also applied quite comfortably and quite suited on plant materials that are thermolabile (Ingle *et al*.,2017).

●**INFUSION.** This is also an extraction process like maceration. The drug material is run into fine powder, and then introduced into a clean container. The extracting solvent is poured on top of the drug material hot or cold, and then soaked or kept for a short period of time.This method suits well with readily soluble bioactive constituents. Moreover, a solvent is an appropriate use during the preparation of fresh extract before the using8. Normally, the ratio of solvent to sample is 4:1 or 16:1 depending on the intention3d use.(Ingle et al.,2017).

●**DIGESTION:** This is a moderate heat extraction where one place the solvent of extraction in clean container and powdered drug material follows. The mixture is placed over water bath or in an oven at a temperature about 50o C to before being filtered and the solvent evaporated to obtain a crude extract.Heat was applied all through the process of extraction to lower the viscosity of extraction solvent, and improve in removing secondary metabolites from the solid matrix. This method is suitable for plant materials that are readily soluble. (Ingle et al.,2017).

●**DECOCTION.** Here, extraction of continuous hot has been done through volume of water as the solvent. A dried, grinded, and powdered material from a plant is placed in a clean container whereby water is poured and stirred through. Throughout this process, heat application is made to accelerate the extraction.The process usually takes only short duration, usually about 15min. The ratio of solvent to crude drug is usually 4:1 or 16:1. It is used for extraction of water soluble and heat stable plant material (Azwanida, 2015)

●**PERCOLATION**. A percolator is the apparatus used in this technique. It's a narrow conical shaped glass vessel open at both ends. Dried, grinded and finely powdered plant material is moistened with the solvent of extraction in a clean container. More quantity of solvent is added, and the mixture is kept for a period of 4h. This was then transferred into a percolator with its lower end closed and subsequently allowed to stand for the period of 24h.The solvent of extraction is then poured at the top till the drug material is completely saturated. Then the lower part of the percolator is opened and the liquid allowed to drip slowly. Some quantity of solvent in taken into the continuous add and the extraction took place by gravitational force pushing the solvent through the drug material downward.The process was stopped when the volume of added solvent reached to be 75% of intended quantity of the entire preparations. Separation of additional solvent is then carried out by filtration followed by decantation. Then, marc is expressed and the final amount of solvent is added to get required volume. (Azwanida, 2015)

●**SOXHLET EXTRACTION:** This process is otherwise referred to as continuous hot extraction. The apparatus is called Soxhlet extractor made up of glass. It consists of a round bottom flask, extraction chamber, siphon tube, and condenser at the top. Put into a porous bag (thimble) which is made up of clean cloth or strong filter paper and tightly closed, place a dried, ground and finely powdered plant material. The solvent is continued to heat from the bottom flask, evaporation and passes through the condenser where condensed and flowed down to the extraction chamber and extracts the drug by the contact. When the level of the solvent in the extraction chamber goes up to a height above the siphon, then the solvent and the already extracted plant material are flown back to the flask. This continues on until the drug is completely extracted, a point in the process where a solvent flowing from extraction chamber does not leave any residue. It is suitable for soluble plant material in the solvent chosen, to which impurities insoluble in it exist only if they are not thermolabile. Its advantages are that large amounts of drug can be extracted with a smaller amount of the volume of solvent. It is also applicable to heat stable plant material. No requirement of filtration, and high amount of temperature could apply. The demerits are that it cannot shake regularly, and it is not an apt method for thermolabile materials. (Pandey and Tripathi 2014)

●**MICROWAVE ASSISTED EXTRACTION:** It is one of the conventional manipulation methods in preparation of medicinal plants. Mechanism is by dipole rotation and ionic transfer through displacement of charged ions existing in the solvent and drug material. This is a suitable procedure for flavonoid extraction. It uses electromagnetic radiations of frequencies present between 300 MHz and 300 GHz and wavelength between 1cm and 1 m.The microwaves applied at frequency of 2450 Hz equated to energy lying between 600 and 700W. The technique used bombardment of an object with microwave radiation, which can absorb electromagnetic energy and convert it into heat. Subsequently, the heat produced facilitates the movement of solvent into the drug matrix. With the assist of a polar solvent, there is dipole rotation and migration of ions which therefore makes the penetration of solvent enhanced and the process of extraction assisted. However, when non-polar solvent is used, the microwave radiation released will produce minimal heat; thus this method does not favor use of non-polar solvents.The Advantages implements; Microwave-assisted extraction has special advantages such as minimization of solvent and time of extraction as well as an increase in the outcome. Disadvantages. This method can be applied for phenolic compounds and flavonoids only. Some of the compounds which are tannins and anthocyanins may get degraded due to high temperature involved. (Pandey and Tripathi 2014)

●**ULTRASOUND-ASSISTED EXTRACTION**: Here, sound energy will be of very high frequency greater than 20 KHz and it breaks the plant cell wall by creating molecular cavitation in the liquid medium, thus increasing the area of the drug to be extracted. This is followed by an increase in the

secondary metabolites in contact with the solvent. In this method, plant material should dry first, grinded into fine power, and sieved properly. The prepared sample is then mixed with and appropriate solvent of extraction and packed into the ultrasonic extractor.The high sound energy applies hasten the extraction process by reducing the heat requirements. Advantages. Ultrasound-assisted extraction is suitable for small sample; time of extraction and amount of solvent used can be shortened, in contrast, yield can be maximized. Disadvantages. This method is hardly to be duplicated; at the same time, high amount of energy applied may lower the phytochemical by producing free radical. (Azwanida,2015)

1.2 SOLVENT EXTRACTION OF PHYTOCHEMICALS FROM PLANT EXTRACT

Fractionation is a process of separating plant extracts into various solvent fractions based on their polarity. This process separates the fractions into portions consisting of a number of compounds until a pure compound is isolated (Ingle *et al*, 2017). During fractionation, the solvent is added according to the order of increasing polarity, starting from n-hexane which is the least polar to more polar solvents such as water (Pandey and Tripathi, 2014). When several solvents are used during fractionation it should be added according to the order of increasing polarity. Fractionation of plant extracts can be separated using the separation funnel method. During the fractionation process, when four different solvents(n-hexane, chloroform, acetone, and n-butanol) is selected for the fractionation process, the process begins by dissolution of the crude extract into the required volume of water to be used. The dissolved crude extract is then transferred into a separating funnel, shaken, and allowed to settle. When the content settles properly the bottom of the separating funnel is opened to remove the aqueous layer. The remaining content in the separating funnel is poured into a clean container to get the n-hexane fraction(Ingle *et al*, 2017).Equal volume of n-hexane is added again, shaken, and separated. This process is done for other solvents such as chloroform, acetone, and n-butanol fractions. The remaining portion left after fractionation is called residual aqueous fraction since the crude extract was first dissolved in the most polar solvent such as water.

1.3 CHROMATOGRAPHIC SEPARATION

Plant extracts contains various type of bioactive compounds which has different polarities and the bioactive compounds are separated using different chromatographic separation technique. This separation technique includes: thin layer chromatography, high performance liquid chromatography, paper chromatography, column chromatography, gas chromatography, high performance thin layer chromatography, and optimum performance laminar chromatography are used to obtain either pure compounds or a group of compounds with similar polarity. The pure compounds can then used for determining the structure and biological activity(Ingle *et al*, 2017). Chromatography is a technique that enables the separation, identification and purification of components of a mixture for quantitative and qualitative analysis (Ingle *et al*, 2017). The various separation techniques are discussed below.

●**Thin layer chromatography (TLC):** Thin layer chromatography is a method of separation or identification of a mixture of components into individual components by using finely divide adsorbent solid/(liquid) spread over a plate and liquid as a mobile phase (Ingle *et al*, 2017).TLC is one of the easiest and most versatile methods used because of its low cost, simplicity, quick development time, high sensitivity, and good reproducibility . This separation technique is mostly used for the separation of low molecular weight compounds. The adsorbent used to separate the various compounds includes silica gel which is used to separate amino acids, alkaloids, sugars, fatty acids and lipids;alumina which is used to separate alkaloids, phenols, steroids, vitamins and carotenes;celite which is used to separate steroids and inorganic acids; cellulose powder which is used to separate amino acids, food dyes and alkaloids; starch which is used to separate amino acids and Sephadex which is used to separate amino acids and proteins.

●**High Performance Thin Layer Chromatography:**High performance thin layer chromatography (HPTLC) is a technique that is used widely in pharmaceutical industries for process development, identification, and detection of adulterants in herbal products. HPTLC has several advantages over the more widely used high performance liquid chromatography because of its high throughput (multiple samples can be analyzed at once), economy in use of mobile phase and cost effectiveness.

●**Paper chromatography:** Paper chromatography is a technique used for the separation of compounds based on the differential solubility in the stationary phase and mobile phase. One of the advantages of paper chromatography is that the separation is carried out on sheets of filter paper, which acts as both support and a medium for separation (Harborne, 1998). In paper chromatography the filter paper is used as a solid phase which is the inert phase. A sample is placed near the bottom of the filter paper and the filterpaper is placed in a chromatographic chamber with a solvent. The solventmovesforward by capillary action carrying soluble molecules along with it. Low porosity paper will produce a slow rate of movement of the solvent and thick papers have increased sample capacity.

●**High Performance Liquid Chromatography (HPLC):** High performance liquid chromatography is an analytical technique used to separate the compounds in a mixture, to identify each component, to quantify and purify the component. High performance liquid chromatography separates compounds based on their interaction with solid particles of tightly packed column and solvent of the mobile phase. High performance liquid chromatography is useful for compounds that cannot be vaporized or that decomposes under high temperature, and it also provides a good complement to gas chromatography for detection of compounds. It is a very expensive but highly efficient procedure.

●**Column chromatography:**Column chromatography involves ion exchange, molecular sieves, and adsorption phenomenon. It is a simple technique used in the separation and purification of compounds. In column chromatography both solids and liquids can be separated and purified, it consists of a stationary phase that absorbs and separates the compounds passing through it with the help of a liquid mobile phase. The various stationary phases used are silica, alumina, calcium phosphate, calcium carbonate, starch, magnesium and different solvent compositions based on the nature of the compound to be separated and isolated.

●**Gas chromatography:**Gas chromatography(GC) is a technique used for separating highly complex mixtures based on the difference of their boiling point/ vapor pressure and polarity (Ingle *et al*, 2017). In gas chromatography volatile compoundsis carried by a moving gas stream through a tube packed with a finely divided solid that may coated with a film of a liquid.In gas chromatography the specie distributes between gas and a liquid phase.The gas phase is mobile phase and the liquid phase is stationary. Gas chromatography involves a sample which is vaporized and injected onto the head of the chromatographic column. The sample is the transported through the column by the flow of inert, gaseous mobile phase. The column contains a liquid stationary phase which is adsorbed onto the surface of an inert solid**.**

2. MATERIALS AND METHODS

Here chapter outlines the method used in the analysis of Leaves of *Euphorbia Heterophylla.*

●**MATERIALS:** Grinder, Weighing balance, Measuring cylinder, beaker, spatula, beaker, filter paper, funnel, sample bottles, Water bath, beaker and filter paper, Separating funnel, Percolated TLC Plate, Chromatographic tank, capillary tube, UV lamp, UV Visible Spectroscopy ,Aluminium foils

●**REAGENTS USED:** Methanol, Hexane, Ethyl Acetate, DPPH(2,2-diphenhydramine-1-picrylhydrazyl).

2.1 METHODOLOGY

Fresh/ Healthy Leaves of *Euphorbia Heterophylla* were Collected in NnamdiAzikiwe University Awka on October 2023, The sample was air dried under room temperature, pulverized and weighed

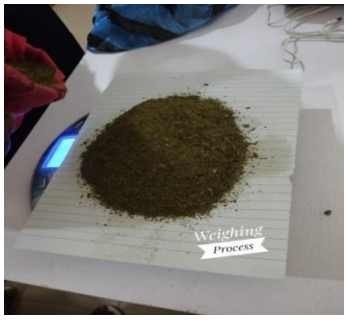

Figure 3:Pulverised E.H leaves Figure 4:Pulverised E.H leaves on weighing balance

The plant material was extracted using cold maceration method. The Leaves of *Euphorbia Heterophylla* sample was weighed to 200g into 5 batches using a weighing balance. Each of the 200g weighed sample was filled into a 1-liter glass bottle. In each of the glass bottle 200ml of a solvent methanol which was measured using a measuring cylinder was filled into them. After the methanol was added, each of the glass bottles was shaken vigorously for even distribution of the methanol in the sample and for better extraction of the plant sample. The sample was left for 24hours before being decanted. Methanol was used for the first extraction process because it is a polar solvent which picks most of the secondary metabolites. After 24 hours each of the glass bottles containing the methanol which has already extracted the secondary metabolites is decanted into another empty glass bottle and then covered. This process is repeated using 300ml methanol which was shaken vigorously and left again for another 24hours before it was decanted. This process was done repeatedly to ensure all secondary metabolites were extracted from the plant material. A total of 4 litres of methanol was used to extract 1kg of the pulverized of Leaves of *Euphorbia Heterophylla.* The extract was filtered using a funnel and a filter paper. The extract was poured into different 250ml beakers which was placed into a water bath at a temperature of 45 ºC - 50 ºC to evaporate the methanol which is the solvent and leave just the extracted sample.

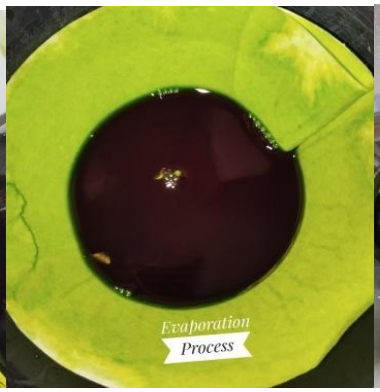

Figure 5: Extraction using Methanol Figure 6: Filtation process Figure 7: Filtrate in a waterbath

2..2 FRACTIONATION

This is a separation process whereby a certain mixture is divided during a phase transition into a smaller quantity which varies according to a gradient. Fractionation process using a separating funnel was carried out on the methanol extract of the leaves. Water was filled into the extract solution to about 150ml and was stirred. The 150ml extract solution was shared into half and was poured into a separating funnel. A total 120ml of hexane was poured into the first separating funnel containing the 75ml solution, was shaken vigorously and was set up on a retort stand which was left to separate. Another 120ml of hexane was added into the separating funnel containing the 75ml extract solution which was shaken vigorously and was set up on a retort stand and left to separate. After 5mins the aqueous layer would have separated from the hexane layer. The aqueous layer was collected from the bottom of the separating funnel into a beaker and the hexane layer was collected in a separate beaker. This process was repeated continuously using hexane to separate the hexane layer from the aqueous layer. The total solvent used was 490ml hexane and the total leaves extract was 450ml. The hexane layer was left to evaporate for sometime before carrying out TLC.

Figure 8: Fractionation using Hexane

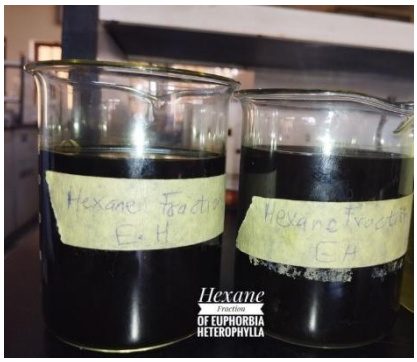

Figure 9: Hexane fraction of E.H

2.3 CHROMATOGRAPHY

2.3.1 THIN LAYER CHROMATOGRAPY

TLC was carried out using a aluminium pre-coated plate, capillary tube, chromatographic tank and a suitable solvent. Hexane and ethyl acetate was prepared into three different ratios, 9:1, 8:2, and 7:3 which was used as the solvent system used in carrying out the TLC. UV light was used to visualize the invisible spots on the plates. The retention factor value for each spot on the different TLC plates was calculated using the formula above.

Rf value = distance travelled by solute

distance travelled by solvent, on the image below.

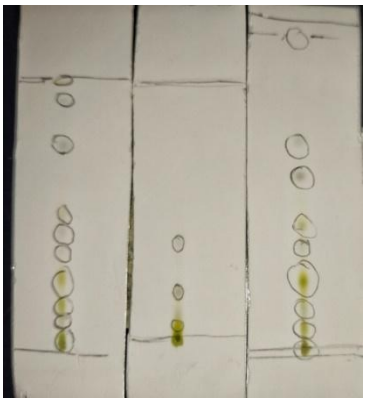

Figure 10: Thin layer Chromatography

3. PHYTOCHEMICAL STUDIES OF THE HEXANE FRACTION OF THE LEAVES OF *EUPHORBIA HETEROPHYLLA*

3.1 TEST FOR ALKALOIDS (MEYER'S TEST)

Alkaloid under acid condition reacts with mercuric chloride and potassium iodide to give a cream colour or PPT, the dissolve Meyers Reagent used is 1.4g of Mercuric Chloride in 60ml of distilled water and 4.5g of potassium iodide in 20ml of distilled water. The two solution is mixed and diluted to a

100ml of distilled water. Pipette 1.0ml of titrate into a test tube, also pipette 10ml of Meyers reagent into the same test tube. Mix properly and observe a colour change, Green colour precipitate indicates the presence of alkaloids.

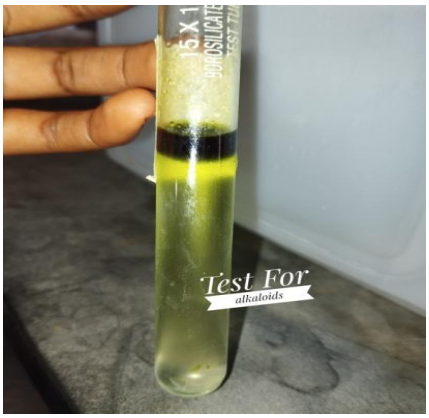

Figure 11: Test for alkaloids

3.2 TEST FOR SAPONINS (FROTHING TEST)

Saponins when heated and shaken in water maintains stable forth when shaken in water. We add 3.0ml of distilled water to 1.0ml of filtrate into a test tube and shake the solution vigorously and observe a stable forth on standing.

Figure 12: Test for saponin

3.3 TEST FOR TANNIS:

3.3.1 ACID TEST:

Phlobotannis under acid condition reacts with dilute HCl to give a red colour or precipitate, 1% HCl is pipetted of 100ml of Concentrated HCl, make up to 100ml with distilled water, we add 3.0ml of extract to 2.0ml of HCl and observe Presence of red colour or precipitation.

Figure 13: Test for tannis

3.3.2 LEAD ACETATE TEST

Phototannis reacts at room temperature with lead acetate to give a dark blue to black ppt. We take 5% lead acetate and weigh 5.0g of the lead acetate and dissolve in 100ml distilled water. We pipette into a test tube 2.0ml of extract, then add 3 drops of 5% lead acetate solution to the extract. and noticed for colour change.

Figure 14: Test for tannis

Subsequent test for flavonoids, terprenoids, steroids and cardiac glycosides test was done. The presence of flavornoid in the sample was investigated using the method described by Harbone (1998), while the Salkowsi's test was used to test the presence of terpenoids. Where 5 ml of extract was mixed with 2ml of chloroform followed by a few drops of concentrated sulphuric acid, a reddish brown precipitate indicates the presence of terpenoids. The Liebermann-Burchard'stest was used to test for steroids. Where 1ml of each extract was treated with 0.5ml of acetic anhydride and cooled. This was later mixed with 0.5mL of chloroform and 1ml of concentrated sulphuric acid was carefully added using a pipette, there was the formation of a reddish brown ring which indicated the presence of steroids. The Keller killani test was use for test of cardiac glycosides(Mariyaraj J, 2019), where 5ml of the filtrate was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was under played with 1ml of concentrated sulphuric acid. A brown ring of the interface indicates 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 gradually throughout thin layer.

3.3.3 ANTI- OXIDANT STUDIES

In order to check the antioxidant property, 2,2- diphenyl-1-picrylhydrazyl (DPPH) assay was employed.

3.3.4. SCAVENGING EFFECT ON DPPH:

0.1mM 0f DPPH solution in methanol was prepared and mixed with the extract at varying concentration (1mg/ml, 0.5mg/ml, 0,25mg/ml, 0.125mb/ml, 0.625mg/ml. 0.5ml of DPPH was added to each except the last concentration which 1ml was added to each of the reaction mixture was incubated in the dark at a room temperature for 30 minutes.The absorbance was taken at a suitable wavelength of 517 using a UV-Vis spectrophotometer. A negative control was prepared using 5ml methanol and 0.5ml of DPPH was added to it then a positive control was carried out using vitamin C into varying concentrations (1mg/ml, 0.5mg/ml, 0,25mg/ml, 0.125mb/ml, 0.625mg/ml), % DPPH Scavenging was calculated using the formular (1 - Absorbance of sample/ absorbance of control) x 100. A graph of DPPH scavenging % against concentration was plotted and from the graph, the concentration of antioxidant required to scavenge 50% of DPPH radicals from the dose response curve was calculated.

4. RESULTS AND DISCUSSION

4.1 RETENTION FACTOR

RETENTION FACTION (R.F) is the ratio of the distance travelled by the solute to the distance travelled by the solvent, it is given or calculated as: RF= Distance travelled by solute / Distance travelled by solvent.

The spots was viewed under a UV Lamp and all spots were coloured

4.1.1 FOR THE HEXANE ETHYL ACETATE RATIO OF 7:3

SOLVENT FRONT (S) =6.3CM

$R_{fl\,A}$ = 0.3/ 6.3 = 0.048cm

$R_{fl\,B}$= 0.7/6.3= 0.111cm

$R_{fl\ C}$= 1.3/6.3= 0.206cm

R_{flD} =1.8/6.3= 0.286cm

R_{flE} =2.3/6.3=0.365cm

R_{flF} =3.2/6.3= 0.508cm

R_{flG} =3.9/6.3 =0.619 cm

R_{flH} =6.0/6.3= 0.952cm

R_{flA} is More Polar and travels slowly while R_{flH} is less Polar and travels faster

4.1.2 FOR THE HEXANE ETHYL ACETATE RATIO OF 8:2

SOLVENT FRONT (S) =5.4CM

R_{flA} =0.7/5.4= 0.130cm

$R_{fl\ B}$= 1-0/5.4= 0.185cm

$R_{fl\ C}$= 1.4/5.4=0.259cm

$R_{fl\ D}$= 2.0/5.4= 0.370cm

$R_{fl\ E}$= 2.3/5.4= 0.425cm

$R_{fl\ F}$= 2.7/5.4= 0.500cm

$R_{fl\ G}$= 4.0/5.4= 0.740cm

$R_{fl\ H}$= 5.0/5.4= 0.925cm

4.1.3 FOR THE HEXANE ETHYL ACETATE RATIO OF 9:1

SOLVENT FRONT (S) =5.0CM

$R_{fl\ A}$= 0.3/5.0= 0.060cm

$R_{fl\ B}$= 1.0/5.0=0.200cm

$R_{fl\ C}$= 1,8/ 5.0= 0.360cm

4.2 PHYTOCHEMICAL TEST

Table 1: **Qualitative Phytochemical analysis of the hexane fraction of leaves of euphoria heterophylla showing observation and inference**

	TEST	**OBSERVATION**	**INFERENCE**
1	Sample+ Meyer's reagent	Cream Colour Ppt	Alkaloid Present
2	Sample +distilled water	Stable forth on standing	Saponin present
3	Sample +2ml of HCl	No Red colouration	Tannis absent
4	Sample + Lead Acetate	No Red colouration	Tannis absent
5	Sample + FeCl2	Greenish brown ppt	Flavornoids Present
6	Sample +Liebermann Burchard test	Reddish brown ring	Steroids present
7	Sample +kellerkillani test	A violet ring appears below a brown ring	Glycosides present
8	Sample + salkowski's test	A reddish brown ppt	Terpenoids present

Table 2: **Qualitative Phytochemical analysis of the hexane fraction of leaves of euphoria heterophylla**

Sample	**N-Hexane Extract**

Sample	**N-Hexane Extract**
Alkaloids	+
Saponin	++
Tannis	-
Flavournoid	+
Glycosides	+
Phenolics	-
Steroids	++

Key: += low abundance, ++ =Moderate abundance, - = Absent

4.3 ANTIOXIDANT STUDY

UV RESULT FOR SAMPLE

Solution concentration	Absorbance	%DPPH Scavenging
A 1ml	0.956	14.5
B 0.5ml	1.036	7.3
C 0.25ml	1.097	1.9
D 0.125	1.124	-0.5
E 0.0625	0.914	18.2

Table 3: UV Result of the sample using DPPH

FOR VITAMIN C

Solution	Absorbance	%DPPH Scavenging
A 1ml	0.436	61.00
B 0.5ml	0.365	67.35
C 0.25ml	0.231	79.34
D 0.125	0.227	79.70
E 0.0625	0.132	88.193

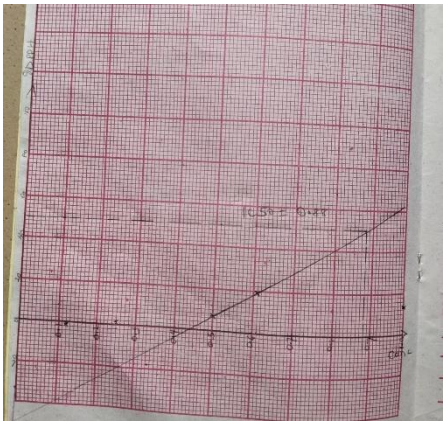

Fig 16 Graph of sample against antioxidant activity

IC_{50}=0.88

4.4 DISCUSSION

4.4.1 SEPARATION

The hexane ethyl acetate ratio of7:3 separates more than the hexane ethyl acetate ratio of 9:1 clearly showing the constituents when viewed in UV

7:3 Ratio: This ratio results in the most significant separation.

8:2 Ratio: Intermediate separation compared to 7:3 and 9:1.

9:1 Ratio: The least separation observed.

4.4.2 SOLVENT POLARITY:

The increasing separation trend from 9:1 to 7:3 suggests that the compounds in Hexane Fraction are likely more soluble in moderately polar to polar solvents.

The 7:3 ratio provides a more polar environment, allowing enhanced separation, while the 9:1 ratio leans towards non-polarity, resulting in reduced separation.

4.4.3 EXPECTED POLARITY OF COMPOUNDS:

It follows that the Hexane Fraction should present compounds with different polarity.

To be clearer, separation 7:3 represents moderately polar compounds present in the crude extract that can be extracted by a quite less polar solvent, though not so efficiently.The reduced separation at 9:1 suggests the presence of compounds with lower polarity or non-polar characteristics.

4.4.4 POSSIBLE TYPES OF COMPOUNDS:

7:3 Ratio: This region could contain some flavonoids, alkaloids or any other conjugated systems which are of average polarity.

8:2 Ratio : The separation still holds good in this ratio and is provided to a lesser extent and shows that polarity is of intermediate compounds.

Ratio 9:1: Limited resolution suggestive of less polar compounds or hydrocarbons that are saturated or those with fewer polar functional groups.

4.4.5 REASONING:

This observed separation trend is in keeping with the tenet that moderately polar to polar solvents enhance the separation of compounds of higher polarity.

In the Hexane Fraction, there are probably the so-called compound of more polar functional groups or even extended conjugated systems that's contributing to the coloration and separation observed.

4.4.6 PHYTOCHEMICAL DISCUSSION

For the Phytochemical's, the result of qualitative phytochemical analyses in tables showed that the flavonoids, alkaloids, glycosides and steroids are contained in the extracts.

4.4.7 FLAVONOIDS:

Flavonoids have been observed to possess antibiotic activities by causing the disfunctionality of microorganisms. (Anarado, 2020). They are a heterogeneous class of compounds further characterized by this property - antioxidant. They are most frequently distributed in moderately polar to the polar fractions. From this, it can be assumed that it mostly has flavonoids which are contributing to these features by coloration and their separation majority within the 7:3 ratio.

4.4.8 ALKALOIDS

Anti-tumor, diuretic, antiviral, analgesics and anti-inflammatory properties are descriptions reported about alkaloids (Dieu-Hien et al, 2019). The most often nitrogen-containing compounds as well as variable biological activities are alkaloids. They exist as polarities. The reported partitioning based on the 7:3 and 8:2 solvent ratios could in part be associated alkaloids presence suggesting that alkaloids are moderately polar solvents solubility.

4.4.9 STEROIDS :

Steroids are normally not such polar or weakly polar compounds. Lowering the separation to 9:1 means that the less polar fractions could contain such steroids.

5. FUTHER DISCUSSIONS :

Flavonoids, alkaloids, glycosides and probably other polar to moderately polar compounds may have significantly contributed to the more distinct separation in the 7:3 and 8:2 ratios.

Since less separation was observed with the 9:1 ratio, there were likely some compounds within the mixture being of different polarity yet mostly composed of relatively less polar compounds such as the steroids.

5.1 ANTIOXIDANT:

The sample has antioxidant activity, but at 0.125mg/ml concentration , the fraction has no activity.

The IC50 of ascorbic acid from the graph is 0.36mg/ml, while that of the sample is 0.88mg/ml. This is moderately close to the activity of the ascorbic acid

6. CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

The whole results of the phytochemical and antioxidant screening could explain the enthusiasm of traditional healers for these plants as drugs. This research aimed to unravel the phytochemical composition and antioxidant potential of the Hexane Fraction derived from *Euphorbia heterophylla* leaves. The chromatographic analysis, particularly in Thin Layer Chromatography using varying hexane:ethyl acetate ratios, revealed a nuanced separation pattern. Notably, the 7:3 ratio exhibited the most significant separation, indicating the prevalence of moderately polar to polar compounds in this fraction. The phytochemical screening confirmed the presence of saponins, flavonoids, alkaloids, glycosides and steroids in the Hexane Fraction. These findings align with the observed coloration and separation patterns, suggesting a diverse array of bioactive compounds contributing to the fraction's characteristics. The antioxidant study further underscored the potential health benefits of the Hexane Fraction, as evidenced by its notable antioxidant activity. The presence of flavonoids and other identified phytochemical likely contributes to the observed antioxidant effects, highlighting the fraction's potential as a natural source of antioxidant compounds. Therefore, it is of great interest to carry out further screening of these plant extracts in order to reveal all their active ingredients by isolation and characterization of their constituents.

6.2 RECOMMENDATION

This research was limited to the phytochemical and antioxidant studies of the hexane fraction of *euphorbia heterophylla.* However, further works needs to be carried out on the plant in other to determine the other active chemical constituents responsible for some of its activities.

These include:

●Further Compound Isolation and Characterization: Considering the diversity of phytochemicals identified in the Hexane Fraction, future studies should focus on the isolation and detailed characterization of individual compounds. Techniques such as chromatography coupled with spectroscopy (NMR, MS) would provide a more comprehensive understanding of the chemical constituents.

●Synergistic Interactions Exploration : Investigate potential synergistic interactions among the identified compounds. Studying the combined effects of flavonoids, phenolics, alkaloids, and other phytochemicals could provide insights into the cooperative antioxidant mechanisms within the Hexane Fraction.

●Biological Activity Studies: Expand research to explore the biological activities of the isolated compounds or the Hexane Fraction as a whole. Conduct assays to assess specific therapeutic effects, such as anti-inflammatory or anticancer properties, to further establish the potential medicinal applications.

●Optimization of Extraction Techniques: Explore alternative extraction methods to enhance the yield of bioactive compounds. Different extraction techniques, such as ultrasound-assisted extraction or supercritical fluid extraction, could be employed to optimize the extraction process.

●Public Awareness and Conservation: Promote public awareness about the sustainable use and conservation of *Euphorbia heterophylla*. Emphasize the importance of ethical harvesting practices and conservation efforts to maintain the ecological balance of this plant species.

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