Chemical Composition Of Phytochemicals And Essential Oil Of Euphorbia Heterophylla

Wellington, E.O, Peters, D. E, Onyeike, E.N.

Department of Biochemistry, Faculty of Science,
University of Port-Harcourt Choba, Rivers State, Nigeria.

Initial: Wellington, E.O, Department of Biochemistry, Faculty of Science,
University of Port-Harcourt Choba, Rivers State, Nigeria. +234(0)9074578612
ewellington41@yahoo.com

Abstract: Introduction: Medicinal plants are very rich in bioactive source of drugs of traditional system of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. E. heterophylla is widely used in folklore medicine for the treatment of several bacterial infections. In traditional system of medicine, aqueous extract or decoction of the leaves that is used to prepare food such as yam porridge or taken directly to "wash out the bowels" or as a purgative. **Objective:** To qualitatively and quantitatively determine the phytochemical and essential oil composition of the aerial parts of E. heterophylla. **Methodology:** Phytochemical and essential oil screening and quantification of the aerial parts of E. heterophylla were carried out using high performance liquid chromatography (HPLC) and gas chromatography (GC). **Results:** Phytochemicals characterized include 719.19mg/100g alkaloid (euphorbin-A and C), 376.20mg/100g flavonoid (quercitrin and myricitrin), 139.80mg/100g saponin (sapogenin, and saponine), 80.60mg/100g glycoside (rutin and kaempferol-3-glucopyranoside), 33.93 sterol (sistosterol and campesterol), 25.32 phenolic acid (vanillic and protocatechuric acid), 19.32mg/100g isoflavan (daidzein and genistein), 6.11mg/100g anthocyanins (Cyaniding-3-(6,6'caffeonyl-p-hydroxybenzoylso and cyaniding-3-(6,6'dicaffeonylsophoroside)-5-glucos), 1.92mg/100g terpenoid (B-amyrin and clovandiol), 1.19mg/100g anthraquinones (2,3-dimethoxybenzoquinone and heterophylline), $1.72x10^{-1}$mg/100g lignan (seroisolariciresino and epipinoresinol) in decreasing order and 100% essential oil. **Conclusion:** The presence of these essential oil and phytochemical constituents in E. heterophylla could be responsible for the therapeutic activities elicited by the plant.

Keywords: Phytochemical, Euphorbia heterophylla, essential oil, HPLC, Gas chromatograph.

1. INTRODUCTION

Extracts of plant materials have recently become of great interest owing to their versatile application. Medicinal plants are very rich in bioactive source of drugs of traditional system of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [1].There are 380,000 species of plants in the world of which 280,000 belong to higher plant and 10% of this higher plant has been studied to an extent. An analysis on western medicine showed approximately one fourth of all medicine now in use are derived from over 2500 flowering plants [2]. The medicinal properties of these plants lie in the bioactive phytochemical constituent that produces definite physiological effect on human body. These natural substances formed the base of modern drugs that are in use [3,4]. The reliance of societies on herbal-based therapies is as a result of their effectiveness, affordability, availability, low toxicity and acceptability [5]. Euphorbia heterophylla originate from South America but now pantropically distributed [6]. Over the last two decades, E heterophylla was regarded as an agricultural problem resulting from changes in management practices, especially the widespread adoption of chemical weed control, coupled with its resistance to most herbicides [7]. E. heterophylla is widely used in folklore medicine for the treatment of several bacterial infections [8]. In traditional system of medicine, aqueous extract or decoction of the leaves that is used to prepare food such as yam porridge or taken directly to "wash out the bowels" or as a purgative [9,10]. The genus Euphorbia attracted the attention of many researchers worldwide, from natural products, bioactivity, and ecological perspective [11]. The essential oils (EOs) of Euphorbia heterophylla are poorly studied. The essential oils from aerial parts of E. heterophylla have been extracted and characterized using hydrodistillation and analyzed via GC-MS. The antioxidant activity has also been determined based on scavenging of the free radicals, 1,1-Diphenyl-2-picrylhydrazyl and H_2O_2 [12]. Extract from the leaves of Euphorbia heterophylla has been shown to mediate synthesis of multifunctional Zinc oxide nanoparticles (ZnO NPs) by green chemistry approaches which showed that Euphorbia heterophylla is an effective reducing agent for the formation of ZnO NPs with significant antibacterial and cytotoxicity properties [13]. This research determine the chemical composition of phytochemicals and essential oils of Euphorbia heterophylla

2.1 EXPERIMENTAL

2.2 Equipments/chemicals

High performance liquid chromatography (1200 DAD System, Agilent Technology, Ericino, Califonia, USA), gas chromatography (Model 8610C-SRI), Soxhlet machine (S-1829, Soxhlet-Giant, USA), centrifuge, borosilicate glass flask, screw-capped test-tube, Stopperd flask, Whatzman filter paper1 (542 mm), Spectrophotometer (Spectrumlab 752 S) water bath (PURA 22 JULABO USA, Allenntown, PA18109), magnetic rod, round bottom flask, aqueous ammonia (NH_3), chloroform ($CHCl_3$), hydrochloric acid, hexane, sulphoric acid, petroleum ethers, potassium hydroxide(KOH), benzene, ethanol, methanol, deionized water, paraffin oil, nitrogen steam, potassium permanganate ($KMnO_4$), sodium hydroxide (NaOH),

chromatography autosampler vials, phenolic acid standard and ethyl acetate.

2.3 Source and identification of plant material

Fresh aerial parts of E. heterophylla were harvested from Obinze Communities, in Owerri-West Local Government Area of Imo State, Nigeria. The plant sample was identified and authenticated by Dr. Ekeke Chimezie at the Herbarium Unit of the Department of Plant Science and Biotechnology (PSB), University of Port Harcourt.

2.4 Quantitative phytochemical screening using high performance liquid chromatography

The leaves of Euphorbia heterophylla subjected to phytochemical screening which was carried out at Multi Environmental Management Consultants Limited, Memac building, Olujunwo Avenue, Laara-igbe, Ikorodu, Lagos, Nigeria.

2.4.1 Extraction of alkaloids

Extraction of alkaloids was carried out using modified methods [14]. In this method, 5.0 g of the powdered sample was macerated in 25 ml of hexane for 72 hours. The mixture was filtered and residue air-dried and treated with 10% aqueous ammonia (NH_3) mixed with 5ml of chloroform ($CHCl_3$) for 24 hours. The residue was further treated with 75ml of 5 % hydrochloric acid (HCl) and delivered into a round bottom flask. The extract was concentrated to 2ml in vial for chromatography analysis while 1µl of the extract was injected into the port of gas chromatograph for the determination of the chromatograms of all the different alkaloids present in the aerial parts of the plant.

2.4.2 Extraction of glycoside

Glycoside extraction was carried out according to modified method [15]. In this method, 1.0 g of the powdered sample was macerated in 10 ml of 70% alcohol for 24 hours. The mixture was filtered and concentrated to 1ml in the vial for gas chromatography analysis as 1 µl was injected into the injection port of gas chromatograph for the determination of the chromatograms of the different glycoside present in the plant.

2.4.3 Extraction of flavonoids

Extraction of flavonoids was carried out using modified methods [16]. The powdered sample was oven-dried at 105^0C for two weeks of in the laboratory. In this method, 1.0 g of the oven-dried sample was weighed and macerated in 250ml capacity conical flask containing 100ml of methanol and distilled water in the ratio 70:30 for 1 hour. The mixture was filtered and the filtrate was concentrated to 1ml in vial for chromatography analysis while 1µl of the extract was injected into the port of gas chromatograph for the determination of the chromatograms of the different sub-members in the plant expressed in mg/100 g.

2.4.4 Extraction of saponin

Extraction of saponin was carried out based on modified methods [17]. In this method, 2.0 g of the powdered sample was macerated in 20 ml of methanol for 20 minutes. The mixture was filtered and the filtrate was concentrated to form a syrup which was suspended in water. The suspension was then filtered and concentrated to 2 ml in the vial for gas chromatography analysis while 1 µl was injected into the port of gas chromatograph for the determination of the chromatograms of the different saponin present in the aerial parts of the plant expressed in mg/100 g.

2.4.5 Phenolic extraction

Two stage of extraction procedure were employed for the effective removal of the polyphenols/phenolic compounds.

STAGE 1: A weight of 50.0 mg of the sample was extracted using 5ml of 1 M sodium hydroxide for 16 hours on a shaker at ambient temperature. After 16 hours, the mixture was centrifuged at 5000 rpm twice and supernatants was placed in a not reusable glass test tube and heated at 90^0C for 2 hours to release the conjugated phenolic compounds. The compounds were titrated against 4M hydrochloric acid to pH <2.0, diluted to 10 ml, with deionized water.

STAGE 2: The residue from stage 1 above was extracted with 5 ml of 4M sodium hydroxide, heated to 160^0C in Teflon. After cooling, the mixture was filtered and the supernatant collected and the residue washed with water (deionized). The supernatant was adjusted to pH <2.0 with 4M HCl and the filtrate were combined for further purification.

2.4.6 Extraction of lignans

Extraction of lignan was carried out using the methods [16]. In this method, 1.0g of the sample was weighed into 100 ml borosilicate glass flask. Then, 75 ml of 3 M solution of sulphoric acid was added to the solution and stirred carefully using a magnetic stirrer for about 1 hour and the mixture was filtered with Whatman filter paper 1 (542 mm). Then, 25 ml of the filtrate was collected and then titrated at 80^0C – 90^0C against 0.1M solution of potassium permanganate ($KMnO_4$) to the end point. While, 2ml of the solution was then put in vial for chromatography analysis and 1µl of the solution was injected into the port of gas chromatograph for the determination of the chromatograms of the different lignan present in the plant.

2.4.7 Extraction of sterols

Extraction of sterols was performed using modified methods [18]. In this method, 3.0 g of the powdered sample was weighed into 250 ml beaker and petroleum ether was gradually added until the powder was fully soaked for 24 hours. The mixture was filtered and concentrated to 1ml in vial for chromatography analysis while 1µl was injected into the port of gas chromatograph for the determination of the chromatograms of all the different sterol present in the plant expressed in mg/100 g.

2.4.8 Extraction of anthrocyanin

Extraction of anthrocyanin was carried out following modified methods [19]. In this method, 5.0 g of the sample was weighed into 50 ml borosilicate beaker. Twenty mililiter (20 ml) of 50% methanol was added to the mixture and covered with paraffin in a water bath at

80^0C for 1 hour. The mixture was stirred using a magnetic rod to prevent lumping. The mixture was then filtered using a doubled-layer Whatzman filter paper1 (542 mm) into a 100ml volumetric flask. The filtrate was concentrated to 2 ml in the borosilicate vial for the gas chromatography analysis; 1.0 µl was injected into the injection port of the gas chromatograph for the determination of the chromatograms of all the different anthocyanins present in the plant expressed in mg/100 g

2.4.9 Extraction of anthraquinone

Extraction of anthraquinone was carried out using the method as explicated by [20]. In this method, 5.0 g of the powdered sample was weighed and transferred to Stopperd flask and 20ml of petroleum ether was added until the powder was fully soaked. The flask was shaken six hourly for 24 hours. This process was repeated for three days and then the extract filtered. The filtrate was collected and evaporated to dryness by using nitrogen steam. Then, 0.5 g of the residue was added to the screw-capped test-tube. The sample was saponified at 95^0C for 30 minutes by using 3ml of 10 % potassium hydroxide (KOH) in the ethanol to which 0.20ml of benzene was added to ensure miscibility. Then, 3 ml and 2 ml of de-ionized water and hexane were added respectively for the of extraction non-saponifiable anthraquinones. The extract was concentrated to 2 ml in Agilent vial for gas chromatography analysis while 1µl of the extract was injected into the port of gas chromatograph for the determination of the chromatograms of the different anthraquinone present in the plant expressed in mg/100 g.

2.4.10 Extraction of terpenoid

Extraction of terpenoid was performed based on the modified methods [21]. In this method, 3.0 g of the powdered sample was macerated in 250 ml of redistilled chloroform for 24 hours. The mixture was filtered and the filtrated was extracted with 10ml of chloroform for 15minutes. The mixture was filtered and concentrated to 1ml in the vial for chromatography analysis while 1µl of the extract was injected into the injection port of gas chromatograph for the determination of the chromatograms of the different terpenoids present in the plant.

2.4.11 Extraction of essential oil

Extracted of essential oil was carried out using modified method as explained by [22]. In this method, 1.0 g of the powdered sample was macerated into 10 ml of hexane in 50 ml borosilicate glass flask for 24 hours. The mixture was filtered and the filtrate was treated with 75ml of 3M solution of sulphoric acid which was stirred with a magnetic rod for 1 hour. The mixture was again filtered and the filtrate concentrated to 2ml in vial for chromatography analysis while 1 µl of the extract was injected into the port of gas chromatograph for the determination of the chromatograms of the different essential oils present in the plant expressed in percentage.

3.1 RESULTS AND DISCUSSION

Table 1 *Quantitative phytochemical composition of alkaloids in the aerial parts of Euphorbia heterophylla*

Alkaloids	Concentration (mg/100g)
3,4-Di-O-galloylquinic acid	25.32
2,4.6-Tri-O-galloyl-D-glucose	73.06
1,2,3,4,6-penta-O-galloyl-beta-D-glucose	79.24
Euphorbin-A	135.02
Euphorbin-B	110.87
Tannic acid	4.89
Euphorbin-C	115.94
Euphorbin-D	93.37
Euphorbin-E	81.47
TOTAL	719.19

Table 2 *Quantitative phytochemical composition of glycoside content in the aerial parts of E. heterophylla*

Glycoside	Concentration (mg/100g)
Alfzelin	17.37
Arbutin	1.36×10^{-6}
Salicin	1.10×10^{-5}
Allantoin	9.41×10^{-6}
Amygdalin	4.49×10^{-5}
Kaempferitrin	3.18×10^{-5}
O-sitosterol-3-B-glycoside	3.81×10^{-5}
Rutin	39.93×10^{-4}
B-siterol-glycoside	1.06×10^{-6}
Costusoside I	2.98×10^{-6}
Coustusoside J	1.66×10^{-6}
Kaempferol-3-O-glycopyranoside	23.30
TOTAL	80.60

Table 3 *Quantitative phytochemical composition of flavonoid content in the aerial parts of E. heterophylla*

Flavonoids	Concentration (mg/100g)
(+)-catechin	9.59×10^{-4}
(+)-gallocatechin	2.97×10^{-5}
Coumarin	5.36×10^{-2}
Dihydroxycoumarin	3.69
Apigenin	9.45×10^{-4}
Butein	2.32×10^{-5}
Naringenin	6.44×10^{-3}
Kaemferol	12.53
Luteolin	3.19×10^{-4}
(-)-epicatechin	3.71×10^{-2}
Quercitrin	170.97
(-)-epigallocatechin	6.04×10^{-5}
Myricitrin	120.68
Quercetin	59.70
(-)-epicatechin-3-gallate	3.76×10^{-3}
Hysperin	1.05×10^{-3}
(-) (-)epigallocatechin-3-gallate	1.24×10^{-5}
Isorhamnetin	1.66×10^{-5}
Robinetin	6.45×10^{-3}
Abzelin	5.46×10^{-4}
Myricetin	8.49
Baicalein	9.26×10^{-5}
Isoquercitrin	4.30×10^{-5}
Baicalin	2.92×10^{-6}
Silymarin	3.24×10^{-7}
TOTAL	376.20

Table 4 *Quantitative phytochemical composition of sterol content in the aerial parts of E. heterophylla*

Sterol	Concentration (mg/100g)
Cholesterol	1.28×10^{-6}
Cholestanol	2.67×10^{-3}
Tinyaloxin	2.72
Daucosterol	3.10
Ergosterol	2.04×10^{-3}
Campesterol	4.63×10^{-1}
Stigmasterol	4.23
Stavenasterol	9.37×10^{-1}
Sitosterol	17.53
TOTAL	33.16

Table 5 *Quantitative phytochemical composition of anthrocyanin content in the aerial parts of E. heterophylla.*

Anthrocyanin	Concentration (mg/100g)
Cyanidine-3sophoside-5-glucoside	5.35×10^{-1}
Peonidin	3.13×10^{-1}
p-hydroxybenzolated (cyaniding-3-sophoroside-5-glucose)	5.61×10^{-1}
Caffeoylated (cyaniding-3-sophoroside-5-glucose)	2.68×10^{-1}
p-hydroxybenzolated (peonidin-3-sophoroside)	2.56×10^{-1}
Feruloylated (peonidin-3-sophoroside-5-glucose)	1.78×10^{-1}
Cyanidin-3-(6,6'-caffeoyl-p-hydroxybenzoylso	2.11
Cyanidin-3-(6,6'-dicaffeoylsophoroside)-5-glucose)	1.01
Cyanidin-3-(6-caffeoylsophoroside)-5-glucoside)	3.11×10^{-1}
Cyanidin-3-(6,6'-caffeoylferuloylsophoroside)	3.34×10^{-1}
Peonidin-3-(6,6'-dicaffeoylsophoroside)-5-glucose)	1.17
Peonidin-3-(6.6'cafeoyl-p-hydroxybenzoyylso)	6.65×10^{-1}
Peonidin-3-(6-caffeoylsophoroside)-5-glucose)	1.31×10^{-1}
Peonidin-3-(6.6'-caffeoylferuloylsophoroside)	1.79×10^{-1}
TOTAL	6.52

Table 6 *Quantitative phytochemical composition of anthraquinone content in the aerial parts of E. heterophylla.*

Anthraquinones	Concentration (mg/100g)
-2,6-dimethoxybenzoquinone	4.08×10^{-1}
6-methoxyquinolin-1-oxide	1.20×10^{-3}
Soranjidiol	2.90×10^{-1}
Damnacanthal	9.22×10^{-3}
Damnacanthol	1.13×10^{-3}
Heterophylline	3.00×10^{-1}
TOTAL	1.19

Table 7 *Quantitative phytochemical composition of terpenoid in the aerial parts of E. heterophylla*

Terpenoids	Concentration (mg/100g)
Euphorbioside-A	$2.44X\ 10^{-2}$
Euphorbioside-B	$2.49\ X\ 10^{-2}$
Taraxerol	$2.66X\ 10^{-1}$
Taraxerone	$5.21X\ 10^{-2}$
Alpha-amyrin	$2.39\ X\ 10^{-3}$
Deglucosyleuphorbioside–A	$1.37\ X\ 10^{-1}$
Clovandiol	$3.31\ X\ 10^{-1}$
Beta-amyrin	$9.59\ X\ 10^{-1}$
Lupeol	$1.89\ X\ 10^{-3}$
Ephaginol	$1.19\ X\ 10^{-1}$
Beuerenol acetate	$3.78\ X\ 10^{-3}$
TOTAL	1.92

Table 8 *Quantitative phytochemical composition of lignan content in the aerial parts of E. heterophylla*

Lignan	Concentration (mg/100g)
2-allyl-5-ethoxy-4-methoxyphenol	$2.28\ X\ 10^{-7}$
Laricresinol	$2.10\ X\ 10^{-3}$
(9E,12E,15E)-9,12,15-octadecatrien-1-ol	$1.60\ X\ 10^{-6}$
Matairesinol	$1.31\ X\ 10^{-2}$
Apigenin-4,7-dimethyl ethers	$3.49\ X\ 10^{-5}$
Epipinoresinol	$4.02\ X\ 10^{-2}$
Pinoresinol	$3.07\ X\ 10^{-2}$
Seroisolariresinol	$4.85\ X\ 10^{-2}$
Dehydroabietic acid	$1.68\ X\ 10^{-4}$
Retusin	$3.68\ X\ 10^{-2}$
Galgravin	$4.29\ X\ 10^{-4}$
Epieudesmin	$5.73\ X\ 10^{-6}$
Sakuranin	$9.32\ X\ 10^{-5}$
TOTAL	$1.72\ X\ 10^{-1}$

Table 9 *Quantitative phytochemical composition of phenolic acid content in the aerial parts of E. heterophylla*

Phenolic acids	Concentration (mg/100g)
Salycicylic acid	$3.66\ X\ 10^{-6}$
Cinnamic acid	$1.17\ X\ 10^{-7}$
Protocatechuic acid	5.79
P-Coumaric acid	$6.08\ X\ 10^{-6}$
Vanillic acid	8.48
O-Coumaric acid	$3.21\ X\ 10^{-6}$
p-hydroxybenzoic acid	$6.52\ X\ 10^{-2}$
Gallic acid	5.54
Ferulic acid	$7.26\ X\ 10^{-2}$
Syringic acid	$1.90\ X\ 10^{-2}$
Caffeic acid	$9.70X\ 10^{-2}$
Piperic acid	$1.30\ X\ 10^{-7}$
Sinapinic acid	$3.65\ X\ 10^{-6}$
Ellagic acid	5.25
Chlorogenic acid	$3.45\ X\ 10^{-5}$
Rosmarinic acid	$7.20\ X\ 10^{-5}$
TOTAL	25.32

Table 10 *Quantitative phytochemical composition of saponin in the aerial parts of Euphorbia heterophylla.*

Saponin	Concentration (mg/100g)
Hispogenine	1.08×10^{-4}
Saponin B	3.29×10^{-4}
Saponin C	1.05×10^{-4}
Solagenin	2.72×10^{-5}
Diosgenin	$2.45X\ 10^{-4}$
Justicisaponin-1	4.15×10^{-4}
Tigogenin	1.88×10^{-2}
Neochlorogenin	8.42×10^{-1}
Hecogenin	1.89×10^{-4}
Sapogenin	126.03
Tribuloin	4.31×10^{-4}
Yanogenin	4.37×10^{-4}
Conyzorgin	$1.73X\ 10^{-4}$
Gracillin	2.73×10^{-6}
Saponine	12.90
TOTAL	139.80

Table 11 *Quantitative phytochemical composition of essential oil in the aerial parts of Euphorbia heterophylla.*

Essential oil	Concentration (%)
Xylene (o,m,p)	6.95
2,4-dimethylhexane	0.09
Cis-1,3-dimethylcyclohexane	0.07
3,5-bis (1,1-dimethylethylphenol	8.01
Beta pinene	0.18
Benzylalcohol	0.14
Alpha, alpha-dimethyl-14-benzenedimeti	8.26
Isoartemisia	0.05
1,8-Cineole	0.14
Eugenol	0.07
Linalool	0.02
Alpha terpineol	0.07
Terpinen-4-ol	0.07
13-Octadecenal	2.29
Thymylmethylether	0.08
Trans-decahyronaphthalene	0.03
Undecanoic acid- ethyl ester	0.04
Linalyl acetate	0.11
Ethyl cinnamate	0.14
Beta bisabolene	1.07
Beta -caryophyllene	2.64
Dodecanoic acid	4.45
14-methylpentane decanoic acid methyl	3.21
Pentadecane	0.12
Gama-cadinene	0.07
Beta-elemene	0.03
Beta-cadinene	0.04
Hexadecane	0.11
2,6,10,15-tetramethylheptadecane	0.09
9,12-octadecanoic acid	19.51
Hexadecanoic acid	18.78
Hexadecanoic acid methylester	10.64
10-Octadecanoic acid, methylesters	8.40
Phytol	0.04
Heneicosane	0.06
Tetracosane	0.07
Eicosanoic acid ethylester	3.63
Hexacosane	0.07
Heptacosane	0.09
Alphamuurolene	0.04
TOTAL	100.00

Medicinal plants constitute the main source of new pharmaceuticals and healthcare products [23]. Extraction and characterization of several active phytonutrients from these green factories have given birth to some high activity profile drugs [23]. It is believed that crude extract from medicinal plants are more biologically active than isolated compounds due to their synergistic effects [24]. Phytochemical screening of plants has revealed the presence of numerous chemicals including alkaloids, tannins, flavonoids, steroids, glycosides and saponins etc. Secondary metabolites of plants serve as defense mechanisms against predation by many microorganisms, insects and herbivores [25]. Qualitative and quantitative phytochemical screening of the aerial parts of E. heterophylla showed the presence nine alkaloid phytochemicals with total concentration of 719.18mg/100g as shown in Table 1. Euphorbia heterophylla is reported to possess and elicit antifungal and antibacterial potential [26]. The high alkaloid concentration (719.18mg/100g) in the plant could be

responsible for the antifungal and antibacterial potentials elicited by Euphorbia heterophylla. Emmanuel, 2010 offer similar claim on the phytochemical composition, bioactive and wound healing potential of Euphorbia heterophylla leaf extract. Table 3 showed twenty five different flavonoids with total concentration 376.20mg/100g which is the second most abundant phytochemical in the plant followed by saponins (fifteen different members) with total concentration 139.80mg/100g and twelve different sterol with total concentration 33.16mg/100g. Saponins, flavonoids and sterols are known to be responsible for membrane-permeabilizing, immune-stimulating, hypocholesterolaemic and anticarcinogenic properties of medicinal plants [27]. Saponins, flavonoids and sterols are phytochemical constituents found in many species of Euphorbia heterophylla and have been known to kill protozoans and molluscs, produce an effect on cold blooded animals, elicits analgesic, antioxidant activity, to impair the digestion of protein, to cause hypoglycemia and to act as antifungal and antiviral agents [25]. The anti-inflammatory, antioxidant and anti-tubular cytotoxicity of Euphorbia species could be due to the presence of saponin, flavonoids and sterols. This result is also in agreement with the report of Mei-Fen and Jong-Yuh, 2012 on the therapeutic application of Euphorbia heterophylla as anti-inflammatory and antioxidant agent . Anthocyanins are natural colorants belonging to the flavonoid family and are widely distributed among flowers, fruits, and vegetables [28]. Anthrocyains such as aglycons delphinidin, cyanindin, pelargonidin, malvidin, peonidin, and kuromanin (cyanidin-3-glucoside). Kuromanin had the highest oxygen reabsorption antioxidant activity (ORAC) which was 3.5 times stronger than vitamin E analogue [6]. Table 5 in this study showed the presence of twelve different anthrocyanins with a total concentration of 6.52mg/100g which may also be responsible for the antioxidant property of Euphorbia heterophylla. Anthraquinone possess therapeutic properties including laxative potential [26]. Table 6 in this study indicated the presence of six different anthraquinones with a total concentration of 1.19mg/100g which may be responsible for the purgative potential of Euphorbia heterophylla as reported by Oksuz et al., (1995) and Falodun et al., (2003). The data in this study also agrees with the Maryam, et al., (2017) on the use of anthraquinones and their derivatives as a antimicrobial agents. Phenolic acids are the largest and most ubiquitous group of metabolites that have aromatic ring containing one or more hydroxyl constituents [29]. Phenolic acids are widely found in the secondary products of medicinal and other edible plants [30]. They are responsible for free radical scavenging, metallic ions chelation, and changing enzymatic activity. Moreover, these compounds exhibit antiviral activity, anti-inflammatory activity, diuretic, anti-allergic, cholagogic and choleretic activities [31]. In this research, sixteen different types of phenolic acids with a total concentration of 25.32mg/100g were characterized which may be responsible for antiviral, antibacterial and anti-durectics properties of Euphorbia heterophylla. This result agrees with the report of Singh et al., 2007 on evaluation of antioxidant potential of ethyl acetate extract/fractions of Acacia auriculiformis. Essential oils are volatile and liquid aroma compounds from natural sources, usually plants [32]. Essential oils are regarded chemical weapons of plants in terms of functionality and protect plant against bacterial and fungal attack [32]. In this study, Fourty different types of essential oils (100%) were characterized from the aerial parts of Euphorbia heterophylla. The yield of essential oil from leaves of E.heterophylla reported by Ivan and Christopher, 2018 was 0.1816%w/w which was slightly different from what Adedoyin et al., (2013) obtained from the leaves of same plant which was 0.21%w/w. This could be attributed to external factors such as climate, nature of the soil, age of the plant, and time of collection but also mode of extraction [33].

CONCLUSION

From this research, the qualitative and quantitative screening of the aerial parts of Euphorbia heterophylla showed the presence of teeming different phytochemical constituents and essential oils with various therapeutic properties that are precursors in most western therapeutic agents.

ACKNOWLEDGMENT

The authors wish to express their gratitude to the staffs of Multi environmental management and Mr. Ighariemu Victor of Environmental Toxicology Unit in the department of Biochemistry, University of Port Harcourt Rivers, state for guiding me through preparation of this manuscript.

CONFLICT OF INTEREST

The authors declare that they do not have any conflict of interest

REFERENCES

[1] Ncube, N.S., Afolayan, A. J and Okoh, A. I. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. African Journal of Biotechnology, 2008, 7 (12): 1797-1806.
[2] Dutta choudhury, M. Ethno-medico botanical aspects of Reang tribe of Asam: A comprehensive study. PH.D thesis of Gauhati University, 1999.
[3] Edeoga, H.O., Okwu, D., Mbaebie, B.O. Phytochemical constituents of some Nigerian Medicinal plants. Afri. J. Biotechnol, 2005, 4(7):685-688.
[4] Rout, S.P., Choudhury, K.A., Kar, D.M., Das, L., Jain, A. Plants in traditional medicinal system-future source of new drugs. Internl. J. Pharmacy Pharmaceurical. Sci, 2009, 1(1):1-23.
[5] Akharaiyi, F.C., Boboye, B. Antibacterial and phytochemical evaluation of three medicinal plants, J Nat Prod, 2010 3:27-34.
[6] Hong, W., Guohua, C and Ronald, L. Oxygen Radical Absorbing Capacity of Anthocyanins. J. Agric. Food Chem, 1979, 45 (2), pp 304–309.
[7] Yorinori, J.T. Biological control of milk weed (Euphorbia heterophylla) with pathogenic fungi. In: Delfosse ES (ed). Proceedings of the Sixth International Symposium on Biological Control of Weeds 1985: 667–681.
[8] Falodun, A., Agbakwuru, E. O., and Ukoh, G. C. Antibacterial activity of Euphorbia heterophylla Linn

(Family-Euphorbiaceae). Pakistan Journal of Scientific and Industrial Research, 2003, 4(3):31-42.
[9] Oksuiz, S., Gil, R.R., Heebyung, C and Pezzuto, J.M. Biologically Active Compounds from the Euphorbiaceae; 2. Two Triterpenoids of Euphorbia cyparissias. Planta Medica, 1995, 60(6):594-6.
[10] Elshamy, A.I., Abd El-Gawad, A.M., El Gendy, A.E.G and Assaeed, A.M. Chemical characterization of Euphorbia heterophylla L. essential oils and their antioxidant activity and allelopathic potential on Cenchrus echinatus L. Chem Biodivers. 2019
[11] Lingaraju, K., Raja, N., Nagabhushana, G and Nagaraju, G (2019). Euphorbia heterophylla (L.) mediated fabrication of ZnO NPs: Characterization and evaluation of antibacterial and anticancer properties. Biocatalysis and Agricultural Biotechnology, 2019, Volume 18, 100894.
[12] Ngounou, L., Kuete, V., Ngameni, B., Simo, C.C, Tankeu, R. K., Ngadjiu, B.T and Meyer, J.M. Extraction and isolation of Alkaloids from the Stem Bark of Garcinia cowa. Phytochemistry, 2005, 39,124-144.
[13] Kolb, N., Herrera, J.L and Fereyra, D.J. Analysis of Sweet Diterpene Glycosiedes from Stevia rebaudinana. Improved High Performance Liquid Chromatography Method. European Food Research and Technology, 2001, 231, 581-588.
[14] Victorio, C.P., Lage, C.L.S., Kuster, R.M and Quimica, E . Flavoid extraction from Alpinia zerumbet (Pers.). Ecletica Quimoica, 2010, 35, 15-46.
[15] Lough, G.N., Lannang, A.M., Ambazoa, C.D and Tangmoue, J.G. Extraction and isolation of Saponin Phytochemical constituents from the wood trunk of Garcinia polyanantha Oliv. Phytochemistry, 2009, 9, 1013-1017.
[16] Day, B.K and Underwood, J.C. Extraction and isolation of Essential oil using High Performance Liquid Chromatography (HPLC) and Gas Chromatography (G.C). Phytochemistry, 1986, 52, 32-47.
[17] Goad, T and Akihisa, W. Validation of a quantitative procedure for the extraction of Sterol from edible oils using radiolabelled compounds. Article in Food Science, 2012, 32, 4-17.
[18] Xiaoxi, W. Extraction of Anthocyanin from Black Bean Canning wastewater with Macrowave-assisted Resins. Article in Journal of Food Science, 2014, 79(2):245-31.
[19] Hemwimon, S., Pavasant, P and Shotiprak, A. Microwave-assisted extraction of
[20] Antioxidant Anthraquinone from the root of Morinda citrifolia, 2007, 54, 44-50.
[21] Ivanova, D., Gerova, D., Chervenkov, T and Yankova, T. (2005). Polyphenols and antioxidant capacity of Bulgarian medicinal plants. Journal of Ethnopharmacology, 2005, 96: 145-150.
[22] Mandal, V., Mohan, Y and Hemalatha, S. Microwave assisted extraction: An innovative and promising extraction tool for medicinal plant research. Pharmacognosy Reviewed, 2007, 1: 7-18.
[23] Jana, S and Shekhawat, G.S. Phytochemical analysis and antibacterial screening of in vivo and in vitroextracts of Indian medicinal herb: Anethum graveolens. Res. J. Med. Plant, 2010, 4: 206-212.
[24] Cowan, M.M. Plant products as antimicrobial agents. Clinical Microbiology, Rev., 1999, 12: 564-582.
[25] Ibtisam, M .A. Antimicrobial Activity and Phytochemical Screening of Sarcocolla Gum Resin. Pakistan Journal of Biological Sciences, 2017, Volume 20(11): 571-576.
[26] Mei Fen, S and Jong Yuh, C. Potential Applications of Euphorbia hirta in Pharmacology. In book: Drug Discovery Research in Pharmacognosy,2012,
[27] Anthon, R and Hang, B.M. Therapeutic uses of Natural anthraquinon for other than Laxative action. Pharmacology, 1980, 20:(suppl.1):104-112.
[28] Maryam, M., Rita, S and Olga, M. D. S. Anthraquinones as potential antimicrobial agents-A review. A. Mendez-Vilas, 2017, pp.55-6.
[29] Singh, R., Singh, S. , Kumar, S and Arora, S. Evaluation of antioxidant potential of ethyl acetate extract/fractions of Acacia auriculiformis A. Cunn. Food Chem. Toxicol., 2007, 45: 1216-1223.
[30] Hagerman, A.E., Riedl, K.M. , Jones, G.A. , Sovik, K.N. , Ritchard, N.T. , Hartzfeld, P.W and Riechel, T.L. High molecular weight plant polyphenolics (Tannins) as biological antioxidants. Journal of Agricultural Food Chemistry, 1998, 46: 1887-1892.
[31] Atoui, A. K., Mansouri, A., Boskou, G and Kefalas, P. Tea and herbal infusions: Their antioxidant activity and phenolic profile. Food Chemistry, 2005, 89, 27-36.
[32] Hamid, A. A. Aiyelaagbe, O. O and Usman, L. A. Essential oils: its Medicinal and Pharmacological Uses. International Journal of Current Research, 2011, Vol. 33, Issue, 2, pp.086-098.
[33] Ocheng, F., Bwanga, F., Almer, B.E., Joloba, M., Borg-Karlson, A.K., Yucel-Lindberg, T and Gustafsson, A. Essential Oils from Ugandan Medicinal Plants: In Vitro Cytotoxicity and Effects on IL-1β-Induced Proinflammatory Mediators by Human Gingival Fibroblasts. Evidence-Based Complementary and Alternative Medicine : eCAM, 2016, 5357689. https://doi.org/10.1155/2016/5357689.
[34] Emmanuel, T.F. Phytochemical composition, bioactivity and wound healing potential of Euphorbia heterophylla (Euphorbiaceae) leaf extract. International Journal of Research and Pharmaceutical and Biomedical Sciences, 2010, 1(1).