CHEMICAL COMPOSITION, ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF VOLATILE OIL OF EUPHORBIA GRAMINEA JACQ FROM NIGERIA

[1]EJIKE O. OKPALA, [2]GANIYAT K. OLOYEDE, [3]PATRICIA A. ONOCHA

[1,2,3]Natural Products/Medicinal Chemistry Unit, Department of Chemistry, University of Ibadan, Nigeria.
E-mail: [1]okpalaejike2015@gmail.com, [3]oloyedegk@gmail.com, [3]triciaonocha@yahoo.com

Abstract - Hydro-distillation of the air dried and pulverized whole plant of Euphorbia graminea yielded colourless volatile oil which was analysed by gas chromatography-mass spectrometry. Isopropyl palmitate (45.81%), Oleic acid (14.09%), Palmitic acid (6.65%) and Trans-2-Pentenal (5.68%) were the major constituents in the oil. The volatile oil revealed good antimicrobial activity against ten tested strains of clinical isolates of bacteria and fungi. It was observed that the potency of the volatile oil increased with increase in concentration. High zones of inhibition were observed across all the micro-organisms tested at 100µg/ml. Antioxidant activity of the volatile oil tested using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical assay revealed a moderate activity (40%) at concentration of 1000 µg/ml. The present study confirms the traditional use of E. graminea in folk medicine for dermatological diseases and microbial infections.

Keywords - Euphorbia Graminea, Volatile Oil, Gas Chromatography-Mass Spectrometry, Antioxidant Activities

I. INTRODUCTION

Medicinal plants have various effect on living system ranging from analgesic, antipyretics, cardio-protective, anti-inflammatory, antioxidant, antispasmodics to anticancer and anti-human immune virus (1-5). Scientific studies revealed that volatile oils are released by some plants and trees to act as antimicrobial, antifungal, and antibacterial agents against a variety of organisms that may threaten their survival. Essential oils have been reported to have antioxidant properties capable of scavenging free radicals that are known to cause oxidative damages to biological macromolecules. Essential oil chemistry therefore, may provide the basis for the therapy of diseases resulting from oxidants (6-9). Euphorbia graminea (Euphorbiaceae), a grassleaf spurge is an annual herb (10) which though native to northern Mexico, Colombia, and Venezuela, has been present in Nigeria as early as 1993 (11-12).

The chemical composition, antioxidant and antimicrobial activities of volatile oil of Euphorbia graminea has not been reported in literature. This study is aimed at characterizing the volatile oil of E. graminea and investigating their biological activities based on their ethno-medicinal uses as antimicrobial and anticancer agents.

II. MATERIALS AND METHODS

A. Plant Materials

Euphorbia graminea was collected from Ibadan, Oyo State. The plant was identified and authenticated at the Forest Herbarium Ibadan, Nigeria, where a voucher specimen was deposited as FHI-110653.

B. Extraction of the Essential Oils

The air dried and pulverized whole plant of Euphorbia graminea was weighed and subjected to hydrodistillation for three hours using all glass Clevenger distillation unit designed according to the British pharmacopoeia specification (13). The volatile oil trapped in 2.0 ml of hexane was collected in a pre-weighed sample vial and refrigerated at 4^{o}C until the time of analysis.

C. Gas chromatography-mass spectrometry (GC-MS) analysis

The volatile oil was analysed using GC-MS Agilent Technologies, Model-7890A Gas Chromatograph, coupled with a 5975C mass spectrometer Agilent Technology. The gas chromatograph capillary column type was a HP-5MS, with column length 30 m, internal diameter 0.320 mm and film thickness 0.25 µm. The carrier gas used was helium at a constant flow rate of 1.4123 mL/min and an average velocity of 43.311 cm/sec, while the pressure was put at 1.5 psi. Temperature programming started with an initial column temperature set at 80 ^{o}C for 2 mins and was increased to 240 ^{o}C at the rate of 10 ^{o}C/min. The volume of the sample injected was 1 µL and the split ratio was 50:1 and split flow at 70.615ml/min. The total chromatogram was auto-integrated and the constituents were identified by comparison of the GC-MS data with published mass spectral database (NIST 11.L) and literature data.

D. Antimicrobial assay

1. Preparation of graded concentration of the samples
A solution of the volatile oil was prepared by dissolving 200 µg of the oil in 2.0 ml of

Dimethylsulfoxide (DMSO) to give 100 µg/ml. From the 100 µg/ml solution, 1 ml was taken into another sample bottle and 1 ml of solvent (DMSO) was added to give 50 µg/ml. This was serially diluted until a 6.25 µg/ml concentration was obtained. Two other sample bottles contained the negative control (DMSO solvent) and the positive controls (Gentamicin 10 µg/ml for bacteria and Tioconazole 70% for fungi).

2. Agar diffusion: Pour Plate Method for Bacteria

The volatile oil was screened against two gram positive bacteria: Staphylococcus aureus and Bacillus subtilis; four gram negative bacteria: Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae and Salmonella typhi; obtained from Department of Pharmaceutical Microbiology. An overnight culture of each organism was prepared by taken a loop full of the organisms from stock (slope) and inoculated each into the sterile nutrient broth of 5 ml each incubated for 18-24 hrs at 37 ^{o}C. From the overnight culture, 0.1 ml of each organism was taken and put into 9.9 ml of sterile distilled water to get 1:100 (10^{-2}) dilution of the organisms. From the molten diluent (10^{-2}), 0.2 ml was taken into the 20 ml of freshly prepared sterile Nutrient Agar, and then shakes gently for uniformity, aseptically poured into sterile petri-dishes, allowed to solidify for about 30-50 minutes. Using a sterile cork borer of 6 mm diameter and wells were made in the set Nutrient Agar plate according to the number of graded concentrations of the samples.

In each well, 0.02 ml of the different graded concentrations of the oils were introduced in the well using Pasteur pipette. This was done in triplicate. The plates were allowed to stay on the bench for 1 hrs for pre-diffusion. The plates were incubated uprightly in the incubator for 18-24 hrs at 37^{o}C. Then the observed zones of inhibition were measured (16-17).

3. Agar diffusion: Surface Plate Method for Fungi

The anti-fungi potential of the volatile oil was determined against four fungi strains: Candida albicans, Aspergillus niger, Rhizopus stolonifer and Penicillum notatum; obtained from Department of Pharmaceutical Microbiology. A sterile Sabouraud Dextrose Agar (62g/L) was prepared and aseptically poured into the sterile plate in triplicates and allowed to set properly. The same procedure described for antibacterial assay was carried out. In each well, the graded concentrations of the oils were introduced into the wells including the controls. The plates were left on the bench for 120 minutes so as to allow volatile oil to diffuse properly into the agar i.e. pre-diffusion. The plates were incubated uprightly in the incubator for 48 hours at 26-28 ^{o}C (16-17).

E. Antioxidant assay

The antioxidant activity of the volatile oil was determined using the DPPH (2, 2-diphenyl-1-picryl hydrazyl) free radical scavenging ability method described by Shimadu et al., (1992) with some modifications. Various concentrations (1000µg/mL, 500µg/mL, 250µg/mL, 125µg/mL and 62.5µg/mL) of the essential oil was mixed with 100µM methanolic DPPH solution (2.0 mL), prepared by dissolving 3.94 mg of DPPH in 100 ml of methanol to give a purple solution. The mixture was shaken vigorously and left to incubate for 30 minutes in the dark at room temperature and the absorbance was then measured at 517 nm and recorded as $A_{(sample)}$ using a GS UV-12, UV-VIS Spectrophotometer. In its radical form, DPPH absorbs, but upon reduction by antioxidant species, its absorption reduces. A blank experiment was carried out applying the same procedure without essential oil (DPPH + Methanol) and the absorbance was recorded as $A_{(control).}$ Ascorbic acid, Butylated Hydroxyl Anisole (BHA) and α-Tocopherol were used as standards for comparison (14-15). The free radical scavenging activity of the essential oil was then calculated as percentage inhibition according to the following equation:

$$\%\text{Inhibition} = (Abs_{control} - Abs_{sample})/(Abs_{control}) \times 100$$

III. RESULTS AND DISCUSSION

1. Chemical Composition of volatile oil

The yield of the volatile oil (% w/w) from E. graminea plant was 0.94g corresponding to a percentage yield of 0.88%. Four compounds were identified in the volatile oil representing a total percentage composition of 72.23%. All the compounds were non-terpenoids. Isopropyl palmitate (45.81%) was the most abundant compound and other compounds present in the E. graminea plant volatile oil include: Oleic acid (14.09%), Palmitic acid (6.65%), and Trans-2-Pentenal (5.68%).

S/N	RI	COMPOUNDS	% Composition
1	75	Trans-2-Pentenal	5.68
2	1621	Isopropyl palmitate	45.81
3.	2104	Palmitic acid	6.65
4.	2057	Oleic acid	14.09
		Total	72.23

Table 1: Chemical composition of volatile oil of Euphorbia graminea

RI = Calculated Retention index; Compounds listed in order of elution from a HP-5MS column.

2. Antioxidant activity of the volatile oil

The activity for the scavenging ability on DPPH radicals of the volatile oil from the whole plant of E. graminea assays are summarized in Tables 2 and 3. The antioxidant activity of the volatile oil of E. graminea plant exhibited a moderate antioxidant activity when compared to ascorbic acid and butylated hydroxyl anisole, known antioxidants used as standards at all concentrations, at 1000-62.5 µg/ml

measured absorbance values of the volatile oil increases from 0.793 ± 0.005 to 0.914 ± 0.001 (Table 2) which correspond to calculated percentage inhibition (Table 3) of 40-31% in comparism to that of two known antioxidants Ascorbic acid and Butylated hydroxyl anisole.

Conc. (µg/ml)	Euphorbia graminea	Ascorbic Acid	Butylated Hydroxyl anisole
1000	0.793 ± 0.005	0.063 ± 0.001	0.076 ± 0.002
500	0.865 ± 0.002	0.065 ± 0.001	0.076 ± 0.002
250	0.884 ± 0.001	0.066 ± 0.003	0.076 ± 0.001
125	0.898 ± 0.003	0.067 ± 0.002	0.080 ± 0.004
62.5	0.914 ± 0.001	0.282 ± 0.001	0.087 ± 0.005

Table 2: Absorbance values from scavenging effect of volatile oil from E. graminea and standard compounds at 517nm of DPPH method of antioxidant assay

Absorbance values in mean ± standard error; Absorbance of control (DPPH + Methanol =1.32 1nm)

Conc. (µg/ml)	E. graminea	Ascorbic Acid	Butylated Hydroxyl anisole
1000	40	95	94
500	35	95	94
250	33	95	94
125	32	95	94
62.5	31	79	93

Table 3: Percentage inhibition of volatile oil from E. graminea and standard compounds

3. Antimicrobial activity of the volatile oil

Table 4 present the calculated mean zones of inhibition (mm) of the volatile oil of the whole plant of Euphorbia graminea. The mean zones of inhibition of growth of the isolates are a function of relative antibacterial and anti-fungal activities of the volatile oil. The zone of inhibition is simply the area on the agar plate that remains free from microbial growth. The size of the zone of inhibition is usually related to the level of antimicrobial activity; a larger zone of inhibition usually means that the antimicrobial is more potent. It was observed that the potency of the essential oil of E. graminea increased with increase in concentration. High zones of inhibition were observed across all the micro-organisms tested at 100µg/ml. Also, the volatile oil showed a broad spectrum of activity (recorded zone of inhibition of 10mm and above) on both two gram positive bacteria: Staphylococcus aureus and Bacillus subtilis: four gram negative bacteria: Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumonia and Salmonellae typhi used.

Test Micoorgan-isms	Conc.(µg/ml) / Zones of Inhibition (mm)								
	100	50	25	12.5	6.25	3.125	DMSO	Gen. (+ve)	Tio. (+ve)
S. auerus	26	24	20	12	14	10	-	36	-
E. coli	24	20	18	16	14	10	-	38	-
B. subtilis	24	22	20	16	12	10	-	40	-
P. aeruginosa	26	22	18	16	14	10	-	38	-

S. typhi	24	20	18	16	14	10	-	40	-
K. pneumona	24	20	18	16	14	10	-	38	-
C. albican	20	18	16	12	12	10	-	-	28
A. niger	20	18	16	12	12	10	-	-	28
P. notatum	18	16	14	10	10	10	-	-	28
R. stolonifer	18	16	14	10	10	10	-	-	28

Table 4: Antimicrobial activity of volatile oil of Euphorbia graminea Plant

DMSO = negative control (Dimethylsulphoxide), Gen. (+ve) = positive control (Gentamicin at 10µg/ml for bacteria), Tio. (+ve) = positive control (Tioconazole 70% for fungi), - = no inhibition, S. aureus = Staphylococcus aureus, E. coli = Escherichia coli, B. subtilis= Bacillus subtilis, P. aeruginosa = Pseudomonas aeruginosa, S. typhil = Salmonella typhi, K. pneumonae = Klebsiellae pneumona, C. albicans = Candida albicans, A. niger = Aspergillus niger, P. notatum= Penicillum notatum. R. stolonifer = Rhizopus stolonifer.

Isopropyl palmitate which is the most abundant constituent of the volatile oil, is the ester of isopropyl alcohol and palmitic acid. Palmitic acid has been reported to possess antioxidant properties while some studies have revealed the beneficial effect of oleic acid on cancer, autoimmune and inflamatory diseases (18). The presence of these constituents in the volatile oil in addition to the antimicrobial and antioxidant assay results of the volatile oil of the whole plant supports its ethno-medicinal uses for the treatment of dermatological diseases, microbial infections and as anticancer agent.

IV. CONCLUSION

Euphorbia graminea is used traditional for the treatment of cancer, skin infections and other ailments. Chemical constituents, antibacterial, antifungi and antioxidant activities of the volatile oil from the plant is reported for the first time. E. graminea volatile oil exhibited good antimicrobial activity against tested strains of clinical isolates of bacteria and fungi. The present study justifies the traditional use of E. graminea in folk medicine for dermatological diseases and microbial infections.

REFERENCES

[1] Cassel and R.M.F Vargas, “Experiments and modeling of the Cymbopogon winterianus essential oil extraction by steam distillation,” J Mexican Chem Soc, Vol.50, pp.126-9, 2006.

[2] P. Di Leo Lira, D. Retta, E. Tkacik, J. Ringuelet, J. D. Coussio, C. Van Baren and A. L. Bandoni, “Essential oil and by-products of distillation of bay leaves (Laurusnobilis L.) from Argentina,” Ind. Crops Prod Vol. 30,pp.259-64, 2009.

[3] F. Bakkali, S. Averbeck, D. Averbeck, and M. Idaomar, “Biological effects of essential oils- a review,” Food Chem Toxicol. Vol. 46, pp. 446-75, 2008.

[4] A. A. Mohamed, G. A. El-Emary, and H. F. Ali,” Influence of some citrus essential oils on cell viability, glutathione-s-transferase and lipid peroxidation in Ehrlichascites Carcinoma cell,” J. Am Sci, Vol. 6, pp.820-826, 2010.

[5] D. E Okwu,. and V. Ezenagu, “Evaluation of the phytochemical composition of Mango (Mangifera indica Linn) stem bark and leaves,” Int. J. Chem. Sci.,Vol. 6(2), pp.705-716, 2008.

[6] W. P. Lindsay, C. J. Lamb, and R. A. Dixon, “Microbial recognition and activation of plant defense systems. Trends Microbial,”Vol.1, pp.181-187,1993.

[6] R. A. Dixon, :Natural products and plant disease resistance,” Nature, Vol.411, pp. 843-7, 2001. H. O. Edeoga, D. E. Okwu, and B. O. Mbaebia, “Phytochemical constituents of some Nigerian medicinal plants,” African journal of Biotecnology, Vol. 47, pp.685-688, 2005.

[7] G.K. Oloyede, and P.A. Onocha, “ Oxygenated chemical constituents, brine shrimp toxicity and free radical scavenging activity of leaf essential oils of Alchornea cordifolia (Schumach. & Thonn.) Mull. Arg.,” International journal of Essential Oil Therapeutics Vol. 4, pp. 104-107, 2010.

[8] E.I. Aigbokhan, and O. Ekutu, “Aspects of the Biology and Ecology of Euphorbia graminea Jacq. (Euphorbiaceae)- A potentially Invasive Herbaceous Plant in Nigeria, “ igerian Journal of Botany ,Vol.25, pp. 35-53, 2012.

D.B. Ward, and C.M. Housel, “A corrected identification and a new combination for a recent Florida introduction: Agaloma graminea (Euphorbiaceae),” Phytologia,Vol. 89 (2), pp. 225, 2007.

A. Danin, S. Cohen-Sivan, and T. Raus, “Euphorbia graminea (Euphorbiaceae). In: Greuter W. & Raus, Th. (ed.): Med-Checklist Notulae, 28,” Willdenowia, ,Vol. 39, pp. 335-345, 2009.

[9] British Pharmacoepia. London: H, M Stationary office, PA.Vol. 2, pp. 109, 1980.

[10] P.A. Onocha, G.K. Oloyede, and O. A. Bamkole,“Volatile Oil Composition, Cytotoxicity and free radical scavenging of leaves and twigs of the Chenille plant (Acalypha hispida),” Inter J Essen Oil Therapeutics, Vol. 4, pp. 86-89, 2010.

[11] G.K. Oloyede, P.A. Onocha, J. Soyinka, O. Oguntokun, and E. Thonda, “Phytochemical screening, antimicrobial and antioxidant activities of four Nigerian medicinal plants,” Annals of Biological Research,Vol. 1(2), pp.114-120, 2010.

[12] P.A. Onocha, G.K. Oloyede, and Q. A. Afolabi, “Chemical composition, cytotoxicity and Antimicrobial Activity of Essential Oils of Acalypha hispida flowers,” International Journal of Pharmacology, Vol. 7, pp. 144-148, 2011.

[13] National Committee for Clinical Laboratory Standards

(NCCLS), "Performance Standards for Antimicrobial Susceptibility Testing in: 11th Informational Supplement, M100-S11, Wayne, PA," pp. 48-52, 2001.

[14] H. Sales-Campos, P. Reis de Souza, B.C. Peghini, J. Santana da Silva, and C.R. Cardoso, "An overview of the modulatory effects of Oleic acid in health and diseases," Mini-Reviews in medicinal chemistry, Vol. 13 (2) 2013. DOI: 10.2174/1389557511313020003.

★★★