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QUANTITATIVE PHYTOCHEMICAL ESTIMATION AND PROXIMATE ANALYSIS OF THE WHOLE PLANT OF *EUPHORBIA HETEROPHYLLA LINN.*

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ABSTRACT

Natural products of plant origin offer a vast resource of newer medicinal agents with potential in clinical applications. *Euphorbia heterophylla* is an herb and belongs to the family Euphorbiaceae. It is an important and widely used medicinal plant especially in the treatment of fungi. This study aims to analyse the quantitative phytochemicals and proximate chemical composition of the whole plant of *E.heterophylla* using standard methods. The quantitative phytochemical constituents was separately analysed in n-hexane, ethyl acetate, methanol and aqueous fractions in order of increasing polarity. The results indicated that phenol has the highest percentages in the overall quantitative phytochemical estimation as shown in the aqueous (12.30± 0.70%), methanol (11.20± 0.20%) and n-hexane (9.65±2.35%) fractions. This was followed by tannin with percentages of (10.00± 0.20%), (9.00± 0.50%) and (7.00± 0.00%) in aqueous, methanol and ethyl acetate fractions respectively. The lowest percentages of the quantitative phytochemical estimation were recorded in saponin n-hexane and ethyl acetate fractions with values of (0.50± 0.05%) and (0.80±0.00%) respectively. This was replicated in the values obtained from the alkaloid ethyl acetate and methanol fractions and flavonoid n-hexane fraction with each having a percentage of (1.00± 0.00%). The presence of these bioactive compounds is an indication of its therapeutic uses. The moisture content was higher in the fresh plant sample (83.06±2.42%) and lower in the dried plant sample (11.74±1.91%). Also, the carbohydrate content in the dried plant sample was higher (75.92±0.81%) and lower in the fresh plant sample (10.74±1.82%). The ash content, fat, protein and crude fibre all varies accordingly from the fresh to the dried plant sample which further confirms its application in medicine.

KEYWORDS: *Euphorbia heterophylla*, quantitative phytochemicals, proximate analysis, medicinal applications.

INTRODUCTION

The use of medicinal plants is wide spread in many parts of the world, Nigeria inclusive.[1] Traditional medicine is the oldest method of curing diseases and infections and various plants are used in different parts of the world to treat human diseases and infections.[2,3,4,5] In Nigeria, many plants are used against infectious diseases, which today are frequent due to very poor hygienic conditions, cost and microbial resistance to the time-honoured antibiotics.[6] The continuing increase in the incidence of fungal infections together with the gradual rise in resistance of bacterial and fungal pathogens for antibiotics and antifungals highlights the need to find alternative sources from medicinal plants.[7] The selection of these plants for evaluation was based on ethnomedical information obtained from traditional healers who used the plants for treatment of dermatophytic infection. Medicinal plants are widely spread in nature and are used to maintain and promote healthy life, prevent diseases and cure ailments.[8] Their use in traditional medicine has been supported by the World Health Organization provided they are proven to be efficacious and safe.[9] The medicinal value of these plants is as a result of the bioactive phytochemical constituents present in them which produce definite physiological action on the human body.[10] Some of the most important bioactive phytochemical constituents are alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, phenolic compounds etc.[11]

Euphorbia heterophylla is an herb and belongs to the family Euphorbiaceae. It is an annual plant which is sparsely-branched and it is harvested from the wild for local use as a medicine. The plant has sometimes been used for the production of rubber. It is occasionally grown as an ornament.[12] The parts of the plant that grow above the ground are used to make medicine. *Euphorbia heterophylla* is used for treating breathing disorders including asthma, bronchitis, and chest congestion. It is also used for reducing mucus in the nose and throat, throat spasms, hay fever and tumors. An

extract of the aerial parts given orally showed moderate activity against several intestinal nematodes and fungi.

Phytochemicals are non-essential nutrients, that is, they are not required by the human body for sustaining life. They are non- nutritive plant chemicals that have protective or disease preventive properties. Plants produce these chemicals to protect themselves but recent research demonstrates that they can also protect against diseases.[8]

This present study tends to investigate the quantitative phytochemical estimation and the proximate analysis of the chemical composition of the whole plant of *Euphorbia heterophylla* Linn.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

The fresh whole plant of *Euphorbia heterophylla* were collected in Zaria, Kaduna State in June 2016. Samples from the plant were taken to the herbarium section and was identified and authenticated by Mallam Mohammed Sule of the Department of biological sciences, Ahmadu Bello University, Zaria.

Extraction of Plant Sample

The collected plant was clean and air-dried under the shade for four weeks at room temperature. The plant samples were ground to powdered form using a mortar and pestle. The ethanol crude extract of the plant sample was prepared by soaking 200g of the dried powdered samples in 1200ml of 96% ethanol for 2 weeks. The mixture was filtered using Whatman No. 1 filter paper and the filtrate was collected and concentrated using rotary evaporator at a temperature of 40°C. The extracts were macerated sequentially using 500ml each of n-hexane, ethyl acetate, methanol and aqueous solvents after which they were stored in a refrigerator at 4°C in a closed container to protect from light and moisture.

Quantitative Phytochemical Analysis

The qualitative phytochemical analysis of *Euphorbia heterophylla* revealed the presence of saponins, tannins, flavonoids, carbohydrates, phenolic compounds, etc.[13] Hence based on qualitative analysis, quantitative tests were carried out on the whole plant of *E. heterophylla*.

Determination of Alkaloids

About 5g of the sample is weighed into a 250ml beaker and 200ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue was the alkaloid, which was completely dried and weighed.[14]

Determination of Flavonoids

About l0g of the plant sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The solution was filtered through whatman filter paper No. 42. The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed until a constant weight is obtained.[15]

Determination of Saponin

In the determination of saponin content,[16] 20g of the sample was weighed and transferred into a conical flask and 200ml of 20% aqueous ethanol was added. The sample was heated over a hot water bath for 4 hours with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200ml of 20% ethanol. The combined extracts were reduced to 40ml over water bath at 90°C. The concentrate obtained was transferred into a 250ml separatory funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and 60ml of n-butanol was added. The combined n-butanol extracts was washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution is heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight and the saponin content was calculated as percentage.

Determination of Tannin

In the determination of the total tannin, a 500 mg of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 hour in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filterate was pipetted out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.I N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min.[17] The values obtained were plotted against various diluted concentrations of gallic acid.

Determination of Phenol

500 mg of the extract of the sample was weighed accurately and dissolved in 100 ml of triple distilled water (TDW). 1 ml of this solution was transferred to a test tube, then 0.5 ml 2N of the Folin-Ciocalteu reagent and 1.5 ml 20% of Na_2CO_3 solution was added and ultimately the volume was made up to 8 ml with TDW followed by vigorous shaking and finally allowed to stand for 2 hours after which the absorbance was taken at 765 nm. These data were used to estimate the total phenolic content using a standard calibration curve obtained from various diluted concentrations of gallic acid.[18]

Proximate Analysis

The determination of the proximate analysis of the fresh and dried leaf of *Euphorbia heterophylla* was carried out using the standard methods adopted by the Association of Official Analytical Chemists.[19] The moisture content,

crude protein, crude fat, total ash, carbohydrate and crude fiber content were analysed for the plant sample. Carbohydrate was determined by calculation using the difference method [100 – (protein + fat + moisture + ash)]. The nitrogen value which is the precursor for protein of a substance was determined using the kjedahl method. The nitrogen value was converted to crude protein by multiplying with a factor of 6.25, i.e. (% total nitrogen×6.25). Moisture content was determined by heating five grams of the sample to a constant weight in a crucible placed in an oven maintained at 105^0C. Crude fat (lipid) was obtained by exhaustively extracting five grams of the sample in a soxhlet apparatus using petroleum ether (boiling point range 40-60^0C) as the extractant. Ash was determined by the incineration of six grams placed in a muffle furnace maintained at 550^0C for five hours. Crude fiber was obtained by digesting four grams of sample with H_2SO_4 and NaOH and incinerating the residue in a muffle furnace maintained at 550^0C for five hours. Each analysis was carried out in triplicate and reported in percentages.

Statistical Analysis

The data obtained from this study were expressed as Mean ±Standard Error of Mean using statistical package for social sciences (SPSS) version 20.0.

RESULTS AND DISCUSSION

Table 1: Result of the Phytochemical Quantitative Analysis of *Euphorbia heterophylla* in percentages.

Phytochemicals	n-Hexane	Ethyl Acetate	Methanol	Aqueous
Alkaloids	3.00±0.90	1.00±0.10	1.00±0.00	4.00±0.00
Flavonoids	1.00±0.00	2.50±0.50	3.00±0.20	4.00±0.50
Tannins	3.00±0.00	7.00±0.00	9.00±0.50	10.00±0.20
Saponins	0.50±0.05	0.80±0.00	3.00±0.40	3.20±0.20
Phenols	9.65±2.35	6.00±0.00	11.20±0.20	12.30±0.70

Values are expressed as Mean ± SEM.

The result of quantitative phytochemical analysis of the plant showed a significant level of phytochemical constituents which are present as evident from the qualitative analysis data that was earlier carried out.[13,20,21] Euphorbia plants are widespread in nature ranging from herbs and shrubs to trees in tropical and temperate regions all over the world and their use in medicinal therapy has been documented.[22] The aqueous and methanol fractions of the plant showed the presence of phenol with (12.30±0.70%) and (11.20±0.20%) respectively with the highest percentage of the phytochemicals when compared with the other secondary metabolites analysed. The tannins also showed an appreciable presence in the plant with a percentage of 10.00±0.20%, 9.00±0.50% and 7.00±0.00% in the aqueous, methanol and ethyl acetate fractions respectively. Saponins, flavonoids and alkaloids were present in traces in all the fractions except where they showed appreciable presence in the aqueous fraction with percentages ranging from 3.20±0.20 to 4.00±0.00% for saponins, flavonoids and alkaloids respectively. The presence of these phytoconstituents in varying quantities in the plant might be responsible for its biological and medicinal activities. Previous studies have reported the antifungi and antibacterial activity of the leaf of *E. heterophylla*,[23] its anti-inflammatory activity[13] as well as the wound healing potentials[20] which was as a result of the presence of secondary metabolites.

Table 2: Result of the proximate analysis of fresh and dried leaves of *Euphorbia heterophylla.*

Parameters	% Composition Fresh	% Composition Dried
Moisture Content	83.06 ± 2.42	11.74 ± 1.91
Ash Content	0.63 ± 0.35	1.41 ± 0.26
Fat	1.61 ± 0.46	1.83 ± 0.27
Protein	2.01 ± 0.17	6.27 ± 0.11
Crude Fibre	0.77 ± 2.13	2.66 ± 2.01
Carbohydrate	10.74 ± 1.82	75.92 ± 0.81

Values are expressed as Mean ± SEM.

The proximate chemical composition of the fresh and dried leaves of *E.heterophylla* is shown in table 2. The result reveals that both the fresh and dried samples of the plant can be used as food and for medicinal purposes because of the presence of the nutrients that they contain. The percentage of carbohydrate in the fresh sample (10.74 ± 1.82) and the dried sample (75.92 ± 0.81) was highest in the nutrients aside the moisture content of the fresh plant sample which demonstrated that the plant is a good source of energy. The moisture content in the fresh sample is higher (83.06 ± 2.42) compared to the dried sample (11.74 ± 1.91). This was as result of air drying of the plant sample under shade for many days. The higher the moisture contents, the vulnerability of the plant to spoilage when stored. This result agreed with the findings of other researchers.[20] The protein, fat and crude fibre content of the dried sample is higher than the

fresh sample which further shows the importance of the dried plant sample in medicine.

CONCLUSION

The study carried out on the plant has revealed the quantitative phytochemical estimation of the secondary metabolites and the proximate analysis of its chemical composition which will further assist in its use in traditional medicine and in the discovery of novel compounds with pharmacological potentials and importance for the development of drugs for the human populace. In view of the foregoing, studies aimed at isolating and characterizing the chemical compounds present in the plant which was responsible for its medicinal usage should be explored.

REFERENCES

1. Zavala, S.M.A, Perez, G.S, Perez, G.R. M, Antimicrobial Screening of some Medicinal Plants. *Phytother. Res*., 1997; 11: 368-371.
2. Caceres A, Cabrera O, Morales O, Mollinedo P, Mendia P Pharmacological properties of Moringa oleifera. 1: Preliminary screening for antimicrobial activity. *J.Ethnopharmacol*, 1991; 3: 213-216.
3. Venugopal, P.V and Venugopal, T.V. Antidermatophytic Activity of Garlic (*Allium sativum*) *in vitro*. *Int J Dermatol*, 1995; 34(4): 278-279.
4. Nweze, E.I, Okafor, J.I and Njoku, O. Antimicrobial Activities of Methanolic Extracts of *Trema guineesis* (Schumm and Thorn) and *Morinda lucida* Benth used in Nigerian Herbal Practice. *Journal of Biological Research and Biotechnology*, 2004; 2(1): 39-46.
5. Vineela, C.H. and Elizabeth, K.M. Antimicrobial Activity of Marine Algae of Visakhapatnam City, Andhra Pradesh. *Asian Journal of Microbiology, Biotechnology and Environmental Sciences*, 2005; 7(2): 209-212.
6. Ngane NA, *Biyiti* L, Zollo PHA, Bouchet, PH Evaluation of Antifungal Activity of Extracts of two Cameroonian Rutaceae: *Zanthoxylum leprieurii* Gruill et Perr. And *Zanthoxylum xanthoxyloides* Waterm. *J Ethnopharmacol*, 2000; 70(3): 335–342.
7. Berkowitz, F.E Antibiotic Resistance in Bacteria. *South Med J*., 1995; 88(8): 797-804.
8. Olatunde, A and Dikwa, M.A. Qualitative and Quantitative Analysis of Phytochemicals of *Loranthus bengwensis* Leaf. *International Research Journal of Pharmaceutical sciences*, 2014; 5(1): 10-12.
9. Ajuru, M.G, Williams, L.F and Ajuru, G. Qualitative and Quantitative Phytochemical Screening of Some Plants Used in Ethnomedicine in the Niger Delta Region of Nigeria. *Journal of Food and Nutrition Sciences*, 2017; 5(5): 198-205.
10. Gladis Raja Malar, C and Chellaram, C. Phytochemical Screening, Total Flavonoid, Total Terpenoid and Anti-Inflammatory Activity of Aqueous Stem Extract of *Salacia oblonga*. *Journal of Chemical and Pharmaceutical Sciences*, 2017; 10(1): 550-556.
11. Saxena, M, Saxena, J, Nema, R, Singh, D and Gupta, A. Phytochemistry of Medicinal Plants. *Journal of Pharmacognosy and Phytochemistry*, 2013; 1(6): 168-182.
12. Wilson, A. K. "*Euphorbia heterophylla*: A Review of Distribution, Importance and Control". *Tropical Pest Management*, 1981; 27(1): 32–38.
13. Falodun A, Okunrobo LO, Uzoamaka N. Phytochemical and Anti-inflammatory evaluation of methanolic and aqueous extracts of *Euphorbia heterophylla* Linn (Euphorbiaceae) *Afri. J. Biotech*, 2006; 5(5): 529-531.
14. Harborne, J.B. Phytochemical Methods, London, Chapman and Hall Limited, 1973; 49-188.
15. Bohm, B.A, and Koupai–Abyazani, M.R. Flavonoids and Condensed Tannins from Leaves of Hawaiian Vaccinium reticulatum and V.calycinum (Ericaceae). Pacific science, 1994; 48(4): 458 – 463.
16. Obadoni, B.O and Ochuko, P.O. Phytochemical Studies and Comparative Efficacy of the Crude Extracts of some Homostatic Plants in Edo and Delta State of Nigeria" *Global Journal of Pure and Applied Sciences*, 2001; 8: 203 -208.
17. Van-Burden TP, Robinson WC Formation of Complexes between Protein and Tannin Acid. *J. Agric. Food Chem*, 1981; 1: 77.
18. Hagerman, A., Muller, I and Makkar, H. Quantification of Tannins in Tree Foliage. A Laboratory Manual, Vienna: FAO/IAEA, 2000; 4-7.
19. AOAC Official Methods of Analysis (18th edition) Association of Official Analytical, Chemists International, Maryland, USA, 2005.
20. Omale, J. and Emmanuel, T.F Phytochemical Composition, Bioactivity and Wound Healing Potential of *Euphorbia Heterophylla* (Euphorbiaceae) Leaf Extract. *International Journal on Pharmaceutical and Biomedical Research*, 2010; 1(1): 54-63.
21. Fred – Jaiyesimi, A.A and Abo, K.A Phytochemical and Antimicrobial analysis of the curde extract, petroleum either and chloroform fraction of the whole plant of *Euphorbia heterophylla*. Linn. Plant. *Pharmacognosy Journal*, 2010; 12(16): 1-4.
22. Bremer, K. *Asteraceae cludistics* and classification, Timber Press, Portland Oregon, 1994.
23. Falodun, A, Agbakwuru, E.O.P, Ukoh, G.C. Antibacterial Activity of *Euphorbia heterophylla* Linn (Family Euphorbiaceae). *Pak. J. Sci. Res*., 2003; 46(6): 471- 472.