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Qualitative and Quantitative Evaluation of Phytochemical Constituents of *Ageratum conyzoides L.* (Asteraceae)

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ABSTRACT

Ageratum conyzoides L. (Asteraceae) commonly known as Billy goat weed is widely distributed across Tropical Africa and all parts of Nigeria. The plant is well known in folkloric medicine for its anti-haemorrhagic and antimicrobial uses. This research was undertaken to identify the bioactive compounds present in the dried sample of the whole plant using simple chemical tests and to determine their concentrations using gravimetric and spectrophotometric methods. The results of preliminary phytochemical screening showed the presence of alkaloids, tannins, saponins, cardiac glycosides, terpeniods, flavonoids, phenols, phlobatannins, and glycosides. The results of the quantitative analysis were as follows; (mg/100 g): alkaloids 38 ± 5.0 (7.6%), flavonoids 21± 3.9 (4.2%), saponins, 7 ± 3.0 (1.4%), terpenoids, 11±2.5 (2.2%), phenols, 0.073 ± 0.02 (0.146%), tannins 0.0225 ± 0.012 (0.0045%), riboflavin 0.014± .0090 (0.0027%), thiamine 0.007 ± 0.001 (0.00133%) and moisture content 177.0±4.77 (35.4%). The results of this research work indicated that *Ageratum conyzoides* is rich in these essential phytochemicals which may be responsible for its vast biological activities. These findings would also serve as a reference for the concentrations of these phytochemicals in the plant and are reported here for the first time.

Key words: *Ageratum conyzoides,* Riboflavin, Thiamine, Flavonoids, Alkaloids, Phenols.

INTRODUCTION

Ageratum conyzoides L. (Asteraceae) is an annual herbaceous plant with a long history of traditional medicinal uses in many countries in the world, especially in the tropical and subtropical regions. *Ageratum conyzoides L.* belongs to the family Asteraceae which is well marked for their characteristics features and cannot be confused with any other. It is a tropical plant that is very common in West Africa, Australia and some part of Asia and South America. The plant grows commonly in waste and on ruined sites. It has a peculiar odour likened in Australia to that of a male goat and hence its name "goat weed" or "Billy goat weed" (Kohli *et al.*, 2006, Encyclopedia of Life (2016), GISD, 2016, PIER, 2016). The weed have been known since ancient times for its curative properties and have been utilized for treatment of various ailments, such as burns, wounds, headache and dyspnea, pneumonia, leprosy and other skin diseases. A wide range of chemical compounds including alkaloids, flavonoids, sterols and terpenoids, have been isolated from this plant. Extract and metabolites from this plant have been found to possess pharmacological and insecticidal activities.

In Africa and South America *A. conyzoides* is used to treat abdominal ache, burn, colic, collyrium, dyspepsia, emetic, eye problems, wound and uterine disorders, sleeping-sickness, sore, syphilis, as well as lithontriptic and purgative (Githen 1948, Bionet-Earfinet, 2016). The leaves are used for application on cuts, sores (Ahluwalia *et al.,* 1968, Upadhay *et al.,* 1998) as anti-inflammatory agent, haemostatic (Jain *et al.*, 1984, Barnejea *et al.,* 1986, , Suresh *et al.,* 1995), as an insecticide, in headache, in boils, skin diseases, ringworm infection; in typhoid, as an antidote to snake poison (Neogi *et al.*, 1989, Jain *et al.*, 1993). *Ageratum conyzoides L.* (Asteraceae*)* also has a number of magical and superstitious attributes example in Ivory Coast, it has protective fetish properties for snake charmers against snake bite. In Congo, the leaf sap on the hands of card players is believed to improve the luck (Adams, 1963). If sap is spread on the accused in a trial and is then pricked with a needle, pain will be felt only if guilty (Burkill, 1985). The objective of this study was to use direct, simple gravimetric and spectrophotometric methods for the determination of concentrations of phytochemical constituents in *Ageratum conyzoides* which can serve as a reference material for researchers.

MATERIALS AND METHODS

Reagents, collection of plant materials and extraction

All the reagents used were of analytical grade obtained from Sigma Aldrich, USA. The UV was recorded on Pye Unicam, (USA).

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Fresh plants of *Ageratum conyzoides* were collected from a garden in Uyo, Akwa Ibom State, Nigeria and identified and authenticated by Mrs E. G. Udoma, (Taxonomist) of the herbarium section of the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, with the voucher number UUPH 10(a). The fresh plants were thoroughly rinsed with water and dried under shade at room temperature, pulverized and weighed. The powdered plant material was stored in the refrigerator prior to the analysis.

Qualitative Phytochemical Screening of *Ageratum conyzoides L. (*Asteraceae)

The powdered sample (100.0 g) was subjected to cold maceration using 70% aqueous ethanol and the extract used for phytochemical screening using standard methods to test for the presence of Alkaloids, Tannins, Saponins, Glycosides, Anthraquinones, Flavonoids, Phlobatannins, Polyphenols and Steroids (Sofowora, 1993, Trease and Evans, 2009).

Quantitative Determination of the Phytochemcial Constituents

(i) Determination of Alkaloids

The dried powdered plant material (5.0 g) was extracted with 100 ml of 10 % acetic acid in ethanol for 4 h and filtered. The filtrate was then concentrated on a water bath to ¼ of its original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete, allowed to settle and filtered. The precipitate was washed with dilute ammonium hydroxide, dried in an oven and weighed. The determination was done in triplicate. The alkaloid content was determined using this formula (Trease and Evans, 2009).

$$\% \text{ Alkaloid} = \frac{\text{final weight of precipitate}}{\text{initial weight of sample used}} \quad X\ 100\%$$

(ii) Determination of Flavonoids

A standard method was followed with slight modification to quantify the flavonoid content. 10 g of the dried plant material was taken in a 250 ml conical flask and 100 ml of 70% aqueous methanol was added to it, magnetic stirred for 3 hours and filtered. The filtrate was transferred into a crucible and evaporated to dryness in a hot water bath of 60ºC and weighed (Okwu, 2005).

(iii) Determination of Tannins

Dried powdered sample of the plant material (5.0 g) of the sample was added to flask to 50 ml of distilled water in a conical flask and shaken for 1 hour on a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to mark. Then 5 ml of the filtrate was pipetted out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 NH_4Cl and 0.008 M potassium ferric cyanide. The absorbance was measured at 395 nm within 10 minutes. A blank was prepared without the sample and the absorbance was taken at the same wavelength. A standard was also prepared using tannic acid (0.10 mg/ml) and the absorbance read at the same wavelength to obtain a calibration curve. The determinations were done in triplicate (Trease and Evans, 2009).

(iv) Determination of Saponins

Saponins content was determined using slightly modified standard method (Hostettmann and Marston, 1999). Dried powdered sample (5.0 g) was defatted using n- hexane, partitioned with n-butanol exhaustively. The n-butanol fraction was evaporated to dryness on water bath. The dried n-butanol extract was dissolved in 50 ml methanol in a 250 ml beaker and diethyl ether added slowly by the side of the beaker until the precipitation was complete. The precipitate was centrifuged and the solid residue dried to remove the solvent completely. The determination was in triplicate. The saponins content was calculated as follows:

$$\% \text{ Saponin} = \frac{\text{weight of precipitate}}{\text{weight of sample}} \quad X\ 100\%$$

(v) Determination of Thiamine

The dried plant material (5.0 g) was dispersed in 50 ml, 20 % methanol NaOH and stirred over a magnetic stirrer for 3 hours at room temperature. The resultant extract was filtrated through Whatman filter paper number 1 in a 100 ml conical flask. 10 ml filtrate was mixed with equal volume of 2 % potassium dichromate solution. The resultant colour developed was read at 360 nm against a blank prepared without the sample. The blank contain all but lacks the leaves extract (Okwu, 2005).

(vi) Determination of Riboflavin

The experiment was performed according to the standard method with slight modification. The dried sample (5.0 g) was mixed with 50% ethanol in a 250 ml conical flask. The mixture was stirred on a magnetic stirrer for about 10 hours. The solution was filtered and the filtrate mixed with 25 ml of 5% $KMnO_4$ solution. To the mixture, 25 ml of 30% H_2O_2 was added with continuous stirring. The whole mixture was placed on 80 ºC hot water bath for 30 minutes and allowed to cool; then 5 ml of 40% Na_2SO_4 was added to it and the absorbance was measured at 510 nm using UV spectrophotometer (Okwu, 2005).

(vii) Determination of Phenol

Crude powdered sample (5.0 g) was defatted with n-hexane (50 ml) using soxhlet apparatus and the fat free sample was boiled with 50 ml of diethyl ether for the extraction of the phenolic component for 15 minutes. The extract (5 ml) was pipetted into a 50 ml flask followed by the addition of 10 ml of distilled water, 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol and made up to mark. The absorbance of the coloured solution that developed was measured after 30 minutes at 505 nm at room temperature (Okwu, 2005).

(viii) Determination of Total Terpenoids

About 2 g of the plant leaf powder was weighed and soaked in 50 ml of 95% ethanol for 24 hours. The extract was filtered and the filtrate extracted with petroleum ether (60 - 80ºC) and concentrated to dryness cooled and weighed. The weight of dried ether extract was taken as total terpenoids (Harbourne, 1984).

(ix) Determination of Moisture Content

The crucible was weighed empty (W_o). Fresh sample of the plant material was added to the crucible and weighed again (W_1). The crucible and the sample were dried in hot air drying oven at 105ºC for 24 hours and then cooled in a dessicator. The dried sample was further dried in the oven until a constant weight (W_2) was obtained (Trease and Evans, 2009). The moisture content in the sample was calculated as follows:

$$\% \text{ Moisture} = \frac{w_1 - w_2}{w_1 - w_0} \times 100$$

RESULTS

Qualitative phytochemical screening of *Ageratum conyzoides L. (*Asteraceae)

The results indicated that the presence of alkaloids, tannins, saponins, cardiac glycosides, steroidal terpenes, flavonoids, polyphenols, phlobatannins, glycosides while anthraquinones was absent.

The results of the quantitative estimation of the phytochemical composition of are displayed in the table 1.

Table 1 Result of Quantitative Phytochemical determination of *Ageratum conyzoides*

Phytochemical Constituent	Concentration (mg/100 g)	Percentage composition
Alkaloids	38.0±5.0	7.6
Flavonoids	21.0±3.9	4.2
Saponin	7.0±3.0	1.4
Terpenoids	11.0±2.5	2.2
Phenol	0.073±0.02	0.15
Tannin	0.023±0.012	0.0045
Riboflavin	0.014±0.009	0.0027
Thiamine	0.007±0.001	0.00133
Moisture content	177.0±4.77	35.4

The concentration of Riboflavin, tannin total phenol and thiamine were calculated from their standard calibration curves.

DISCUSSION

The phytochemical analysis of *Ageratum conyzoides L.* (Asteraceae*)* showed that it contained alkaloids (7.6%), flavonoids (4.2%), saponins (1.4%), terpenoids (2.2%) and moisture (35.4%). Spectrophotometric analysis method of the plant phytochemicals shows that it contained phenol (0.146%), tannins (0.0045%), riboflavin (0.0027%) and thiamine (0.00133%) per 100 g of the sample.

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties and are considered to be beneficial to human health (Harborne 1984). The qualitative and quantitative determination of the bioactive constituents of *Ageratum conzyoides* indicated that the plant is rich in alkaloids, tannins, flavonoids, saponins and phenols. Alkaloids have a wide range of pharmacological properties including anti-malarial, antiasthma, anticancer properties as reported by Kitta Koop, (2014). They are used in medicinal remedies especially the steroidal alkaloids; because of their pharmacological activity (Davis *et al.*, 1963). They are also reported to have cholinergic, vasodilatory, antiarrhythmic, anti-hyperglycemic activities, (Qui *et al.*, 2014), analgesic and antibacterial properties (Russo *et al.*, 2013, Cushine *et al.*, 2014), though they can be toxic too (Robbers *et al.*, 1996).

Flavonoids help to prevent platelets sickness and hence platelet aggregation (Okwu *et al.*, 2006). They act as antioxidants in biological systems. Other properties of flavonoids include protection against allergies, inflammation, free radicals, microbes, ulcers, viruses and tumors (Okwu *et al.*, 2004).

Phenolic compounds are essential for plant growth, reproduction and as protecting agents against pathogens; they also prevent chronic illnesses such as cardiovascular disease, and diabetes. (Scalbert *et al.*, 2005). Plants that contain phenol such as *Ageratum conyzoides L.* (Asteraceae) could be used as anti-inflammatory immune enhancers and hormone

modulators (Okwu *et al.,* 2005). Phenols also possess the ability to block specific enzymes that can cause inflammation and to prevent disease (Okwu *et al.,* 2004). Tannins possess physiological, constringent and hemostatic properties which hasten wound healing and ameliorate inflamed mucus membrane. They have important roles such as being stable and potent anti-oxidants (, Tyler *et al.,* 1988, Awosike *et al.,* 1991, Trease and Evans 2009). Tannins also form complexes with digestive enzymes thus reducing the digestibility of proteins in foods (Awosike *et al.,* 1991); prevent urinary tract infection by preventing bacteria from adhering to the walls. Tannins are useful in the management of HIV infection and herpes; combination of tannins and anthocyanin can breakdown cholesterol in the blood stream (Awosike *et al.,* 1991). Tannins along with vitamin C can help build and strengthen collagen (Tyler *et al.,* 1988).

Saponins are active as expectorant and are very useful in the treatment of upper respiratory tract inflammations; they also have anti-diabetic and anti-fungal properties (Finaer *et al*, 1989, Trease and Evans 2009). Saponins which are often referred to as natural detergent due to their foamy nature also possess anti-carcinogenic properties, immune modulation activities and regulation of cells proliferation as well as inhibition of the growth of cancer cells and cholesterol lowering activity (Jimoh *et al.,* 2005). The moisture content of the plant is considered high and thus would promote the growth of microorganisms and hence, storage life of the plant will be short.

In conclusion, the plant *Ageratum conyzoides L. (Asteraceae)* might serve as a potential source of useful drugs as indicated by the presence of the following phytochemicals: alkaloids flavonoids, saponins, terpenoids, tannins, cardiac glycosides, polyphenols, phlobatannins and glycosides. The various medicinal uses of the plant may be due to these chemical constituents present in the plant.

REFERENCES

Adams, C. D. (1963). *Compositae*. In: Hutchinson J, Dalziel JM, Hepper F. N., eds. Flora of West Tropical Africa, Volume 2, Second edition. London, UK: Crown Agents

Ahluwalia, K. S. (1968); Medicinal plants of Kerala, *Nagarjun* 11(2): 363-369.

Awosike, F. (1991). Local medicinal plants and health of consumers. *Clinical Pharmacology and Herbal Medicine*. 9(4): 28-29

Banerjee, A. K., Banerjee, I. (1986). A Survey of Medicinal Plants in Shevaroy hills. *J Econ Tax Bot*. 6(8): 271-290.

BioNET-EAFRINET, (2016). Invasive plants key and fact sheets. http://keys.lucidcentral.org/keys/v3/eafrinet/index.htm (accessed 29th Nov 2017)

Burkill, H. M. (1985). The Useful Plants of West Tropical Africa, Royal Botanic Gardens, kew; 1(3): 184-187.

Cushnie, T. P., Cushnie, B., Lamb, A. J (2014). "Alkaloids: An overview of their antibacterial, antibiotic- enhancing and anti-virulence activities". *Int J. antimicrobial Agent.* 44(5): 377-386.

Davis, P., H., and Heywood, V. H (1963). Principles of Angiosperm Taxonomy, Oliver and Boyd, Edinburgh; 22: 164-168.

Encyclopedia of Life (2016). Encyclopedia of Life. http://www.eol.org (accessed 29th Nov. 2017)

Githens T. S. (1948). Drug plants of Africa. *Afr Handbooks*; 8: 59.

GISD, (2016). Global Invasive Species Database (GISD). http://www.issg.org/database/welcome/ (accessed 29th Nov. 2017)

Harborne, Jeffrey B.; Baxter, Herbert; Moss, Gerard P (1999) eds. "General Introduct". Phytochemical dictionary a handbook of bioactive compounds from plants (2nd ed.). London: Taylor & Francis. p.vii.

Hostettmann K.and Marston A., (1995), *Saponins*, Cambridge University Presspp. 143-145.

Jain S, Pand Puri H. S. (1984). Ethnomedicinal plants of Jaunsar-Bawar hills, Utt ar Pradesh. *Indian J. Ethnopharmacol*; 12: 213-222.

Jimoh, F. O. and Oladji, A. T(2005). Preliminary studies on *Pilostigma thonningii* seed: proximate analysis, medicinal composition and phytochemical screening, *African Journal of Biotechnology*. 4(12): 1439-1442.

Kittakoop, P., Mahidol, C., Ruchirawat, S.(2014). "Alkaloids as important Scaffolds in therapeutic drugs for the treatments of cancer, tuberculosis and smoking cessation". *Cur Top Med Chem*. 14(2): 239-252.

Kohli R. K, Batish D. R, Singh H. P, Dogra K. S., (2006). Status, invasiveness and environmental threats of three tropical American invasive weeds (*Parthenium hysterophorus* L., *Ageratum conyzoides* L., *Lantana camara* L.) in India. Biological Invasions,8(7):1501-1510.

Neogi,B.,Prasad,M.N.,Rao,R.R. (1989). Ethnobotany of some weeds of Khasi and Garo hills, Meghalaya, Northeastern India. *Econ Bot* ; 43: 471-479.

Okunade A., L., (1981). Ph.D Thesis, Nigeria: University of Ibadan. p. 84.

Okwu, D. E., (2005) Phytochemicals, Vitamins and Minerals contents of Two Nigerian Medicinal Plants, *International Journal of Molecular Medicine and Advanced Sciences*, 1(4): 375-381

Okwu, D. E. and Emenike, I. N (2006). Evaluation of the phytonutrients and vitamin contents of Citrus fruits. *International J. of Molecular Med. and Advanced Science*. 2(1): 1-6.

Okwu, D. E. and Omodamino, O. D. (2005). Effects of hexane extract and phytochemical content of Xylopia aethiopica and Ocimum gratissimum on uterus of guinea pig. *Bio. Research*. 3(4): 40-44.

Okwu, D. E., (2004). Phytochemicals and vitamin contents of indigenous species of South Eastern Nigeria, *Journal of Sustainable Agriculture and Environment*. 6: 30-37.

Oviedo Prieto R, Herrera Oliver P, Caluff MG, (2012). National list of invasive and potentially invasive plants in the Republic of Cuba - 2011. (Lista nacional de especies de plantas invasorasy potencialmente invasoras en la República de Cuba - 2011). Bissea: Boletín sobre Conservación de Plantas del Jardín Botánico Nacional de Cuba, 6(Special Issue 1):22-96.

PIER, (2016). Pacific Island Ecosystems at Risk. Honolulu, USA: HEAR, University of Hawaii. http://www.hear.org/pier/index.html. Accessed 29th Nov. 2017.

Qiu, S., Sun, H., Zhang, A. H., Xu, H. Y., Yan, G. L., Han, Y., Wang, X. J. (2014).“Natural alkaloids: basicaspects, biological roles, and future perspectives.” *Chinese Journal of Natural Medicine*. 12(6): 401-406.

Quijano, L., Calderson, J. S., Gomez, G. F., Rios, T. (1980). In-vitro cytotoxicity of ßavonoids against MK2 and C6 tumour cells. *Phytochemistry*; 21:29-65.

Quijano, L., Calderson, J. S., Gomez, G. F., SoriaI, E., Rios, T. (1980). Highly oxygenated ßavonoids from *Ageratum conyzoides*. *Phytochemistry*; 19:24-39.

Robbers, J. E, Speedie, M. K., Tyler, V. E. (1996). “Chapter 9: Alkaloids”. *Pharmacognosy and Pharmaco biotechnology*. Philadelphia: Lippincott, Williams and Wilkins. pp. 143-185.

Russo, P., Frustaci, A., Del Bufalo, A., Fini, M., Cesario, A (2013). “Multitarget drugs of plants origin acting on Alzheimer’s disease”. *Curr Med Chem*. 20(13): 1686-1693.

Scalbert, A., Manach, C., Morand, C., Rémésy, C. and Jiménez, L. (2005). Dietary polyphenols and the prevention of diseases. Critical Reviews on Food Science and Nutrition. 45(4): 287-306.

Sofowora, A. (1993). *Medicinal plants and traditional medicine in West Africa*. Spectrum books limited Ibadan, Nigeria: 35-44.

Sumner, J. (2000). *The Natural History of Medicinal Plants*. Timber Press: 16.

Suresh, B., Dhanasekaran, S, Kumar R.V., Balasubramanian, S. (1995). Ethnopharmacological studies on the medicinal plants of Nilgiris. Indian Drugs; 32: 340-352.

Tapsell, L. C., Hamphill, I., Cobiac, L. (2006) “Health benefits of herbs and spices”: The past, the present, the future. *Med. J. Augt*. 185(4): 14-24.

Trease, G. E. and Evans, W. C. (2009). *Pharmacognosy*. 11th edn. Brailliar Tiridel Can. Macmillian publishers: 148-194.

Tyler, V.E., Brady, L., R., and Roberts J., E., (1988). Pharmacology Lea and ferbiger, *Philadelphia*. 8(14): 85-90.

Upadhay OP, Kumar K, Tiwari RK. (1998). Ethnobotanical study of skin treatment uses of medicinal plants of Bihar. *Pharmaceut Biol*; 36: 167-72.