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COMPARATIVE STUDY OF THE PHYTOCHEMICAL CONTENTS OF *COCHORUS OLITORIUS* AND *AMARANTHUS HYBRIDUS* AT DIFFERENT STAGES OF GROWTH

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ABSTRACT

The objective of this study was to carry out a comparative study of the phytochemical contents of Corchorus olitorius L. and Amaranthus hybridus L. at three stages of growth. This was with the aim of determining the stages at which each of the tested phytochemicals were present in each of the two vegetables. Ethanolic extracts from the dried leaves of plant samples harvested at different growth stages was prepared by soaking 5 g of powdered samples in 200 mL of ethanol for two weeks. The leaf extract was then filtered using Whatman's No 1 filter paper. The phytochemical compounds such as steroid, reducing sugars, alkaloids, phenol, flavonoid, saponin, tannin and resin were screened using standard methods. The results obtained showed the presence of alkaloids, steroids and resins at all the growth stages of the two plants. Alkaloid was present at all stages of growth of Amaranthus hybridus. The results also indicated no presence of phenols at all stages of growth of Amaranthus hybridus. This study concluded that most of these phytochemicals were present more at the flowering stage than at the vegetative and fruiting stages.

KEY WORDS: *phytochemicals, vegetables, herbs, antioxidants, Chochorous olitorious*

INTRODUCTION

Phytochemicals are chemical compounds that are of biological importance found occurring naturally in plants. They contribute to colour of plants, their aroma and flavor, as well as contribute to the protection of plants against disease and damage (Hasler & Blumberg, 1999). In general, these plant chemicals protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack (Mathai, 2000). Their importance also includes the protection of human health, when their dietary intake is significant. More than 4,000 phytochemicals have been cataloged and are classified by protective function, physical

characteristics and chemical characteristics (Meagher & Thomson, 1999; Ianovici *et al*, 2017). Of these, about 150 phytochemicals have been studied in detail. Phytochemicals are found in fruits, vegetables, legumes, whole grains, nuts, seeds, fungi, herbs and spices (Mathai, 2000). Phytochemicals are accumulated in different parts of the plants. These include the roots, stems, leaves, flowers, fruits or seeds (Costa *et al*., 1999). Many of these, phytochemicals, particularly the pigment molecules, are often concentrated in the outer layers of the various plant tissues (Ianovici *et al.,* 2009). The variety, processing, cooking and growing conditions determine the level of phytochemicals accumulated by different parts of the plants (King & Young, 1999).

Corchorus olitorius and *Amaranthus hybridus* are well known vegetables with high nutritional values. The consumption of these vegetables gives diversity to daily food. They are underutilized because of inadequate scientific knowledge of their nutritional potentials (Awobajo *et al*., 2010). *Corchorus olitorius* is a very popular vegetable especially in South-Western Nigeria. It is made into a common mucilaginous soup or sauce in some ethnic groups in West Africa as cooking traditions. The leaves are rich in beta carotene, iron, calcium and vitamin C. The plant has an antioxidant activity with a significant tocopherol equivalent Vitamin E. It has been reported that aqueous extracts of the seeds of *Corhorus olitorius* possess peripheral and anti-inflammatory and anti-pyretic activities (Zakaria, 2006). The seeds are used as a purgative while the leaves are used in the treatment of chronic cystitis, gonorrhea, dysuria and toothache (Hillocks, 1998). Plants are useful to man not only as food but also as raw materials for industries as well as sources of medicament (Azoro, 2004).

Amaranthus hybridus is widely cultivated in several parts of the world including South America, Africa, India, China and the United States (He & Cork, 2010). It grows well in semi-arid region such as Southern Africa and its commercial production is increasing throughout the world as an important alternative food source (Kauffman & Weber, 1990). It is cultivated on a commercial scale in Southern Nigeria. *A. hybridus* constitutes a major part of the diet of the people in the middle and southern parts of Nigeria, where they are mostly used in soups, because of their rich source of protein, minerals and vitamin C (Mepba *et al*., 2007). It was also reported that *A. hybridus* seed oil contained squalene which has important beneficial effects on cancers and reduce cholesterol level in the blood (Rao & Newmarj, 1998). *A. hybridus* leaves combined with condiments are used to prepare soup (Maiyo *et al*., 2010). In Congo, their leaves are eaten as spin ach or green vegetables (Dhellot *et al*., 2006).

The consumption of fresh vegetables gives the consumer a variety of compounds that have a positive influence on human health. The phytochemicals found in fresh vegetables and fruits have antiflammatory enzyme inhibiting and bioactive features capable of combating the activities of oxidants. Although, phytochemical contents of *Corchorus olitorius* and *Amaranthus hybridus* have been documented, there is a need to carry out and investigate the stages of growth at which each of these important phytochemicals were present and thereby suggest to consumers when the vegetables are best consumed.

MATERIALS AND METHODS

Raising of Seedlings. Twenty-four plastic pots (of about 10 cm in diameter and 15cm in height) were obtained. Holes of equal diameter were bored at the bottom of each pot to allow for proper drainage and to prevent water logging. The pots were filled near brim with 5 kg of

soil. The seeds of *Corchorus olitorius* and *Amaranthus hybridus* were sown at the rate of four seeds per pots. The pots were then supplied with 200 mL of water in the morning and evening until the seedlings were fully established. After establishment of the seedlings, the pots were divided into two groups, each group containing twelve pots for the two plant species. Four pots each were used to represent the different growth stages (vegetative, flowering, and fruiting) of the two plant species.

Processing of Plant Samples for Phytochemical Screening. The leaves of C*orchorus olitorius* and *Amaranthus hybridus* were harvested, properly washed, oven dried at 40^{o}c and grinded to a powdered form with mortar and pestle. This process was done separately for the three growth stages, and placed in separate, labeled glass bottles.

Preparation of Ethanolic Extract of Plant Samples. The aqueous extract of each plant sample at different growth stages was prepared by soaking 5 g of powdered samples in 200 mL of ethanol for two weeks. The extract was then filtered using Whatman's No 1 filter paper.

Phytochemical Analysis. Qualitative analysis was carried out to ascertain the presence of the different phytochemicals as described by Edeoga *et al*. (2005).

Test for Tannins. Tannin was determined by the Folin-Denis colorimetric method. About 0.5 g of the extracts of each plant were boiled in 20 mL of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added. A brownish green or a blue-black coloration indicated the presence of tannins.

Test for Saponins. 2 mg of the extracts of each plant is boiled together with 20 mL of distilled water in a water bath, vigorously shaken and noted for froth. The appearance and persistence of frothing before and after warming indicated the presence of saponins.

Test for Flavonoids. 5 mL of dilute ammonia solution was added to the ethanolic extract of each plant sample in a test tube, followed by addition of concentrated H_2SO_4. A yellow coloration was observed and disappeared on standing. This indicated the presence of flavonoids.

Test for Phenol. 500 mg of the extract was dissolved in 5 mL of distilled water. To this, few drops of neutral ferric chloride solution was added. A dark green color indicated the presence of phenolic compounds.

Test for Alkaloids. 5 mL solution of the extract and 2 mL of dilute hydrochloric acid were taken in a test tube. Then 1 mL of Dragendroff's reagent was added to this acidic medium. Orange or red precipitate was formed and that was indicated as the presence of alkaloids.

Test for Steroids. 2 mL of acetic anhydride were added to 0.5 g ethanolic extract of each sample with 2 mL H_2SO_4. The change of color from violet to blue or green in some samples indicated the presence of steroids.

Test for Reducing Sugars. About 5 g each of the dried samples was introduced into a test tube and equal amount of Fehling's solution A and B were added. The mixture was boiled over a burner. Observation of colour was made. The colour changed from deep blue to brick red, indicating the presence of reducing sugar.

RESULTS AND DISCUSSIONS

From the result obtained from the phytochemical analysis of *Corchorus olitorius*, alkaloids, steroids and resins were present at all the growth stages. Saponins were only present

at both vegetative and flowering stage, phenols was present at flowering and fruiting stage, reducing sugar and tannin were present only at flowering stage and flavonoid was present only at vegetative stage (Table 1). The result obtained from the phytochemical analysis of *Amaranthus hybridus* showed that alkaloids were present at all stages of growth. Resins and steroids were present both at the vegetative and flowering stages, reducing sugars were present at the vegetative and fruiting stage, saponins were present at flowering and fruiting stage. Tannin was only present at the fruiting stage and flavonoid at flowering stage. The result indicated no presence of phenols at all stages of growth (Table 1).

TABLE 1. Phytochemical content of *Corchorus olitorius* and *Amaranthus hybridus* at different growth stages

	Corchorus olitorius			*Amaranthus hybridus*		
Phytochemical	Vegetative stage	Flowering stage	Fruiting stage	Vegetative stage	Flowering stage	Fruiting stage
Alkaloid	+	+	+	+	+	+
Flavonoids	+	-	-	-	+	-
Saponin	+	+	-	-	+	+
Steroids	+	+	+	+	+	-
Phenol	-	+	+	-	-	-
Tannins	-	+	-	-	-	+
Reducing sugar	-	+	-	+	-	+
Resin	+	+	+	+	+	-

The presence of alkaloids in all the stages of growth of *C. olitorius* and *A. hybridus* showed that alkaloid is the most bioactive compound in these plants at the vegetative, flowering and fruiting stages. Alkaloids are the bioactive constituent of these plants involved in the plant defence system. Literatures have reported flavonoids and phenolics as free radical scavengers that prevent oxidative cell damage, and have strong anticancer activities (Pourmorad *et al*., 2006; Ugwu *et al*., 2013). They might as well be capable of inducing mechanism that affect cancer cells and inhibit tumor invasion (Rafat *et al*., 2008). These attributes could be unconnected with their ability to quench free radicals (Ugwu *et al*., 2013; Omale & Okafor, 2008). The presence of flavonoids at the vegetative stage of *C. olitorius* and at the flowering stage of *A. hybridus* showed that *C. olitorius* can be used as an intoxicant and hallucinogen at the vegetative stage, while *A. hybridus* can serve the same purpose at the flowering stage of growth. Flavonoids are well documented for their biological effects including antimicrobial and anticancer. They have been found in vitro to be effective antimicrobial and anticancer compounds against a wide range of microorganism and cancer cell (Ren *et al*., 2003). Bioactive constituent have been reported to be responsible for medical herbs in China and Japan (Njoku & Oby, 2009).

Plants with tannins as their component are used for the treatment of intestinal disorders such as diarrhoea and dysentery (Bajai, 2001), thus suggesting that *Chochorus olitorius* leaves at flowering stage and *Amaranthus hybridus* at fruiting stage could be used for the treatment of microbial infection. Tannins are known to be useful for the prevention of cancer as well as treatment of inflamed or ulcerated tissues (Okwu & Emineke, 2006; Adegboye *et al*., 2008). This also suggests the possible use of *Chochorous olitorius* leaves at flowering stage as well as *Amaranthus hybridus* at fruiting stage for this purpose (Okwu & Emineke, 2006). Alkaloids are beneficial chemicals to plants serving as repellant to predators and parasites.

Saponins in medicinal plants are responsible for most biological effects related to cell growth and division in humans and have inhibitory effect on inflammation (Just *et al*., 1998; Okwu & Emineke, 2006). The presence of saponin at vegetative and flowering stages of *Chochorous olitorious* suggest that the plants can be of immense medicinal value at these stages of growth.

The presence of steroids and resins at the vegetative and flowering stages of *C. olitorius* and *A. hybridus* showed that these plants exhibit high biological activities such as anti-inflammatory, antimicrobial, anti- agionic at these stages of growth than at the fruiting stage.

The presence of phenols at the flowering and fruiting stage of *C. olitorius* and its absence at the vegetative, flowering and fruiting stages of *A. hybridus* reflects increased growth, yield and photosynthetic pigment accumulation of *A. hybridus* compared to the growth, yield and photosynthetic pigments accumulation of *C. olitorius.* Phenols substantially decrease metabolites and photosynthetic pigments. Also, Shehata *et al*. (2014) reported that phenols inhibited basic physiological processes of plants. The detection of this compound at the flowering and fruiting stage of *C. olitorius* showed the advantage of this plant over *A. hybridus* because of the antioxidant activity and radical scavengers (Ugwu *et al*., 2013) of this bioactive compound.

CONCLUSIONS

The results showed the presence of bioactive constituents like saponin, tannin, flavonoid and alkaloid were present in these plants although at different stages of growth. The results thus suggest that the identified phytochemical compounds may be the bioactive constituents responsible for the efficacy of the leaves of the plants studied. These compounds have also been previously confirmed to have antimicrobial activity (Kavit *et al*., 2012). Hence it could be concluded that the plant extracts if gotten at the right stage of growth of the plants could be a source for the industrial manufacture of drugs that could be used in the treatments of some microbial infection.

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REFERENCES

- Adegboye M. F., Akinpelu D. A., Okoh A. 2008. The bioactive and phytochemical properties of Garcinia kola (Heckel) seed extract on some pathogens. *African Journal of Biotechnology*7 (21): 3934-3938.
- Awobajo F.O., Olatunji-Bello I.I., Obilade T.T., Odugbemi T.O. 2010. Knowledge of the nutritional and medicinal use of some vegetables among a cross section of market women in two major food markets in Lagos State, South West Nigeria. *Pakistan Journal of Nutrition* 9(3): 216-221.
- Azoro C. 2004. Towards effective integration of herbal medicine into Nigerian medical practice. *Nig. J. Res. Prod.,* 3: 132-137.
- Bajai A., Meyers 2001. Effect of natural extract of pineapple on disstibility, performance traits and nitrogen balance of broiler chicks. *Australian Journal of Basic and applied Sciences*. 5(20): 10-30.
- Costa M. A., Zia Z.Q., Davin L.B., Lewis N.G. 1999. Chapter Four: Toward Engineering the Metabolic Pathways of Cancer-Preventing Lignans in Cereal Grains and Other Crops. In Recent Advances in Phytochemistry, vol. 33, *Phytochemicals in Human Health Protection, Nutrition, and Plant Defense*, ed. JT Romeo, New York, 67-87.

- Dhellot J.R., Matouba E., Maloumbi M.G., Nzikou J.M., Safou Ngom, D.G. *et al*., 2006. Extraction, chemical composition and nutritional characterization of vegetable oils: Case of Amaranthus hybridus (Var 1 and 2) of Congo Brazzaville. *African Journal of Biotechnology* 5: 1095-1101.
- Edeoga H.O., Okwu D.E., Mbaebie B.O. 2005. Phytochemical Constituents of some Nigerian medicinal plants. African. *Journal of Biotechnology* 4 (7): 685-688.
- He H.P., Corke H. 2010. Oil and squalene in *Amaranthus* grain and leaf. *Journal of Agriculture and Food Chemistry* 5: 7913-7920.
- Hasler C.M., Blumberg, J.B. 1999. Symposium on Phytochemicals: Biochemistry and Physiology. *Journal of Nutrition* 129: 756-757.
- Hillocks R.J. 1998. The potential benefits of weeds with reference to small holder agriculture in Africa. Integrated. *Pest Management Review* 3: 155-167.
- Ianovici N., Latis A.A. 2017. Foliar traits of *Juglans regia, Aesculus hippocastanum* and *Tilia platyphyllos* in urban habitat. Romanian Biotechnological Letters. 22 (2), 12400-12408
- Ianovici N., Novac I.D., Vlădoiu D., Bijan A., Ionaşcu A., Sălăşan B., Rămuş I. 2009. Biomonitoring of urban habitat quality by anatomical leaf parameters in Timişoara, *Annals of West University of Timişoara, ser. Biology*, 12:73-86.
- Just M. J., Recio M. C., Giner R. M., Cuellar M. J., Manez S., Bilia A. R., Rios J. L. 1998. Anti-inflammatory activity of unusual lupine saponins from Bupleurum fruticescens, *Plant Medicine* 64: 404-407.
- Kavit M., Patel B.N., Jian B.K. 2012, Phytochemical Analysis of Leaf Extract of *Phllanthus fratenus*, *Res. J. Recent. Sci.* 2:12-15.
- Kauffman C.S., Weber L.E. 1990. Grain amaranth. In: Janick, J., Simmon, J.E. (Eds.), Proceedings of the 1st National Symposium on New Crops, Indianapolis, IN, October 1988. Timber Press, Portland, OR, pp. 127-139.
- King A., Young G.1999. Characteristics and Occurrence of Phenolic Phytochemicals. *Journal of the American Dietetic Association* 24: 213-218.
- Maiyo Z.C., Ngure R.M., Matasyoh, J. C., Chepkorir R., *et al*. 2010. Phytochemical constituents and antimicrobial activity of leaf extracts of three Amaranthus plant species. *African Journal of Biotechnology* 9: 3178-3182.
- Mathai K. 2000. Nutrition in the Adult Years. In Krause's Food, Nutrition, and Diet Therapy, 10thed., ed. L.K. Mahan and S. Escott-Stump, 271: 274-275.
- Meagher E., Thomson C. 1999. Vitamin and Mineral Therapy. In *Medical Nutrition and Disease*, 2nded., G Morrison and L Hark, Malden, Massachusetts: Blackwell Science Inc, 33-58.
- Mepba H.D., Eboh, L., Banigo D.E.B. 2007. Effects of processing treatments on the nutritive composition and consumer acceptance of some Nigerian edible leafy vegetables. *African Journal of Food Agriculture and Nutritional Development* 7: 1-18.
- Njoku V.N., Obi C. 2009. Phytochemical Constituents of Some Selected Medical Plants. *Afr. J. Pure Appl. Chem*. 3(11): 228-233.
- Omale J., Okafor P.N. 2008. Comparative antioxidant capacity, membrane stabilization, polyphenol composition and cytotoxicity of the leaf and stem of *Cissus multistriata*.*African Journal of Biotechnology*. 7(17): 3129-3133.
- Okwu D.E., Emenike I.N. 2006. Evaluation of the phytonutrients and vitamin contents of Citrus fruits. *International Journal of Molecular Medicine and Advance Sciences* 2: 1–6.
- Pourmorad F., Hosseininelir S. J., Shahabimajd N. 2006. Antioxidant activity, phenol and flavonoid content of some selected Iraninan medicinal plants. *African Journal of Biotechnology* 5: 1142-1145.
- Rafat H.S., Cillard B.S., Cilliad, N.T. 2008. Hydroxyl Radical Scavenging activity of flavonoids. *Journal ofPhytochemistry* 26(9): 2489-2491.
- Ren W., Qiao Z., Wang H., Zhu L., Zhang L. 2003. Flavonoid: Promising Anticancer Agents. *Med. Res. Rev*. 23: 519-534.
- Rao C.V., Newmarj, H.L.1998. Chemopreventive effect of squalene on colon cancer. *Carcinogenesis* 19: 287-290.
- Shehata A.N., Mahmoud A.E., Abdou H.M. 2014. Quantification of total phenolic and Total flavonoid Contents in Extracts of Some Egyptian Green leaves and Estimation of Antioxidant Activity. *Research Journal of Pharmaceutical, Biological and Chemical Sciences 5(6), 266-273.*
- Ugwu Okechukwu P. C., Nwodo Okwesili F. C., Joshua Parker E., Bawa Abubakar, Ossai Emmanuel C., Odo Christian E. 2013. Phytochemical and Acute Toxicity Studies of Moringa oleifera Ethanol Leaf Extract. *International Journal of Life Sciences Biotechnology and Pharma Research* 2(2), 66-71.
- Zakaria Z.A., Safarul M., Valsala R., Sulaiman M.R., Fatimah C.A., MAT-JAIS A.M. 2005. Influence of temperature on the opioid-mediated antinociceptive activity of *Corchorus olitorius* L. in mice. Naunyn Schmiedeberg's. Arch. *Pharmacology* 372: 55-62.