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Comparative Screening of Phytochemical Compounds in Scent Leaf (*Ocimum gratissimum*) and Bitter Leaf (*Vernonia amygdalina*) Extracts

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Abstract

Phytochemical screening of the leaves of *Ocimum gratissimum* and *Vernonia amygdalina* was revealed the presence of alkaloids, flavonoids, steroid, tannins, and caroternoid. The best solvent of extraction for both plants was the hot water followed by the cold water even though they were unable to liberate flavonoids from bitter leaf extracts. The washed bitter leaf extracts contained more alkaloids than the extracts made from the unwashed leaf though the differences were not significant (washed bitter leaf- 7.32% from hot water and 6.83% from cold water, unwashed bitter leaf- 6.12% from hot water and 5.32% from cold water). The ethanolic extracts of bitter leaf liberated the flavonoids while the hot water was also able to liberate it from the scent leaf. Carotenoids were liberated from the extracts of both plants though the quantities were not significantly different. The study showed that bitter leaf and scent leaf contain similar antimicrobial compounds but former contains more quantity.

Keywords: Phytochemical scent leave; Bitter leaf; Flavonoids; Tannins

Introduction

Phytochemical screening is a process of tracing the medicinal value of plants constituents in some chemical substance that produce a definite physiologic action on the human body [1,2]. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins, saponins, glycosides, cardenolides, bufadionolides and polyphenolic compounds [3]. Knowledge of the chemical constituents of plants is desirable not only for the discovery of therapeutic agents but also because such information may be of value in disclosing new sources of such economic materials as tannins, oils, gum, precursors for the synthesis of complex chemical substances [3]. Bitter leaf *(Vernonia amygdalina)* is derived from the leaves of a small ever-green shrub found all over Africa belonging to the family Asteraceace. It is well known as a medicinal plant for diabetes and fever [4]. *Vernonia amygdalina* (Del.) commonly called bitter leaf is the most widely cultivated species of the genus *Vernonia* which has about 1,000 species of shrubs [5]. It belongs to the family Astaraceae. It is vegetatively cultivated by stem cutting at an angle of 45° and popular in most of West Africa countries including Nigeria, Cameroon, Gabon and Congo Democratic Republic. Although most popularly used for food, it has also, been traditionally used for its medicinal properties. True to its name, bitter leaf is bitter to taste but surprisingly delicious in meals. Bitter leaf is called Omjunso in East Africa especially Tanzania, Onugbo in Igbo-Eastern Nigeria and Orugbo among the Itsekiri and Urhobo tribes in Nigeria, Ewuro (Yoruba), Etidot (Ibibio), Ityuna (Tiv), Oriwo (Edo), Chusa-doki Shiwaka (Hausa). Scent leaf, *Ocimum gratissimum* is an aromatic perennial herb, with erect stem, much branched, glabrous and woody at the base often with epidermic peeling in strips. *Ocimum gratissimum* is grown for the essential oil in its leaves and stems while engenol and to a lesser extent thymol extracted from the oil are substitutes from clove oil and thyme oil (Table 1). The essential oil possesses antibacterial properties and is also an important insect repellent so also are the leaves when left dry and burnt [6]. They are primarily used as vegetables [7], as spice due to its aromatic nature to spice various kinds of soup (e.g., pepper soup) and other delicious meals like porridge [6]. The whole plant has many applications in traditional medicine especially in Africa and India. The applications include in the treatment of ringworms, gout and fungal infections, malaria, catarrh, aches, colon pain. The juice gotten from squeezing its leaf can be used to cure several stomach related illnesses like cholera, diarrhea, dysentery, vomiting and convulsion [6]. *Ocimum gratissimum* and *Vernonia amygdalina* plants are known to have common phytochemical compounds which are non-nutritive plants and significant used in traditional medicine for the treatment of several ailments and the extracts have been evaluated for their ability to stall the activities of organisms responsible for spoilage of fresh catfish (*Clarias gariepinus*) thereby extending its shelflife [8]. Therefore, the study aims to know the quantity of the phytochemical compounds present in the extracts of scent leaf (*Ocimum gratissimum*) and bitter leaf (*Vernonia amygdalina)* and also to know which solvent of extraction contains more of the phytochemical compounds.

Materials and Methods

Collection of plant materials

Fresh leaves of *Ocimum gratissimum* and *Vernonia amuglidana* were collected from a home garden inside the National Institute of Freshwater Fisheries Research (NIFFR) residential quarters in Kainji, New-Bussa, Niger state.

Preparation of bitter and scent leaf extracts

The bitter leaf (*Vernonia amygdalina*) and scent leaf (*Ocimum gratissimum*) were washed with clean water to remove the dust and dirts. This was macerated and divided into two portions, one part of the

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	Phytochemical	Test	Observation	Inference
1	Alkaloid	Wagner dragendr offs	Brown precipitate which turns intense yellow with the picnic acid	Alkaloid present
2	Tannins	Ferric chloride	Greenish – black precipitate	Tannin present
3	Flavonoid	Ammonium and sodium hydroxide acid	Yellow colour which turns colourless on addition of acid	Flavonoid present
4	Steroids	Liberman Burchard and Salkowkis	Brownish colour Red colour at interference	Steroid present

Table 1: Qualitative analysis of phytochemical compounds.

bitter leaf was unwashed and the other part was washed to remove part of the juice that is responsible for the bitter taste. The extraction was done as follows according to Azu and Onyeagba [9].

1. 300 g of bitter leaf was soaked in 150 mL of 95% ethanol for 24 hours. The pulp obtained was left in clean, sterile glass container and shaken vigorously to allow for proper extraction. Filtration was done using a sterile muslin cloth after which the extract obtained was air-dried and store for use.

2. 300 g of bitter leaf was soaked in 150 mL of cold water for 24 hours and the resultant juice extracted was air dried and stored as done.

3. 300 g of bitter leaf was soaked in 150 ml of hot water for 24 hours and the resultant juice extracted was air dried and stored as done.

The scent leaf extracts were also prepared using the above procedure.

Qualitative analysis of phytochemical compounds

Qualitative analysis was carried out to ascertain the presence of the different phytochemical compounds in the leaves.

Quantitative analysis of phytochemical compounds

Determination of alkaloids: This was done by the alkaline precipitation gravimetric method described by Harborne [10]. A measured weight of the sample was dispersed in 95% acetic acid solution in ethanol to form a ratio of 1:95(95%). The mixture was allowed to stand for 24 hours. The filtrate was concentrated to one quarter of its original volume by evaporation and treated with drop wise addition of concentrated aqueous NH_4OH until the alkaloid was precipitated. The alkaloid precipitated was received in weighed filter paper, washed with 1% ammonia solution dried in the oven at 80°c. Alkaloid content was calculated and expressed as a percentage of the weight of sample analysed.

Determination of flavonoids: This was determined according to the method of Harborne [10]. 300 gram of the same was boiled in 150 mL of 2 m HCL solution for 30 min under reflux. It was allowed to cool and then filtered through Whatman No 42 filter paper. A measured volume of the extract was treated with equal volume of ethyl acetate starting with a drop. The flavonoids precipitated were recovered by filtration using weighed filter paper. The resulting weight difference gave the weight of flavonoids in the sample.

Determination of carotenoids: A measured weight of each sample was homogenized in ethanol using a laboratory blender. A 1:10 (1%) mixture was used. The homogenate was filtered to obtain the initial crude extracts. 20 mL of ether were added to the filtrate to take up the carotenoids mixed well and then treated with 20 mL of distilled water in a separating funnel. The other layer was recovered and evaporated to dryness at low temperature (35-50°C) in a vacuum dessicator. The dry extract was then saponified with 20 mL of ethanolic potassium hydroxide and left over night in a dark cupboard. The next day, the carotenoid were taken up in 20 mL of ether and then washed with two portions of 20 mL - distilled water. The carotenoid extract (ether layer) was dried in a dessicator and then treated with a light petroleum (petroleum spirit) and allowed to stand overnight in a freezer (-10°C).

	Alkaloids	Flavonoids	Steroids	Tannin
HWE	+	-	+	+
CWE	+	-	+	-
EE	+	+	+	-

EE: Ethanolic Extract; HWE: Hot Water Extract; CWE: Cold Water Extract

+ = Present **-** =Absent

Table 2: Qualitative screening of washed bitter leaf extracts.

The next day the precipitation steroid was removed by centrifugation and the carotenoid extracts was evaporated to dryness in a weighed evaporation dish, cooled in a dessicator and weighed. The weight of carotenoid was determined was expressed as a percentage of the sample weight [11].

Results

Table 2 shows the phytochemical compounds present in the extracts of washed bitter leaf. Flavonoids were absent in the hot water extracts while alkaloids, tannins and steroids are present. Also flavonoids and tannins were absent in the cold water extract while steroids and alkaloids were present. Tannins were not present in the ethanolic extract while the alkaloids, steroids and flavonoids were present. Table 3 shows the screening of unwashed bitter leaf extract. Flavonoids and steroids were absent in the hot water extract while it was only the flavonoids that were absent in the cold water extract. The ethanol extract was able to liberate flavonoids, alkaloids and tannins with the exception of the steroids. Table 4 shows the phytochemical compounds present in the extract of washed scent leaf. Steroids and tannins were absent in the ethanolic extract while tannins were absent in the hot water extract. The cold water extract was able to liberate the alkaloids and steroids. Table 5 shows the phytochemical compounds present in the extract of unwashed scent leaf. Steroids were absent in the hot water extract while only alkaloids were present in the cold water extract. The ethanolic extract showed the presence of alkaloids and tannins. Table 6 summarizes the result of quantitative screening of phytochemicals present in washed bitter leaf extracts. Alkaloid has the highest quantity 17.08% which present in ethanolic. Cold and hot water extract, also flavonoids as 2.32% present in ethanolic extract only while carotenoids has 0.135% in ethanolic, cold and hot water extracts. Table 7 summarizes the quantity of phytochemicals found in unwashed bitter leaf extracts. Alkaloids has the highest amount 14.56% in ethanolic, hot and cold water extract and flavonoids has 2.42% in ethanolic, cold and hot water extracts only followed by carotenoid which has 0.24% in ethanolic, cold and hot water extracts. Table 8 shows the quantity of phytochemical compounds in washed scent leaf extracts. Alkaloids has 5.86% in ethanolic, cold and hot water extract which flavonoids has 3.28% in ethanolic, and hot water and not found in cold water extract and carotenoids has 0.2% in ethanolic hot and cold water extracts. Table 9 shows the quantity of phytochemical compounds in the extracts of unwashed scent leaf. Alkaloids has 7.82% in ethanolic, cold and hot water extracts and flavonoids 2.11% in hot water extract and not found in ethanolic and cold water extracts while carotenoids has 0.22% in both ethanol, hot and cold water extracts.

Discussion

The results obtained from this study showed that hot water, cold

	Alkaloids	Flavonoids	Steroids	Tannin
HWE	+	-	-	+
CWE	+	-	+	+
EE	+	+	-	+

EE: Ethanolic Extract; HWE: Hot Water Extract; CWE: Cold Water Extract

+ = Present **-** =Absent

Table 3: Qualitative screening of unwashed bitter leaf extracts.

	Alkaloids	Flavonoids	Steroids	Tannin
EE	+	+	-	-
HWE	+	+	+	-
CWE	+	-	+	-

EE: Ethanolic Extract; HWE: Hot Water Extract; CWE: Cold Water Extract

+ = Present **-** =Absent

Table 4: Qualitative screening of washed scent leaf extracts.

	Alkaloids	Flavonoids	Steroids	Tannin
HWE	+	+	-	+
CWE	+	-	-	-
EE	+	-	-	+

EE: Ethanolic Extract; HWE: Hot Water Extract; CWE: Cold Water Extract

+ = Present **-** =Absent

Table 5: Qualitative screening of unwashed scent leaf extracts.

	Alkaloids	Flavonoids	Carotenoids
EE	2.93	2.32	0.045
CWE	6.83	--	0.04
HWE	7.32	--	0.05

EE: Ethanolic Extract; HWE: Hot Water Extract; CWE: Cold Water Extract

Table 6: Quantitative screening of washed bitter leaf extract in %(g/100 g).

	Alkaloids	Flavonoids	Carotenoids
EE	3.12	2.42	0.14
CWE	5.32	--	0.06
HWE	6.12	--	0.07

EE: Ethanolic Extract; HWE: Hot Water Extract; CWE: Cold Water Extract

Table 7: Quantitative screening of unwashed bitter leaf extract % (g/100 g).

water and ethanolic extracts of the plants have varying contents of the phytochemical compounds. It also shows that even though the two plants contain similar phytochemical compounds, the quantities differ. The aqueous extracts of the bitter leaf contained the highest quantities of alkaloids (Tables 6 and 7). This was obvious in the washed and unwashed bitter leaf (washed: Cold water extract-6.83% and Hot water extract-7.32%; unwashed: Cold water extract-5.32% and Hot water extract-6.12%). Therefore, it can be concluded that alkaloids are more revealed in bitter leaf extract when it is washed, although an appreciable quantity can also be gotten when it is not washed. It can also be deduced from this experiment that alkaloids are not heat labile since the highest quantity was liberated from the hot water extract. The ethanolic extracts contained the least quantities (washed: 2.93% and unwashed: 3.12%). This means that as much as ethanol is an organic solvent and is an excellent phytochemical compound liberator, it was unable to exhibit those characteristics in the extraction of alkaloids from bitter leaf extracts. This is unlike the findings of Epraim [12] who got more alkaloids from the ethanolic extracts of black pepper seeds and pawpaw seeds. The study also discovered that the hot water extracts of the two plants (black pepper and pawpaw seeds) liberated more alkaloids than the coldwater extracts. The carotenoids were also not well liberated by the aqueous extracts (cold and hot water). Bitter leaf (washed and unwashed) extracts contained very low content of flavonoids compared to the alkaloids. Hot water extract retained more

	Alkaloids	Flavonoids	Carotenoids
EE	2.56	1.88	0.1
CWE	1.88	--	0.04
HWE	1.4	1.4	0.06

EE: Ethanolic Extract; HWE: Hot Water Extract; CWE: Cold Water Extract

Table 8: Quantitative screening of washed Scent leaf extract in %(g/100 g).

	Alkaloids	Flavonoids	Carotenoids
EE	1.75	--	0.11
CWE	1..43	--	0.03
HWE	4.64	2.11	0.08

EE: Ethanolic Extract; HWE: Hot Water Extract; CWE: Cold Water Extract

Table 9: Quantitative screening of unwashed Scent leaf extract in % (g/100 g).

of the vitamin compared to cold water extracts. Carotenoid levels increased in hot water extracts 0.05 g/100 g while the content was reduced in cold water extracts 0.04 g/100 g. The resistance of carotenoid to the effect of heat is in line with the earlier report of Anderson, that phytochemical compounds are not affected by processing. It can thus be said about bitter leaf that the phytochemical compounds are better liberated after washing using either the hot or cold water as solvents of extraction while the flavonoids content can only be revealed in the ethanolic extract.

Scent leaf contains the same phytochemical compounds as the bitter leaf but it was revealed from the results of this study that more of these compounds can be found in the latter. Unlike the bitter leaf, the aqueous extracts did not liberate much alkaloids (washed: Cold water extract-1.88% and Hot water extract-1.40%; unwashed: Cold water extract-1.43% and Hot water extract-4.64%) while the ethanolic extract liberated 2.56%. This means that to use hot water as solvent of extraction for flavonoids, the scent leaf should not be washed while it should be washed and ethanol used as solvent of extraction when alkaloids are been screened for. Erinle [3] reported that the pigment taste of scent leaf is contributed by its high content of alkaloid. The hot water extract of the unwashed scent leaf can also be depended upon for flavonoids even though the ethanolic extract of the washed sample can also be screened for it. This result thus infers that flavonoids in the scent leaf are not well liberated and are not heat labile.

Generally, heating did not significantly affect the carotenoid content of the leaf. This indicates that carotenoid (colored pigment) remained stable during heating, though there was slight reduction in carotenoids content in ethanolic extracts and hot water extracts.

Conclusion

The results from this work has shown that the ethanolic, hot and cold water extracts of the plants under study contains many phytochemical compounds which include alkaloids, steroids, flavonoids, carotenoids and tannins which are responsible for their various activities and also may account for medicinal benefits. It can be concluded from this work that the different solvents of extraction have varying abilities to liberate these compounds and the quantities that each liberate as been ascertained. The selected plants contain substantial amount of phytochemical which are helpful in the prevention of some deadly diseases. Scent leaf, (*Ocimum gratissimum*) and bitter leaf (*Vernonia amygdalina*) leaves could help fulfill the growing demands of plant based foods for human nutrition.

References

1. Jigna P, Nehel K, Sumitra C (2006) Evaluation of Antibacterial and Phytochemical Analysis of Bauhinia variegate L bark. 9: 53-56.

2. Allero AA, Afolayan AJ (2006) Antimicrobial Activity of Solanum tuberosum. Afri J Biotechnol 5: 269-272.
3. Erinle OD (2012) Phytochemical Screening of Scent leaf (Ocimum gratissimum) and bitter leaf (Vernonia amygdalina) extracts. ND Project, FCFFT, New Bussa, Niger State: 1-5.
4. Adenuga W, Olaleye ON, Adepoju PA (2010) Utilization of bitter vegetable leaves (Gongronema latifolium, Vernonia amygdalina) and Garcinia kola extracts as substitutes for hops in sorghum beer production. African Journal of Biotechnology 9: 8819-8823.
5. Muanya C (2015) Scent and Bitter Leaves Retard Growth of Cancers, Boost Immune System. Guardian Newspaper Publication.
6. Health Facts NG (2015) Health Facts. Designed by iQube Labs.
7. Sulistiarini D (1999) Ocimum gratissimum. Oyen LM, Nguyen LPA, Xuan, Duan editors. Plant Resources of South East Asia: Essential-oils plants. Prosea foundation bojor, Indonesia: 140-142.
8. Oladosu-Ajayi RN, Dienye HE, Ajayi CT, Agha IU (2016) Vernonia amygdalina (Bitter leaf) extracts as preservative for catfish (Clarias gariepinus). International Journal of Nutritional Science and Food Technology 3: 2471-7371.
9. Azu NC, Onyeagba RA (2007) Antimicrobial Properties of Extracts of Allium cepa (onions) and Zingier officinale (ginger) on Escherichia coli, Salmonella typhi and Bacillus subtilis. The Internet Journal of Tropical Medicine 3: 2.
10. Harborne JB (1973) Phytochemical Methods. London, Chapman and Hall Ltd: 49-188.
11. Okafor JC (1987) Identification and Conservation of plants used in traditional medicine. Paper presented at the International Workshop on Evaluation of traditional Medicines. University of Nigeria, Nsukka, Nigeria: 62-68.
12. Ephraim RN (2010) Antimicrobial Properties of extracts Carica papaya and Piper guineese on Microorganisms Associated with Catfish, Clarias gariepinus spoilage. M.Sc. Dissertation; University of Agriculture, Abeokuta (UNAAB) Ogun State, Nigeria: 87.