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 carotenoid. 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, bufadienolides 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, gums, 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 Asteraceae. 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 Asteraceae. 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 Itsikiri and Urhobo tribes in Nigeria, Ewuoro (Yoruba), Etidot (Ibibo), 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 dirt. This was macerated and divided into two portions, one part of the...
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 stored 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 NH4OH 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).

### Table 1: Qualitative analysis of phytochemical compounds.

| Phytochemical   | Test (Reagent)       | Observation                             | Inference                  |
|-----------------|----------------------|-----------------------------------------|----------------------------|
| 1 Alkaloid      | Wagner dragnet 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           |

The table above was modified from the presentation in the original text to make it more readable and clear. The data is presented in a table format with the following columns: Phytochemical, Test (Reagent), Observation, and Inference. The table is used to compare different phytochemicals and their presence or absence in the samples.

### Table 2: Qualitative screening of washed bitter leaf extracts.

| Phytochemical | Observation | Inference |
|---------------|-------------|-----------|
| Alkaloids     | HWE: +      | Present   |
| Flavonoids    | CWE: +      | Present   |
| Steroids      | EE: +       | Present   |
| Tannins       | HWE: +      | Present   |

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.

### 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.

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 desiccator and weighed. The weight of carotenoid was determined was expressed as a percentage of the sample weight [11].

### Discussion

The results obtained from this study showed that hot water, cold
Firstly, the results from this work has shown that the ethanolic, hot and cold 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 extract (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: Ethanol extract-1.75% and Hot water extract-1.48%; unwashed: Ethanol extract-1.43% and Hot water extract-0.64%) 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 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 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 taste of scent leaf is contributed by its high content of alkaloid.

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.

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