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THE DEBITTERING EFFECT OF POTASH ON METHANOLIC EXTRACT OF BITTERLEAF (*Vernonia amygdalina*)

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Abstract

The current study was aimed at evaluating The Debittering Effect of Potash on Methanolic Extract of Bitterleaf (Vernonia amygdalina) using hot treatment process. The extract was analyzed of its qualitative phytochemicals using standard analytical methods before and after treatment with potash salt. The results revealed the presence of alkaloids (+++), flavonoids (++), phenols (++), tannins (++), saponins (++), steroids (++), terpenoids (+) and cardiac glycoside (+) in the sample without potash treatment, while the sample treated with potash showed the presence of alkaloid (+), flavonoids (+), saponins (+), terpenoids (+), steroids (+), cardiac glycoside (+), but there was no presence of phenol and tannins. Phenols and tannins have been reported to be the compound causing bitterness in vegetables. Alkaloid from bitter leaf (V. amygdalina) inhibits phosphodiesterase – 5, and oxidative stress in rat's penile tissue. Phenols and tannins

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which have been reported to be the compound causing bitterness in vegetables were totally absent in the treated extract. Therefore, the process is recommended as a preparative method of bitter leaf for use when cooking, contrary to a belief that squeezing and washing alone will be sufficient to remove anti nutritional factors contributing to the bitter taste of V. amygdalina.

INTRODUCTION

Leafy vegetables constitute an indispensible constituent of human diets in Africa generally and Nigeria in particular (Agomuo *et al.,* 2015).*Vernonia amygdalina*, variously known as bitter leaf (English), oriwo (Edo), ewuro (Yoruba), shikawa (Hausa), and Onugbu (Igbo), is a tropical shrub, 1-3m in height with petiole leaf of about 6mm in diameter, and elliptic in shape (Abioye *et al.,* 2014).
The leaves are dark green coloured with a characteristic odour and bitter taste due to its chemical contents which are responsible for its medicinal and anti microbial activities (Akachukwu, 2001). The common medicinal uses of *V. amygdalina* include, anti malarial, anthelmintic, antidiarrheal, treatment of venereal diseases, gastrointestinal problems and wounds. It is also very useful in the management of diabetes owning to its ability to reduce blood sugar drastically and its ability to repair the pancreas (Nwanjo, 2005).
Nutritionally, bitter leaf is an important vegetable containing significant quantities of zinc-a mineral important in enzyme function (Abioye *et al.,* 2014). The vegetable is consumed after a debittering process that removes the astringent component of the bitter leaf. Thus, the leaf is subjected to various local processing aimed at increasing its palatability however, significant portions of the beneficial components inherent in the leaf are lost during these processing treatments.

The major problem that hinders utilization of *V. amygdalina* is its bitterness which has been attributed to the presence of phytochemical substances, thus, making it less palatable. However, recent studies have focused on the use of common salt and other processes in removing the bitterness of vegetables and fruits but no study has been carried out using potash to remove the bitterness in the bitter leaf.

The research will provide information on the phytochemical composition of methanol extract of bitter leaf debittered with potash salt. It is expected that the findings from the study can also serve as guide to education programs for industries that may utilize the extract in production of commodities like drugs.

MATERIALS AND METHODS

Collection of Raw Materials

Vernonia amygdalina (bitter leaf) was collected from a local farmland in Oko, Orumba North Local Government Area of Anambra State, Nigeria. The bitter leaf was packaged in a clean polyethylene bag and was taken home. The bitter leaf was spread to dry in a room temperature (25°C), ground to powder and was taken to the laboratory for further processing and analysis.

Sample Preparation

Preparation of Bitter Leaf

The combined methods described by Agomuo *et al.*, (2015) and Rashima *et al.* (2017) were used in the preparation of bitter leaf. The fresh bitter leaves were sorted, de-stalked and rinsed in water to remove dust and dirt, and were left to drain. The bitter leaf was soaked in 3.5% (w/v) potash salt solution (1:5) at room temperature (25°C) for 1:30 min. The leaves were air dried after which pulverization ensured using a warring blender.

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Another set of bitter leaf were not treated and it serves as the control.

Preparation of Methanol Extract of Bitter Leaf

The methanol extract of the leaves were prepared in line with the modified method of Molehin and Adefegha (2014) for ethanol extraction. Five grams of the already treated bitter leaves were soaked in 100ml of methanol for about 48hours in an air-tight container; the mixture was filtered. The extract obtained was concentrated for subsequent analysis. All the extracts were stored in a sealed vial at 4 ^{o}C for further analysis.

Qualitative Analysis of Phytochemicals

Qualitative analysis of the crude extracts was carried out as described previously (Abioye *et al.,* 2014) to identify the presence of secondary metabolites.

Test for Alkaloids

One (1) gram of the sample was measured into a test tube, 5.0ml of 2% HCl of the filtrate was treated by adding 5 drops of Wagner's reagent and shake. It was placed on a steam bath for 10mins. A reddish brown colouration indicating the presence of alkaloids.

Test for Saponins

One (1) gram of the sample extract was boiled with 5.0ml of distilled water in test tube for 5minutes in water bath. It was decanted while still hot. The filtrate was used for the following test. 1.0ml of the filtrate was diluted with 4.0ml of distilled water and shaken vigorously for stable froth on standing. The stable froth was observed for 2 minutes indicating the presence of saponins.

Test for Flavonoids

One (1) gram of the sample extract was measured into a test tube, 1.0ml of 10% lead acetate was added and shaken for 30seconds and kept to stand. Formation of yellow precipitate was taken as a positive result for flavonoid.

Test for Tannins

One (1) gram of sample extract was measured into a test tube and 1ml of 5% bromine water was added and shaken. The formation of greenish to red precipitate was recorded as evidence for the presence of tannin.

Test for Terpenoid

Five (5) grams of the sample extract was measured into a test tube, 2 ml of chloroform was added, and 2 ml of concentrated H_2SO_4 was added carefully by the side of the test tube to form a layer. A reddish brown colouration at the interface was formed, indicating presence of Terpenoid.

Test for Phenol

One (1) gram of the sample extract each was measure into a test tube, 1 ml of 10% ferric chloride was added and shaken. The formation of a greenish brown colouration was taken as evidence for the phenolic.

Test for Steroids

One (1) Millilitre of the sample was mixed with 3 ml of concentrated sulphric acid, the colour at the interface was observed and it indicates the presence of steroids in the sample.

Test for Cardiac Glycosides

One (1) Millilitre of the extract was added 10 cm^3 of 50% H_2SO_4 and was heated in boiling water for five minutes. 10 cm^3 of Fehlings solution (5 cm^3 of each solution A and B) was added and boiled. A brick red precipitate indicating the presence of glycosides was observed.

Results and Discussion

The result of debittering effect of potash on the methanolic extract of *Vernonia amygdalina* (bitter leaf) is shown on table 4.1

The result revealed the presence of phytochemicals such as alkaloids (+++), flavonoids (++), phenols (++), tannins (++), saponins (++) and steroids (++) while terpenoids (+) and cardiac glycosides (+) were at lower concentration in the methanolic extract of *V. amygdalina*

without potash treatment. The bitter taste in *V. amygdalina* is as a result of the presence of phenolic compounds and tannins.

However, the result when treated with potash showed the presence of alkaloid (+), flavonoid (+), saponin (+),steroids (+), but terpenoids (+), and cardiac glycoside (+) remained at the same level, while the presence of phenols (-) and tannins (-) which have been reported to be the major cause of bitterness in some vegetables (Adams and Carmen, 2000) were not detected after potash treatment. This is as a result of the minerals; potassium, chlorine, zinc and sodium present in potash. This study agrees with the report of Argheore *et al.,* (1998) on the study of the leaf extracts of *Vernonia amygdalina* on detection of phytochemicals. Phytochemicals in *Vernonia amygdalina* contains bioactive compounds which are anti-viral in nature as well as having prophylactic and therapeutic effect against cancer cells (Noumedem *et al*., 2013). While the report of Ghamba *et al*. (2014) establishes the presence of phytochemicals in *Vernonia amygdalina*. Udochukwu *et al.,* (2015) evaluated the concentration (mg/100g) of some of this aforementioned phytochemicals and observed *Vernonia amygdalina* to contained higher levels of bioactive compounds.

However, the results obtained in this study have shown that potash salt can significantly reduce and remove some phytochemicals responsible for the bitterness of *Vernonia amygdalina* leaves in order to increase its palatability. According to Adam and Carmen (2000), phenolic compounds and tannins are the phytochemicals responsible for the bitterness and astringency of many foods and beverages. These compounds (phenols and tannins) were totally removed from bitter leaf on treatment with potash salt. Bitterness in plant foods has been described as a sensory defect with a major economic effect (Van-Doorn *et al.,* 2008). The degree of bitterness depends on the cultivar, strain, ripening, and storage conditions of fruits and vegetables including herbs and shrubs (Adam and Carmen, 2000).

Responding to taste-driven consumer demand, the food industry generally removes phenolic compounds, flavonoids, isoflavones, terpenes and tannins from foods destined for human consumption. Because of such efforts, the current food supply is less bitter than it might otherwise be (Rouseff, 2000).

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Potential approaches to removing bitter phytochemicals include selective breeding of new and less bitter cultivars, use of different processing methods, application salts and other chemicals etc. (Fenwick *et al.,* 2003). Bitter taste had been identified as the main reason for avoidance of vegetables and was reported as being the least well tolerated for vegetables, spinach, squash, and onions. The current health-oriented push toward selective breeding of phytonutrient-rich and therefore more bitter varieties counters the published studies on vegetables and consumer acceptance (Adam and Carmen, 2000).

Table 4.1: The Debittering Effect of Potash on the Methanolic Extract of *Vernonia amygdalina* (Bitter leaf).

Parameters	Without Potash	With Potash
Alkaloids	+++	+
Flavonoids	++	+
Phenols	++	-
Tannins	++	-
Saponins	++	+
Terpenoids	+	+
Steroids	++	+
Cardiac glycoside	+	+

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