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ANALYSIS ON PHYTOCHEMICAL, PROXIMATE AND MINERAL CONTENTS OF BITTER LEAF (VERNONIA AMYGDALINA) AND ITS POTENCY IN BLOOD SUGAR REDUCTION

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ABSTRACT
This study investigates the phytochemical, mineral and nutritional composition of bitter leaf (Vernonia amygdalina) and its potency in blood sugar reduction. Sample collection and preparation was carried out using the method prescribed by association of analytical chemist (AOAC), (2016). Phytochemical contents of bitter leaf was analyzed using Harborne JB., (1973) method. The study revealed the presence of alkaloids (8.94 mg/kg), flavonoids (0.98 mg/kg), saponins (26.32 mg/kg), tannins (12.00 mg/kg), phenols (20.06 mg/kg), steroids (19.33 mg/kg), and glycosides (4.50 mg/kg). proximate analysis carried out on bitter leaf using standard laboratory method revealed the presence of carbohydrate (37.10 ± 1.60 mg/100g), crude protein (28.10 ± 1.30mg/100g), ash content (9.30 ± 0.23 mg/100g), crude fiber (11.50 ± 0.40 mg/100g), fats (5.45 ± 0.28 mg/100g) and moisture content (8.40 ± 0.04 mg/100g). Also mineral composition analyzed on bitter leaf revealed the presence of Iron (15.00 mg/100g), phosphorous (60.20 mg/100g), Copper (5.50 mg/100g), zinc (8.90 mg/100g), magnesium (85.70 mg/100g), potassium (61.00 mg/100g), and Calcium (65.50 mg/100g). The study proved bitter leaf rich in phytochemicals, minerals and nutritional components essential for human health and blood sugar reduction. The bioactive compounds present in bitter leaf such as saponins, phenols and steroids plays significant role in blood sugar reduction by protecting pancreatic cells while alkaloids enhance insulin sensitivity. Magnesium enhances the activity of insulin thereby reducing blood sugar. These reveals that bitter leaf (V. amygdalina) has effective potentials needed for blood sugar reduction. Hence further investigation will reveal hypoglycemic effects as well as the cytotoxic effects induced by *V. amygdalina* extract.

Keywords: Phytochemical, Mineral, Proximate

INTRODUCTION
In the South-Eastern region of Nigeria, the plant known as bitter leaf is prevalent. Vernonia amygdalina grows throughout tropical Africa and has been domesticated in some parts of Nigeria. It is a herb or shrub that grows as a small tree between 1 and 3 meters tall in Madagascar and Asia (Ojiako and Nwanjo, 2006). The leaves are petiolated in shape with a bitter taste of which its name "Bitter leaf" spring up. The presence of glycosides, alkaloids, saponins, and tannins had been linked to the bitter taste (Wong *et al*. 2013). Farombi and Owoeye (2011) investigated and

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proved that these made them function as a bittering agent and a hop substitute used to control microbial contamination in beer brewing without lowering the quality of the malt. For seven years, this plant can be harvested twice per month. They are frequently utilized in traditional medicine and food.

Their characteristic; odour and bitter taste can be reduced either by washing in several changes of water or by boiling before consumption (Nwaoguikpe, 2010). It has also been used in soup (Ogbono and Onugbo soups) in Nigeria and Cameron (Ndole dish) (Oguwike *et al.* s2013). Oduah (2012), stated that the decoctions of the leaves and roots have been used in ethnomedicine to treat stomach disorders, fevers, kidney issues, and hiccups. A number of studies on this plant suggested that it contains a variety of bioactive compounds, such as terpenes, steroidal glycosides, triterpenoids, flavonoids, alkaloids, tannins, phenolics, and several types of sesquiterpene lactones (Luo *et al.,* 2017). These bioactive compounds made them possess different pharmacological properties like antimicrobial, antimalarial, antithrombotic, antioxidant, anti-diabetic, laxative, hypoglycemic, antihelmintic, anti-inflammatory, cathartic, anticancer, antifertility, anti-fungi, antibacterial, and among others (Alara *et al.,* 2017).

Sofowara (2006), proved glucose necessary for the body to function normally. Diabetes is characterized by an increase in blood glucose levels as a result of a relative or absolute lack of insulin, which prevents glucose from entering the cell. Bitter leaf (Vernonia amygdalina) has long been recognized for its therapeutic properties, particularly those related to diabetes management. Its natural compounds, such as flavonoids, saponins, and tannins, have been shown to help lower blood sugar levels by improving insulin sensitivity and reducing glucose bsorption in the body (Roberts, 2007). The antioxidants in bitter leaf also help reduce oxidative stress, which is often associated with diabetes-related complications (Nwozo and Orojobi 2011).

Regular consumption of bitter leaf, either in its raw form or as an extract, aids in managing blood glucose levels, offering a natural, complementary approach to diabetes management. Bitter leaf's bioactive compounds, such as flavonoids, antioxidants and numerous nutritional contents, not only help lower blood sugar levels but also improve insulin sensitivity and health benefits without the harsh side effects of synthetic drugs (Ali *et al*., 2020). It provides a holistic approach, supporting overall health while effectively managing glucose levels. Hence the quest to ascertain the phytochemical, proximate and mineral contents of bitter leaf and its potency in reducing blood sugar.

LITERATURE REVIEW

Review of Related Works

Ali *et al*., (2020) investigated on "Determination of proximate, phytochemical and mineral composition of bitter leaf" and proved bitter leaf a potential source of vitamins, minerals and bioactive components such as phytochemicals and antioxidants that helps in reducing the risk of diseases.

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Oseghale *et al*., (2024) investigated on "Ethnomedicinal and phytopharmacological aspect of vernonia amygadalina (bitter leaf) utilized as traditional medicinal herb" and proved bitter leaf a therapeutic and remedial potentials due to its phytochemicals contents.

Olusegun *et al*., (2013) investigated on "Assessment of bitter leaf on some selected pathogenic microorganisms" and proved bitter leaf a medicinal plant employed to cure various infection in traditional medicine.

In this present work "Phytochemical, proximate and mineral contents of bitter leaf and its potency in blood sugar reduction" will be studied.

Description of Bitter Leaf (Vernonia Amygdalina)

Bitter leaf (Vernonia amygdalina) is native to tropical Africa and is widely distributed throughout the continent, from West Africa to East Africa. It grows in cultivated areas, savannas, and rainforests, among other ecological zones (Oduah, 2012). The plant is a staple in many African cultures, and has been used for centuries as a food ingredient as well as traditional medicine. Its continuous use in traditional medicine and cuisine extends beyond Africa to other tropical regions, exhibiting its adaptability to various environments and its significant role in diverse cultural practices (Egharevba *et al*., 2014). Vernonia amygdalina has been the most prominent species in the family of Asteraceae that had been studied in Africa. Vernonia amygdalina does not typically produce seeds; however, stem planting is typically used to cultivate this tropical plant. It grows mainly along the drainage, commercial plantation or forest (Yeap *et al*., 2010).

The name "Bitter leaf" emanated from the bitter taste. Vernonia amygdalina are being called different local names which vary from country to country. Awoyinka *et al*. (2007) stated that the presence of glycosides, alkaloids, tannins, and saponins had been linked to the bitter taste. As a result of this bittering agent, they are employed as a hop substitute used to control microbial contamination in beer brewing without lowering the quality of the malt (Farombi and Owoeye , 2011). Bitter leaf has a life span of seven years and can be harvested twice a month (Ghamba *et al*., 2014). They are frequently utilized in traditional medicine and food. Their bitter taste can be controlled by either washing or boiling (Alara *et al*., 2017). Bitter leaf powder can be used for tea for the treatment of malaria (Yeap *et al.,* 2010). In Ethiopia, the bitter leaf is used to make the honey wine known as Tei. The decoctions of the leaves and roots have been used in ethnomedicine to treat stomach disorders, fevers, kidney issues, and hiccups (Oduah, 2012). The medicinal, culinary, and nutritional properties of bitter leaf (Vernonia amygdalina) have attracted widespread use.

Due to its distinct bitter flavor and abundant nutritional content, including vitamins A, C, and E, it has traditionally been used to prepare soups and other meals in numerous African households (Okuda, 2005). Bitter leaf extracts are processed into dietary supplements, teas, and tinctures for diabetes and hypertension management in the herbal medicine industry. The food industry uses

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bitter leaf as a functional food ingredient, enhancing the nutritional content of products like sauces, soups, and beverages. Additionally, bitter leaf extracts are used in skincare products by the cosmetics industry due to their antioxidant and anti-inflammatory properties, which aid in skin health improvement and combat oxidative stress (Yao *et al*., 2004: Evans, 2005). Flavonoids, tannins, alkaloids, saponins, terpenoids, and phenolic compounds are among the most important phytochemicals found in bitter leaf, it also possesses antioxidant, anti-inflammatory, anti-diabetic, and antimicrobial properties (Alara *et al*., 2017).

Bitter leaf is a useful plant for managing diabetes because it contains compounds like vernolide, vernonioside and phytochemicals that help lower blood sugar levels and increase insulin sensitivity (Kendall, 2007). According to Luo *et al.* (2017), its high antioxidant content also shields cells from oxidative damage, enhancing its therapeutic value. It exert its significance effect on the treatment of chronic conditions like diabetes, high blood pressure, and inflammation. It is increasingly being used in natural supplements to help regulate blood glucose levels because of its hypoglycemic properties (Yeap *et al*., 2010). Additionally, bitter leaf's antimicrobial and anti-inflammatory effects make it an effective ingredient in medications aimed at treating infections and promoting immune health (Omale and Okafor, 2008). Bitter leaf extracts are being looked at by the pharmaceutical industry as a way to make drugs that can fight oxidative stress-related diseases like cancer, cardiovascular disease, and neurodegenerative disorders. According to Alara *et al*. (2017), its versatility and effectiveness make it a promising plant for future pharmaceutical research and development.

METHODOLOGY

Sample Collection

The leaves of V. amygadolina (bitter leaf) was purchased in Eke Oko market in Oko town, Anambra State, Nigeria. The leaves were washed, air dried and pounded into fine powder. The powder was stored in air tight container for further use.

Extraction of Plant Material (Bitter Leaf)

30 grams of the powder was soaked in 300 ml of distilled water for 24 hours. The solution was filtered using filter paper to obtain the filtrate. The obtained filtrate was heated for dryness in a water bath at 60°C. The solid extract (sample) was stored in a bottle for further use.

Determination of Proximate Composition

Proximate analysis was carried out on bitter leaf (V. amygadolina) using standard laboratory method. Carbohydrate, crude protein, ash content, crude fiber, fats and moisture content were determined.

Phytochemical Screening

The preliminary phytochemicals present in bitter leaf extract was determined using Harborne JB. (1973) method. The method was employed to determine the alkaloids, flavonoids, tannins, glucosides, saponins, phenols and steroids present.

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Determination of Alkaloids

Five gram (5 g) of the sample was weighed into a 250 ml beaker and 200 ml of 20% acetic acid was added in ethanol and covered. It was allowed to stand for 4 hours at 25^{o}C. The solution was filtered with filter paper and the filtrate was allowed to stand on a water bath so as to evaporate to one quarter of the original volume. Concentrated ammonia was added in drops into the extract until the precipitate was complete.

The whole solution was allowed to settle and the precipitate was then collected pre-weighed filter paper and washed with dilute NH4OH (1 % ammonia solution). The filter paper with the precipitate is then dried in the oven at 80^{o}C for one hour. The alkaloid content was calculated and expressed as a percentage of the weight of the sample analyzed.

% weight of alkaloid = Weight of filter paper with residue – weight of filter paper x 100

Determination of flavonoids

10 grams of the sample was extracted repeatedly (3 times) with 100 mls of 80% aqueous methanol at room temperature. The whole solution was filtered with whatman filter paper. The filtrate was later transferred into a crucible and evaporated into dryness over a water bath. The crucible was cooled in a desiccator and weighed until constant weight is obtained.

% flavonoids = (weight of crucible + residue) – (weight of crucible) x 100

Determination of Saponins

20 grams of the sample was poured into conical flask containing 100ml of 20% ethanol and allowed to stand in a water bath for 24 hours at 50^{o}C. The solution was filtered and the extract was concentrated to one – quarter of the original volume using a water bath. The concentrate was poured into a 250 ml separating funnel, the extract was vigorously shaken with 20 ml of diethyl ether. The aqueous layer is recovered while the diethyl ether layer is discarded and the purification process is repeated. N-butanol 60 ml was added and the solution washed twice with 10 ml of 5% sodium chloride. The remaining solution is then heated in a water bath and after evaporation; the samples are dried in the oven to obtain a constant weight and values are expressed as percentage of extract.

% Saponin content = (Weight of filter paper + residue) – (weight of filter paper) x 100

Tannin Determination using Titration method

100 mls of petroleum ether was added to 20 grams of the sample in a conical flask and covered for 24 hours. The sample was then filtered and allowed to stand for 15 minutes for petroleum ether to evaporate. Thereafter it was soaked in 100 ml of 10% acetic acid in ethanol for 4 hrs, filtered and the filtrate collected with filter paper. The alkaloids were precipitated by addition of 25 ml of NH_4 OH to the filtrate. The alkaloids was heated with electric hot plate to remove some of the NH_4 OH in the solution. 5 ml of the filtrate was taken and 20 ml of ethanol was added to it.

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It was titrated with 0.1M Na0H using phenolphthalein as indicator until a pink end point is reached. Tannin content was then calculated in % ($C_1 V_1 = C_{2v2}$) molarity.

Calculation

Data

C_1 = cone of Tannic Acid

C_2 = cone of Base

V_1 = Volume of Tannic acid

V_2 = Volume of Base

Therefore $C_1 = \frac{C_2 V_2}{V_1}$

% of tannic acid content = C_1 x 100

Determination of steroid content

1.0 g of the sample was added in 100 ml of distilled water in a conical flask and the solution filtered. The filtrate was washed with 0.1 N ammonium hydroxide solution. 2 ml of the eluent was poured in a test tube and 2 ml of chloroform added. 3 ml of ice cold acetic anhydride was added to the mixture in the flask. 2 drops of (200 mg/ dl) standard sterol solution was prepared and treated as described for test as blank. The absorbance of standard and test was measured, zeroing the spectrophotometer with blank at 420 nm.

Determination of Mineral Composition

Atomic absorption spectrophotometer was used to determine the mineral composition of bitter leaf (V. amygadolina). Iron (Fe), phosphorous (P), Copper (Cu), zinc (Zn), magnesium (Mg), potassium (K), and Calcium (K) were analyzed and the result was expressed mg/100g dried powder.

RESULTS

PROXIMATE COMPOSITION

Proximate Composition of Bitter Leaf (V. Amygadolina)

The result of proximate composition of bitter leaf V. amygadolina is presented in table 1

Table 1: **Proximate Composition of Bitter Leaf (V. amygadolina)**

S/N	Nutrients	Composition (mg/100g)
1	Carbohydrate	37.10 ± 1.60
2	Protein	28.10 ± 1.30
3	Ash content	9.30 ± 0.23
4	Crude fiber	11.50 ± 0.40
5	Fats	5.45 ± 0.28
6	Moisture content	8.40 ± 0.04

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PHYTOCHEMICAL CONTENTS

Qualitative Analysis of Bitter Leaf (V. amygadolina)

The result of the qualitative analysis of Bitter Leaf (V. amygadolina) is presented in Table 2

Table 2: **Qualitative Analysis of Bitter Leaf (V. amygadola)**

Parameters	Intensity
Alkaloids	++
Flavonoids	+
Saponins	+++
Tanins	++
Phenols	+++
Steroids	+++
Glycosides	+

Key
+++ —------Highly present
++ —------- Moderately present
+ —---------Fairly present

Quantitative Analysis of Bitter Leaf (V. Amygadolina)

The result of the quantitative analysis of Bitter Leaf (V. amygadolina) is presented in Table 3

Table 3: **Quantitative Analysis of Bitter Leaf (V. amygadolina)**

Parameters	Concentration (mg/kg)
Alkaloids	8.94
Flavonoids	0.98
Saponins	26.32
Tannins	12.00
Phenols	20.06
Steroids	19.33
Glycosides	4.50

MINERAL COMPOSITION

Mineral Composition of Bitter Leaf (V. amygadolina)

The result of mineral composition of Bitter Leaf (V. amygadolina) is presented in Table 4

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Table 4: **Mineral Composition of Bitter Leaf (V. amygadolina)**

S/N	Minerals	Composition (mg/100g)
1	Iron	15.00
2	Phosphorous	60.20
3	Copper	5.50
4	Zinc	8.90
5	Magnesium	85.70
6	Potassium	61.00
7	Calcium	65.50

DISCUSSION

Proximate Composition of Bitter Leaf (V. amygadolina)

The study reveals high content of protein and carbohydrate present in bitter leaf while moisture, ash and fiber were present in moderate amount and small amount of fat. This is in line with the result obtained by Ali *et al.,* (2020). Carbohydrate being present in high amount reveals that bitter leaf is a good source of energy. Also high content of protein in bitter leaf indicates that it is a body building food which possess antibodies that fights against germs (Owu *et al*., 2008). Fiber present restrains carbohydrate intake indicating its reliability in diabetes and cholesterol control (Yeap SK *et al*., 2010). Small amount of fat present proved bitter leaf essential and healthy food for human consumption which justifies the result obtained by Ali *et al.,* (2020).

Phytochemical Screening on Bitter Leaf (V. amygadolina)

Qualitative and quantitative analysis of phytochemical contents of bitter leaf reveals the presence of alkaloids, flavonoids, saponins, tannins, phenols, steroids, and glycosides. Saponins, phenols and steroids are present in high concentration indicating its anti-cancer, anti-diabetic, anti-inflammatory, antioxidant, and cholesterol lowering effect. Tannins were found in considerable amount attributing to its anti-microbial, antioxidant and anti-inflammatory effect. Flavonoids and glycosides were detected in lower concentrations, yet they are recognized for contributing to the plant's bioactivity. These phytochemicals likely work synergistically, with each compound complementing the activity of others. For instance, while saponins improve glucose metabolism, phenols protect pancreatic cells, and alkaloids enhance insulin sensitivity. Such interactions underscore the potential of bitter leaf juice as a comprehensive natural treatment for diabetes. However, this indicate that bitter leaf have nutritional benefits. This study is in line with Nawwar *et al*., (2020): Oladele, (2019), and Olagunju *et al*., (20218) work.

Mineral Composition of Bitter Leaf (V. amygadolina)

Mineral composition analysis on bitter reveals the presence of zinc, copper and iron while phosphorous, potassium, magnesium and calcium were contained in high concentration. This proved bitter leaf nutritionally enriched. Minerals are essential in metabolic processes such as: maintenance of acid balance in the body, production and activity of enzymes and so on (Usunobun and Okolie, 2016). Potassium plays essential role in regulation of osmotic pressure in the body as well as maintaining acid balance in the body (Usunobun and Okolie, 2016). Calcium aid in formation and teeth development. It also play major role in nervous system as well as heart

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functions (Murray *et al.*, 2011). Magnesium promotes the activity of insulin by reducing blood sugar (Igbakin and Oloyede, 2009). Zinc helps in nerve functioning, normal sexual development, improves body immunity and formation of white blood cell (Claude and Paule, 1979). Copper functions as cellular defense, membrane protection and formation of haemoglobin (Claude and Paule, 1979). The mineral analysis reveals that of V. amygdalina provides both nutritional value and medical benefits.

CONCLUSION

The study reveals that bitter leaf are rich in phytochemicals, minerals and nutritional components essential for human health and blood sugar reduction. The bioactive compounds present in bitter leaf such as saponins, phenols and steroids plays significant role in blood sugar reduction by protecting pancreatic cells while alkaloids enhance insulin sensitivity. Magnesium enhances the activity of insulin thereby reducing blood sugar (Igbakin and Oloyede, 2009). These reveals that bitter leaf (V. amygdalina) has effective potentials needed for blood sugar reduction.

RECOMMENDATION

It is recommended that further studies be carried out on bitter leaf to assess its hypoglycemic effects as well as the cytotoxic effects induced by *V. amygdalina* extract. Investigating the mechanisms through which these compounds reduce blood sugar could yield insights into their therapeutic applications.

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