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Phytochemical Analysis of Bitter Kola (*Garcinia kola*)

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Abstract
This study involved the determination of the chemical composition of Garcinia kola using standard methods. To determine the presence of some of the biologically active compounds that are present in the seed of Garcinia using distilled water and n-hexane. Alkaloids, anthraquinones, Tannins, Phlobatannins, flavonoids and Glycoside were found to be present when tested with distilled water. However, when tested with n-hexane Tannins and Glycoside were found to be present. The observed active compound of the extract of Biter Kola proved why Biter Kola is used in traditional medicine. Bitter kola was observed to be used for so many health benefits, like in the treatments of malaria, weight loss, diabetes, and treatment of glaucoma among others. Bitter kola should be sold in pharmaceutical processing factories, cosmetics, agricultural and food industries.

Keywords: Garcinia Kola, Alkaloids, Anthraquinones, Tannins, Phlobatannins

Introduction

Man has been using various parts of plants like leaves, bark and seeds medicinally. Bitter kola is one such plant that has long been used by Man. Bitter kola (*Garcinia kola*) is a tree that is highly cultivated because of its value in Africa where the nuts are edible. Bitter Kola has been used in African traditional medicine for the cure of illnesses like laryngitis, liver diseases, cough, hoarseness of voice, diabetes and other related complications (Adedara & Farombi, 2015). The seed is eaten raw as a stimulant because of its bitter astringent taste, which is followed by a slight sweetness. *G. kola* seed is recommended traditionally for the treatment of diabetes (Adaramoye & Adeyemi, 2006). It is also a potent remedy for liver disorders, and hepatitis, as well as being an aphrodisiac and fertility-enhancing substance (Yakubu & Quadri, 2012). The 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 such as tannins, oils, gums and precursors for the synthesis of complex chemical substances. In addition, the knowledge of the chemical constituents of the plants would further be valuable in discovering the actual value of folkloric remedies. Several phytochemical surveys have been published, including the random sampling approach which involved some plants collected from all parts of the world. (Segelman, 2010). According to Ajibola (2012), Since there is now little evidence to support any potential health benefits, phytochemicals are typically thought of as research molecules rather than necessary nutrients. Major groups such as carotenoids and polyphenols, which comprise phenolic acids, flavonoids, and stilbenes/lignans, can be used to group phytochemicals that are currently being studied. Based on their similar chemical structures, flavonoids can be further classified into groups including anthocyanins, flavones, flavanones, isoflavones, and flavanols. Proanthocyanidins, epicatechins, and catechins are additional classifications for flavanols. Over 25,000 phytochemicals have been identified to date, and most of them are found concentrated in the colourful sections of plants, such as whole grains, fruits, vegetables, nuts, and legumes. According to Yakubu (2014), the scientific classification of the plant is as follows;

Kingdom:	Plantae
Phylum:	Spermatphyta
Class:	Magnoliopsida
Order:	Malpighiales
Family:	Clusiaceae
Genus:	Garcinia
Species:	Kola

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The most common species found in many West African Countries is Cola nitida which has varieties like; Red Kola, Black kola and White kola. The chemical constituents of Bitter kola are; Alkaloids, Tannins and Flavonoids.

In traditional African medicine, Bitter kola is used to treat various ailments. Plant parts like roots, leaves and bark are used for medicinal purposes, while the nuts are mostly eaten. The medicinal uses of bitter kola are;

- Treating malaria: Bitter kola is believed to contain Kolaviron, a chemical that is a natural antioxidant and anti-inflammatory phytochemical, which has a high antimalarial effect (Esiegwu & Udedibie, 2009).
- Weight Loss: Collise (2011), find out that Bitter kola is a natural hunger suppressant and a great thirst stimulant, therefore helps to reduce weight loss. Reduction of food intake and increased water intake is needed by the body to do away with excess fat and remain healthy.
- Diabetes: Hertog and Keselman (2007), believed that seeds of bitter kola are the able to lower blood glucose and so, a potential treatment for diabetes melatus.
- Prevention from glaucoma: an eye drop made from an extract of Garcinia kola has been proven to be effective in the treatment of glaucoma (Farombi, 2005).
- Cold and fever remedy: Bitter kola has the potential to relieve chest colds and the ability to enlarge the alveolar sac and duct (Okwu & Ekeke, 2003).
- Erectile dysfunction treatment: It was found that Garcinia kola (bitter kola), has aphrodisiac effects (causes or increases sexual desire) in males and as such, is used in the treatment of erectile dysfunction (Okwu & Ekeke, 2003).
- Immunity: Bitter kola was found to have the ability to make the body adapt to stress by influencing various regulatory systems including the immune system and also to serve as an anti-infective agent (Collise, 2011).
- Osteoarthritis: Garcinia is a good disease modifier. It plays a vital role in the provision of potential relief in osteoarthritis. This is due to its significant analgesic/anti-inflammatory effects on knee osteoarthritis patients. It's good in the suppressing of inflammation, and pain in immovable joints (Farombi, 2005).
- Healthy lungs: Garcinia kola improve the function of the lungs as it aids the expansion of alveolar ducts and sacs, thereby, strengthening the fibre in the tissue of the lungs. This is because it contains saponin which is an antioxidant (Farombi, 2005).
- In a nutshell, Bitter kola is effective as an Anti-cancerous, anti-parasitic, Anti-Poison, and in Pregnancy, Life Prolongation and snake Repellant (Atawodi , 2011; Emmanuel & Gabriel, 2011; Nair, 2000).

Phytochemical analysis is a process that has to do with the identification and quantification of bio-active chemical substances like Alkaloids, Glycocides, Phenolic acid, Flavonoids, Terpenoids Saponins and Tannins in plant materials like leaves, root, seed and fruit (Laurenta et al., 2016). The common methods used in phytochemical analysis includes: chromatography, spectroscopy, mass spectrometry and solvent extraction (Singh, 2020).

Aim and Objectives of the study

The aim and objectives of the study are to extract the juice from the bitter kola and to analyse the extract so as to determine the presence of Alkaloids, Tannins, Flavonoids, anthraquinones and phlobatannins.

Methods and Materials

The Bitter Kola (Garcinia Kola), were collected at Tudun Wada market and Ladan Sharehu Central Market Zaria, Kaduna state. The epicarp of the collected sample was removed and cut into smaller pieces. It was then winnowed to remove the dirt. The sample was then air-dried for two days in the lab to remove any moisture from it. The dried sample was then pounded to a fine powder using a mortar and pestle. The powdered sample was then measured and transferred into a conical flask and was covered with an aluminium foil for a day. 12.5g of the ground sample was weighed with a weighing balance and transferred into a 250 ml clean and dried conical flask. 62.5 ml of distilled water was added to it and labelled A. Another 12.5g of the sample was weighed and transferred to another 250 ml conical flask labelled B. 62.5 ml n-hexane was added to each of A and B and shaken vigorously. The flasks were covered with Aluminum foil sheet and were allowed to stand for a day to be soaked and moisten. The contents in each of the conical flasks were filtered using filter paper into a clean beaker labeled A and B respectively. This is to separate the filtrates from the residue. $2cm^3$ of filtrates of beaker A were then poured into 6 different clean test tubes and labelled A1, A2, A3, A4, A5 and A6 for set up A. Another 2cm of filtrate in beaker B was poured into a clean test tube labelled B1, B2, B3, B4, B5, and B6 for

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set up B. 2ml of HCl and a few drops of Mayer's reagents were added to A1. 10ml of benzene and 5ml to ammonia mixed to the filtrate A2. A few drops of 0.1% ferric acid were added to A3. 15ml of aqueous HCl was added to A4, and boiled in a water bath for 5 minutes. 5ml of dilute ammonia solution followed by the addition of concentrated H_2SO_4 to A5. And 0.2ml of ferric acid solution was added to A6 and heated in a water bath for 5 minutes. The chemical reactions in all the setups were observed and recorded. While for B, 2ml of HCl and a drop of Mayer's reagent were poured in B1. 10ml of Benzene and 5ml of Ammonia added to B2. A few drops of 0.1% ferric acid were added into B3. 15 ml of Aqueous HCl was added to B4 and boiled in a water bath for 5 minutes. 5 ml of dilute ammonia solution followed by concentrated H_2SO_4 in B5, and lastly, 0.2 ml of Fehling solution was added to B6 and heated in a water bath for 5 minutes. The chemical reactions in all the setups were observed and recorded.

Results

The result obtained from the analyses of the bitter kola extract is as follows;

Findings from distilled water extract (A).

To determine the presence of some of the biologically active compounds that are present in the seed of Garcinia kola using distilled water, six portions of samples in test-tube labelled A1, A2, A3, A4, A5 and A6 were used. Table 1 shows what was observed

Table 1: Phytochemical Test distilled water extract of bitter kola

Test	Observation	Inference
Al+HCl+Mayer's reagents	the white precipitate was observed	Alkaloids are present
A2 + Benzene+ Ammonia solution	The violet colour was observed	Anthraquinones are present
A3 + Ferric Acid	Brownish-green colouration was observed	Tannins is present
A4 + Aqueous HCl	A Red Precipitate was seen	Philobattanins present
A + dil Ammonia solution	Yellow colouration was observed	Flavonoids is present
A6 + Distilled water	A light blue colouration was seen	Glycosides is present

Table 1 shows the result obtained from the Bitter kola extracts using distilled water (A). In test tube A1, 2ml of HCl was added to the filtrate and placed in a water bath for 5 minutes and then treated with a few drops of Mayer's reagents. A creamy white precipitate was observed. In A2, the filtrate is treated with 10ml of benzene and 5ml of 10% ammonia and shake. The presence of violet colour shows the presence of anthraqueousnes. In A3, 2 ml of the filtrate was boiled in 20 ml of distilled water. A few drops of 0.1% ferric acid were added. A brownish green coloration was recorded which shows the presence of tannins. In test tube A4, 2 ml of the filtrate was boiled with 15 ml of aqueous HCl acid. A deposition of a red precipitate was seen which shows the presence of phlobatannins. For test tube A5, 5 ml of 10% dilute ammonia was added to a portion of the filtrate, followed by the addition of concentrated H_2SO_4. A yellowish colouration was observed to indicate the presence of flavonoids. For test tube A6, 2ml of Fehling solution was added and heated for 5 minutes. A light blue coloration was seen which indicates the presence of glycoside.

Findings from the n-hexane extract (B).

In the test of n-hexane (B), six test tubes labelled B1, B2, B3, B4, B5 and B6 are used. In B1, 2ml of HCl was poured and placed in a water bath for 5 minutes. Table 2 shows the result of the test.

Table 2: Photochemical Test of n-Hexane extract of Bitter Kola

Test	Observation	Inference
B1 + HCl	Reddish-brown colouration was observed	Alkaloids is present
B2 + Benzene +Amonia solution	Pink coloration was observed	Anthraquinone is present
B3 + Ternic	No visible reaction	Tannins is absent
B4 + Aqueous Hcl	Yellowish colouration was observed	Phlobatannins is present
B5+Dil.NH4+Conc.H_2SO_4	Reddish-brown coloration was observed	Flavonoids is present
B6 + Distilled water + Fehling's solution	No visible reaction was observed	Glycosides is absent

From Table 2, the portion of the filtrate in B1 was treated with a few drops of Mayer's reagents. A redish-brown coloration was seen which shows the presence of Alkaloids. For test tube B2, 2ml of the filtrate is shaken with

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10ml of benzene and 5ml of 10% ammonia is mixed to the filtrate. A pink colouration shows the presence of Anthraquinones. For test tube B3, 2 ml of the filtrate was boiled in 20 ml of distilled water. A few drops of 0.1% ferric acid were added, and the absence of any visible reaction shows the absence of Tannins. For test tube B4, 2ml of the filtrate was made to react with 15ml aqueous HCl. Yellowish colouration is seen which indicates the presence of Phlobatannis. In test tube B5, 5ml of dilute ammonia was added to a portion of the filtrate, followed by the addition of concentrated H_2SO_4 a reddish-brown colouration was observed which indicates the presence of Flavonoids. For test tube B6, 10ml of distilled water was added to the filtrate and heated for 5 minutes. No visible reaction was observed.
Table 3 compares the result obtained from the analysis with distilled water as a solvent and the result obtained when n-hexane is used as the solvent.

Table 3: comparison of the result obtained from distilled water and n-hexane

Bioactive components	Result in distilled water	Results in n-hexane
Alkaloids	Positive (+)	Positive (+)
Anthraquinones	positive (+)	Positive (+)
Tannins	positive (+)	Negative (-)
Phlobatannins	Positive (+)	Positive (+)
Flavonoids	Positive (+)	Positive (+)
Glycoside	Positive (+)	Negative (-)

Discussion

Table 1 shows that Alkaloids, Anthraquinone, Phlobatannins, Flavonoids Tannins and Glycosides are present in Bitter kola when distilled water is used as a solvent. Table 2 shows the result obtained from the Bitter kola extracts using n-Hexane (B) which indicates that Alkaloids, Anthraquinone, Phlobatannins and Flavonoids are present in Bitter kola while Tannins and Glycosides are absent. Table 3 shows that Alkaloids, Anthraquinoues, Tannis, Phlobatannis, Flavanoids, and Glycosider are all present, while in n-haxene: Tannis, Glycosider, are all absent. This agrees with the findings of Ibedu (2018). It also shows the absence of Glycoside which is similar to the findings of Ezeanya (2012).

Conclusion

Bitter Kola has been used for years in African traditional medicine to cure many illnesses like liver diseases, cough, hoarseness of voice, diabetes and other related complications. This is associated with the chemical contents of the plant. To find out the chemical content the bitter kola, a phytochemical test or analysis of the extract from the Garcinia Kola was conducted. The fruits were collected, cleaned, dried and ground. The sample was treated with distilled water and n-hexane. The phytochemical analysis revealed that with n-hexane there was the presence of Alkaloids, Anthraquinone, Phlobatannins and Flavonoids and the absence of Tannins and Glycosides in Bitter kola. While, with distilled water, Alkaloids, Anthraquinones, Tannis, Phlobatannis, Flavanoids, and Glycosider are all present.

Recommendations

The following recommendations were made.

1. It’s recommended that Bitter kola should be sold in pharmaceutical processing factories, cosmetics, agricultural and food industries.
2. It's also recommended that bitter kola should be used in curing diseases such as wound and hypertension and other related diseases.
3. During the research, it was observed that bitter kola is used in so many health benefits, it is therefore recommended for the treatment of malaria, weight loss, diabetes, treatment of glaucoma among others.
4. It is recommended that further research should be conducted to identify the quantity of the chemicals identified in the Garcinia Kola (Biter Kola).

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