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Comparative Phytochemical Analysis of Fermented and Unfermented Seeds of *Dialium giuneense*

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Abstract

The present study was designed to study the fermentation process and to scientifically evaluate its phytochemical components. Fermentation was carried out using traditional method. Exactly 160 g of the seeds were soaked in water, washed, and cooked for 2 h, after which they were washed, sieved and parboiled for 20 min, the seeds sieved, cotyledon poured into fermenting pot covered tightly to prevent heat escape, fermented for 72 h while still hot, and the final product was gotten and sundried. The phytochemical analysis of *Dialium guineense* was carried out, and the results of the analysis shows the presence of the following phytochemicals in varied proportion across the different samples; saponin, flavonoids and phenolic compounds. Findings from this study suggest that *D. guineense* contains agents (secondary metabolites) capable of ameliorating certain disease conditions, therefore, its use as condiment for food preparation is encourage.

Keywords: *Dialium guineense;* Phytochemical; Fermentation; Anti-oxidants; Nutrition

Introduction

Dialium guineense also known as Black Velvet Tamarind (BVT) is an indigenous tropical forest fruit tree of the family Leguminosae. BVT is an important non-timber multipurpose agroforestry crop with a high potential [1]. The potentials of BVT as food supplement, in herbal medicine and as source of energy are well documented [2-4]. According to Agbani [5], BVT is a lesser known tropical forest fruit with high consumption but given a less priority in terms of research, production, improvement, storage and hence not domesticated. *D. guineense* is also one of food and medicinal plants of Lama Forest reserve [5]. Black velvet tamarind *(D. guineense),* a forest tree, is well known in many localities especially in West Africa. Small black velvet fruits are characteristic of the genus. The tree grows to about 20 m in height, 0.8 m in diameter, low-branching, rarely straight, bearing a compact densely leafy crown but is often shrubby [6].

Phytochemicals are a large group of plant-derived compounds hypothesized to be responsible for much of the disease protection conferred from diets high in fruits, vegetables, beans, cereals, and plant-based beverages such as tea and wine [7]. Based on their chemical structure, phytochemicals can be broken into the following groups; Flavonoids which are the most diverse group of phytochemicals, Phenolic Acids, Stilbenes/Lignans, Alkaloids, Saponinn, Tannin, Steroids, Phenols etc. [8]. Fermentation is a metabolic process that converts sugars to acids, gases and/or alcohol. It occurs in yeast and bacteria but also in oxygen-starved muscle cell as in the case of lactic acid fermentation. The science of fermentation is known as zymology. Fermentation takes place in lack of oxygen (when the electron transport chain is unusable) and becomes the cell's primary means of ATP (energy) production, it turns NADH and pyruvate produced in the glycolysis steps into NAD^+ and various small molecules depending on the type of fermentation. In the presence of O_2, NADH and pyruvate are used to generate ATP in respiration. This is called oxidative phosphorylation and it generates much more ATP than glycolysis alone.

Materials and Methods

Preparation of the sample (*Dialium guineense*)

The African Velvet Tamarind *Dialium guineense* needed for this research was purchased in a local market at Bedia village in Obudu Local Government Area of Cross River State, Nigeria, and transported to Medical Biochemistry laboratory of Cross River University of Technology, Okuku campus for processing.

Processing of *Dialium guineense*

Traditional method of processing *D. guineense* was used with the help of two local scientists specialized in the fermentation of *D. guineense*. The seeds were soaked in water, washed (160 g) of seed sample cooked for 2 h, after which it was washed and sieved, parboiled for 20 min, the seeds sieved, cotyledon poured into native fermenting pot covered tightly to prevent heat escape, fermented for 72 h while still hot, and the final product gotten and sundried.

Phytochemical screening of *Dialium guineense* seeds

The methods and procedures for the phytochemical analysis of *D. guineense* were from Wadood et al. [9].

Determination Flavonoid

1 g of pulverized *Dialium guineense* was put into a test tube and 10 ml of distilled water added and mixed rigorously with whirl mixer and filtered with a filter paper and funnel and the filtrate was divided into 2 test tubes, drops of lead acetate and NaOH was added each yellow and colour precipitate indicates the presence of flavonoids.

Tests for saponin

1 g of pulverized *D. guineense* was measured into a test tube, 10 ml of distilled water was added, and the solution was mixed with whirl mixer then heated to boil on hot plate, it was left to cool and was filtered into a clean test tube and then 1 ml of the filtrate was measured into 2

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test tubes each. In one, filtrate in the test tube ferric chloride solution was added with few drops and into the other, drops of benedict solution and ferric chloride.

Tests for tannin

1 g of pulverized *D. guineense* was measured into test tube and 20 ml of distilled water was added and heated with the help of a hot plate and was allowed to cooled and filtered into a clean test tube. 1 ml of the sample was measured into a test tube and few drops of bromine solution was added showing the presence of tannin by coloured precipitate.

Tests for alkaloids

1 g of pulverized *D. guineense* was measured and 10 ml (10%) HCL was added and mixed rigorously by whirl mixer for about 10 min and it was then filtered, the filtrate was divided into 2 test tubes. Mayer's test and Dragendorff test was carried out in each of the sample respectively.

Tests for steroids

Into a test tube was 1 g of pulverized *D. guineense*) seeds. 10 ml of chloroform was added and then 2 drops of salkowki's reagent was also added.

Tests for phenols

The sample *D. guineense* was weight, 1 g of the pulverized seed was placed into test tube, 10 ml of distilled water was added and mixed, then was filtered into test tubes with filter paper and funnel and 1 ml of the filtrate was measured into test tube and phenol was added, a precipitate indicate the presence of phenolic acid.

Results

This study has revealed the presence of phytochemicals considered as active medicinal chemical constituents. Important medicinal phytochemicals such as flavonoids, saponin, and phenolic compounds were present in the samples. The result of the phytochemical analysis shows the plant is rich in at least one of flavonoids, saponin and phenolic compounds.

Discussion

From the research, it was seen that biologic compounds (phytochemicals) such as flavonoids, saponin and phenolic compounds were present in the three samples in varied proportion whereas alkaloid and tannin were absent. Saponins are extremely poisonous, as they cause hemolysis of blood and are known to cause cattle poisoning [10,11]. They possess a bitter and acrid taste, besides causing irritation to mucous membranes. They are mostly amorphous in nature, soluble in alcohol and water, but insoluble in non-polar organic solvents like benzene and n-hexane. Saponins are also important therapeutically as they are shown to have hypolipidemic and anticancer activity. Saponins are also necessary for activity of cardiac glycosides. The two major types of steroidal sapogenin are diosgenin and hecogenin. Steroidal saponins are used in the commercial production of sex hormones for clinical use. For example, progesterone is derived from diosgenin. From the result obtained, there was a high concentration of saponin in the crude seed (+++) as compared to the fermented seed (+), therefore fermentation reduces the concentration of saponin. Hence fermentation of *Dialium guineense* seed before consumption is advice sable. Concentrations of flavonoids in the fermented seed (++) is higher than that of the crude seed (+). Numerous reports support their use as antioxidants or free radical scavengers [12]. Phenolic compounds are very important to plants and have multiple functions. The most important role may be in plant defense against pathogens and herbivore predators, and thus are applied in the control of human pathogenic infections. Phenolics essentially represent a host of natural antioxidants, used as nutraceuticals, and has been found to have enormous ability to combat cancer and are also thought to prevent heart ailments to an appreciable degree and sometimes are anti-inflammatory agents. These phytochemicals plays unique roles in the biologic system, hence fermented products of *D. guineense* seed should be recommended to our daily dietary intake (Table 1).

Family of Compounds	Aqeous Extract of Fermented Seed	Aqeous Extract of Unfermented Seed	Aqeous Extract of Uncooked Shell
Flavonoids	++	+	+
Saponin	+	+	+++
Tannin	_	_	_
Phenolics	+	_	+
Alkaloids	_	_	_

(-): Negative test
(+): Weakly positive
(+ +): Moderately positive
(+ + +): Strongly positive

Table 1: Phytochemical test of fermented seed, crude seed and uncooked shell of *Dialium guineense*.

Conclusion

The phytochemical analysis of *Dialium guineense* seed shows the presence of various biological secondary metabolites which plays an important role in the normal functioning of the body. Beside their nutritional and protective function, they also serve as medicinal plant. Hence, Nutritionist and pharmacologist should support research on this seed as further research will bring out the potentials in this plant for being processed for drug development and nutritional satiety.

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