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STUDIES ON THE MYCOFLORA AND NUTRITIONAL POTENTIAL OF BLACK PLUM (*VITEX DONIANA*) FRESH FRUITS IN JOS METROPOLIS

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ABSTRACT

Studies were carried out to determine the fungi associated with the surface of black plum (*Vitex doniana)* fresh fruits obtained from two markets in Jos metropolis by filtrate dilution method, using SDA as growth medium. Water obtained from washing the surface of the black plum fruits was used for serial dilution as standard mycological method. The experimental fruits were analyzed for moisture, Nitrogen Free Extract (NFE), crude fat and crude protein using standard analytical methods. The fruits were also analyzed for potassium (k), magnesium (mg), sodium (Na), calcium (Ca), phosphorus (P), iron (Fe) and Zinc (Zn) respectively using AAS machine serial no 2380. Results obtained showed that the surface of the fruits contained species of fungi which included *Fusarium oxysporum, Aspergillus niger, Mucor* sp, *Rhizopus stolonifer* and *Penicillium* sp. The experimental fruits were found to contain 66.72-66.79% moisture, 24.86-26.79% Nitrogen free extract (NFE), 2.60-2.70% Fibre, 1.15-1.25% ash, and 0.40-0.45% crude fat respectively. Elemental composition of the fruits showed 24.72mg/l potassium (k), 3.00mg/1 Magnesium (Mg), 2.91mg/1 sodium (Na), 2.86mg/1 calcium (Ca), 2.20mg/l phosphorus (P), 0.51mg/1 iron (Fe), and 0.05mg/1 zinc (Zn) respectively. A significant difference exists in the colony forming unit of the fungi species, nutrients and element compositions of the fruits obtained from the two markets ($P<0.05$). The findings of the investigation showed that *Vitex doniana* fruits are nutritionally rich and contain some of the essential elements for sustenance of life, however, the fruits must be properly washed before consumption to avoid consumption of surface fungi which are associated with poor hygiene during handling and storage. The nutritionally rich contents of the fruit can be of value to persons with health-related problems.

Keywords: *Vitex doniana*, Potential, Microflora, Markets

INTRODUCTION

Poverty and food shortage are some of the major plagues prevailing in many parts of developing countries, Nigeria inclusive. In recent times, the eastern parts of Nigeria are being confronted with the problem of insurgency due to 'Boko Haram' attacks which have led to the development of many refugee camps. These refugee camps are faced with shortage of food and nutritional inadequacies. The solution to such problems is to identify the nutrient content of several underutilized wild fruits growing in other parts of the country in order to meet the nutritional needs of such refugees. Hyson (2002) reported that adequate intake of fruits entails a consumption of at least 400mg of fruits per day. Unfortunately, in most parts of Nigeria today, fruits which are abundant in the rural areas are not accorded the importance they deserve in diets of many people. Fruits provide nutrients such as vitamins, minerals and anti-oxidants that helps the body to be free of diseases (WHO, 2003). Nonchalant attitude in consumption of fruits may probably be due to ignorance of the nutritive value, cost and difficulty in storage and distribution (Sai, 1997). Fruits in developed countries such as India, USA, etc have long been prized as a source of refreshment for their delightful flavour and aroma. Some are consumed fresh or preserved by being dried, canned, frozen, pickled or cooked with sugary syrups but the knowledge and intake of this essential aspect of nutrition is globally poor. Many people are ignorant of the fact that diets rich in fruits and vegetables could lower the risk of heart disease as reported by Harvard School of Public Health (1998).

Black plum (*Vitex doniana*) belongs to the family Verbenaceae. It is the most abundant widespread of the genus occurring in the Savannah regions. It is a deciduous forest tree of coastal wood, riverine and lowland forests, and deciduous woodland extending as high as uplands grassland. It is a medium-sized deciduous tree 8-18m high, with a heavy rounded crown and clear bole up to $5m^3$. The flowers are small, blue or violet, 3-12cm in diameter, only a few being open at a time. It is the commonest of the *Vitex* species in West Africa (Hutchinson and Dalziel,

1958). Its fruits are plum-like, sweet and edible; they are green when immature and change to dark brown when fully ripe with the pulp surrounded by a hard stone. The species had gained wide application as food and as medicine, especially the leaves. In Plateau state, it is also common in some areas. The dark brown fruits are found throughout the year either as fresh around September–November or dried around November–March being sold in local markets. *Vitex doniana* ripe fruits are usually eaten as snacks either fresh or dried because they have prune-like taste with a lint of chocolate which proves that it has nutritional substances. Children are fond of collecting the ripe fruits from the ground to eat instead of plucking it from the trees, this makes it unhygienic for consumption due to some fungal deposits on the surface of the fruits. Plucking of the fruits by women for sale and consumption with little or no knowledge of the nutritional value of the fruit also poses danger to their health due to poor handling and poor transportation which can increase microbial load on the surface of the fruits. Consumers buy the fruits and eat it directly without washing the fruits which could result to infection. Fungi are known to penetrate fruits through wounds or bruises on the surface – *Vitex doniana* is not an exception. The infection may occur during plucking using dirty hands, sticks or when children pick the fruits directly from the ground. Infection may also occur due to handling or transporting fruits in dirty bags or baskets, storage in dirty bags and other containers. This can pose health hazards to the consumers of the fruits due to toxins produced by the surface fungi. The justification of the research therefore is to scientifically identify fungi species association with the fruits, determine the nutritional and elemental compositions of the fruits in order to give adequate advice to the consumers of the fruits.

MATERIALS AND METHODS

SAMPLE COLLECTION

Two samples of *Vitex doniana* fresh fruits were purchased from two different markets of Jos metropolis (Farin gada and Terminus markets) respectively. Fifty (50) ripe dark brown fruits were purchased during the period of abundance of the fruit in Jos usually in the month of October. The fruits were then taken to the laboratory for analysis.

PREPARATION OF INOCULUM FROM FRUIT SAMPLES

The fruit samples from each location were weighed (10g each). The weighed samples were washed in 10ml distilled water each in sterilized beakers. Fruits were removed and water used in washing the fruits was collected separately in sterile universal bottles labelled accordingly based on the location of the sample. The water were kept for the cultivation of fungi (AOAC, 1990).

ISOLATION OF FUNGI IN THE FRESH FRUITS

Methods of isolation employed are those described by Dubey and Maheshawari (2002). In this method, Sabouraud dextrose agar (SDA) was prepared according to the manufacturers' instruction (45-50^{0}C) in 9ml amounts was used as blank for diluting the water. Agar media were autoclaved at 121^{0}C for 15 minutes and allowed to cool for 1 hour. After cooling, sterile streptomycin 50µg/ml was added to the Sabouraud dextrose agar in order to suppress bacterial growth. It was shaken to allow even mixture. 15ml each of the mixture was poured into six (6) petri-dishes and kept for one day to solidify. 1ml of the water used in washing the fruit was taken with a syringe and injected into the first test-tube containing 9ml of distilled water, shaken vigorously for 1 minute. After that, 1ml of the content shaken was also taken and injected into the second test-tube containing 9ml of distilled water then shaken vigorously for 1 minute. The process continued for the 3rd, 4th, 5th, 6th and 7th bottle. The whole process was repeated for the water sample from the second location. 1ml each of the 1st, 4th and 7th dilutions were taken and spread on the 3 plates of the solidified media labelled 'A' (Faringada market) and the same procedure was carried out for 'B' (Terminus market). The control was set omitting the mixtures from the two locations. The seven plates were labelled according to the locations from where they were obtained (FG1, FG2, FG3, TM1, TM2, TM3) and C respectively. The plates were then ruled horizontally and vertically to form grids behind the petri-dishes, then incubated at room temperature (27^{0}C). Plates were observed at 24 hours, 48 hours, 72 hours, 96 hours, 120 hours, 144 and 168 hours respectively. The colonies formed were counted according to where they were located between the rows and columns of the grids. The different colonies observed after the 7th day of incubation were picked using flame and inoculating needle and inoculated by streaking in newly prepared SDA plates. The sub-cultured plates were further incubated at room temperature (25^0) for 3 days after which, slides and slide covers were thoroughly wiped with cotton wool soaked in ethanol. After 30 seconds, the slides were dried and a drop of lactophenol was dropped on the slides, inoculating needle was flamed and used to pick each of the fungus from the plate in each petri-dish and placed in the drop of lactophenol on the slide. The content was properly smeared with the inoculating needle after which a cover slip was used to cover the slide. The inoculating needle was flamed for 1 minute after each streak of the fungus on a slider. Each slide was labelled according to the plate of the location. Prepared slides were then placed under compound microscope, viewed under low (x4) and high power (x40) objectives. Photomicrographs of fungi viewed were taken. The fungi viewed were compared and identified with the help of a compendium and other relevant textbooks such as Dubey and Maheshawari (2002). Morphology was well noted, control of the experiment was also observed and records made.

DETERMINATION OF NUTRITIONAL COMPOSITION OF THE FRUITS OF *VITEX DONIANA*

Methods employed are those described by the Association of Official Analytical Chemists (AOAC, 1990). Determination for moisture, crude protein, crude fibre, fat, NFE were carried out in duplicates and mean percentages were recorded.

DETERMINATION OF MOISTURE

Three (3) clean aluminium dishes with covers were dried in an oven and cooled in a dessicator with their weight accurately noted (W_1). Exactly 2kg of the *Vitex doniana* fresh fruit was weighed, introduced and spread into the dishes and their weights noted (W_2). The dishes and their contents were transferred into an air oven at 105^0C to dry tongs. The dishes were transferred into a dessicator to cool and weigh and the process was repeated to a constant weight (W_3).

$$\% \text{ Moisture} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

DETERMINATION OF PERCENTAGE CRUDE PROTEIN

The protein content was estimated from organic nitrogen content by digestion, distillation and titration. 2g of sample was weighed into a Kjeldahl flask with 25ml concentrated sulphuric acid added, a blank was also set containing all the components except the sample to be analyzed. The mixed catalyst was added to the flask, heated gently inside the flame cupboard with the flask in an inclined position; the flask was swirled occasionally after the initial vigorous reaction. Heat was increased until the liquid was clear and free from black particles. The flasks were cooled and content removed with distilled water and then transferred into a 500ml flask and 200ml distilled water was added. The nitrogen from protein and other sources are transformed into ammonium sulphate.

PROTEIN DETERMINATION

a. Distillation process
The 500ml capacity flask was connected on a heating mantle to a Markham distillation apparatus; 50ml of 2% boric acid was measured into a 500ml flask with a few drops of methyl red indicator added and placed on the receiver so that the end of the delivery tube dips just below the level of the boric acid. 100ml of sodium hydroxide solution was added to the acid, digest in the 500ml flask to make it strongly alkaline and 50ml of distilled water was added. The mixed content was boiled until 50ml distilled over.

b. Titration
50ml distillate was measured into a 100ml capacity flask and titrated against 0.01M hydrochloric acid and its titre value was noted. The blank was also titrated and the process was repeated.

% Protein was calculated as:

$$\frac{(a + b) \times 0.0014 \times 6.25 \times 100}{W}$$

6.25 = protein conversion factor
a = titre value of digested sample
b = titre value of blank
w = weight of dried sample used

DETERMINATION OF PERCENTAGE CRUDE FAT

This was based on the gravimetric extraction of fat. Exactly 2g of sample was weighed (W_1) around botton flask was weighed (W_2). The extract was transferred into the flask and the weighing dish was rinsed with petroleum ether and poured into the flask. The flask was then plugged and dropped into the extractor and heated for 6 hours to extract into the flask. When the extraction was completed, the solvent was evaporated off on the water bath and the residues dried at 100^0C to remove traces of petroleum ether. The flask and residues were cooled and weighed (W_3).

$$\% \text{ Crude fat} = \frac{W_3 - W_2}{W_1} \times 100$$

DETERMINATION OF ASH

Three dried porcelain crucibles were ignited, cooled in a dessicator, weighed (W_1). 2g of the sample was introduced into the respective crucibles and accurately weighed (W_2). They were placed in a muffle furnace, preheated to 600^0C and held at this temperature for 3 hours. After that, the crucibles were transferred into the dessicator to cool and were immediately weighed (W_3).

$$\% \text{ Ash} = \frac{W_3 - W_1}{W_2 - W_1}$$

DETERMINATION OF FIBRE

Exactly 2g of sample was weighed (W) and transferred into an extraction apparatus and extracted with petroleum ether. 200ml of 5% sulphuric acid was measured in a measuring cylinder and 20ml was poured into 100ml standard conical flask containing the sample and filtered with a reflux condenser with content mixed to produce a cream. The remaining 180ml sulphuric acid was boiled in another flask. The condenser was fitted and the liquid heated to boil for 1 minute. The reflux was continued with occasional shaking for 30 minutes to bring down any particle attached to the side. The flask was then fitted with a Whatmann No 40 filter paper. Boiling water was poured into the funnel and allowed to remain until the funnel was hot, and then the water was drained by applying suction. At the end of 30 minutes, the acid mixture was allowed to stand for 1 minute and then poured immediately into a shallow layer of hot water in the flask; the suction was then adjusted so that the filtration of bulk 200ml is completed within 10 minutes. The recovered residue was washed with boiling water until it was acid-free. It was washed backed into the original flask by means of wash bottle containing 200ml of sodium hydroxide solution at room temperature then brought to boiling point for 30 minutes and allowed to stand for 1 minute and then filtered as before. The whole insoluble material was transferred into the filter with boiling water; the residue was washed with water until the alkaline was free. It was then washed twice with ethanol, and 3 times with ether. The insoluble matter was then carefully transferred into a previously dried and weighed platinum dish (W_1) by a spatula. The platinum dish was dried in the oven at 100^0C for 1 hour, cooled in the dessicator and weighed (W_2). The residue was washed and then weighed (W_3).

$$\% \text{ Crude fibre} = \frac{(W_2 - W_1) - (W_3 - W_1)}{W} \times 100$$

DETERMINATION OF NITROGEN FREE EXTRACT (NFE)

Nitrogen free extract composed of the hexose sugar oligosaccharide, polysaccharide and some water-soluble vitamins. It was calculated as:

100 – (% crude fat + % crude protein + % crude fibre + ash)

DETERMINATION OF ELEMENTS

A working standard solution was prepared for each element from the stock solution. Standard solutions and samples were prepared in solutions containing lanthanum chloride in order to overcome the problem of chemical and ionization interferences. Working standards were then aspirated on the Atomic Absorption Spectrometer Pearkin-Elmer Machine Model with number 2380. The machine was set with calibrations to give its readings in milligrams/litre. Samples were aspirated on the Atomic Absorption Spectrometer and the concentration of each element in the sample solution was read directly from the machine in mg/l. The phosphorus concentration was computed to read mg/100g.

RESULTS

The results of the CFUs/g of fungi load are presented in Table 1 and the mean of CFUs/g are shown in Table 2 respectively. After 6 days of incubation at room temperature, *Vitex doniana* fruits obtained from Farin gada market contained more fungi species than fruits obtained from Terminus market as shown in Table 1. *Fusarium oxysporum* 5.5 x 10^5 CFUs/g recorded, followed by *Aspergillus niger* 4.9 x 10^5, followed by *Rhizopus stolonifer* 3.6 x 10^5 and *Penicillium chrysogenum* 1.12 x 10^5 respectively. *Mucor spp* was absent from all the plates labelled FG1, FG2 and FG3. Terminus market recorded *Aspergillusniger*1.45 x 10^5, *Rhizopus stolonifer* 1.43 x 10^4, *Penicillium chrysogenum* 1.44 x 10^4, *Mucor spp* 2.77 x 10^4 respectively. *Fusarium oxysporum* was absent completely. Control plates did not show the growth of fungi after the 6^{th} day of incubation. The mean colony forming units per gram in Table 2 shows more consistency in the fruits obtained from Terminus market than that obtained from Farin gada market. This difference could be due to the sanitary status of the handlers material used in storage and market environment.

The results of proximate composition of the *Vitex doniana* fresh fruits obtained from two different markets recorded moisture content, FGM (66.79%), TM (66.72%); crude protein, FGM (2.69%), TM (4.07%); crude fibre 2.60 (FGM), 2.70 (TM); crude fat, 0.45 (FGM), 0.40% (TM); % ash for FGM was 1.15 and 1.25% for TM. Nitrogen Free Extract (NFE) was 26.32% (FGM) and 24.86% (TM) respectively. The fruits were found to be rich in moisture and fairly rich in NFE and low in crude fat as shown in Table 3. The mineral analysis showed that the fruit has 24.7248mg/1 potassium, 2.9993mg/1 magnesium, 2.9144mg/1 sodium, 2.8583mg/1 calcium, 0.5155mg/l, and 0.0499mg/l iron and2.176-2.195mg 100g phosphorus respectively. The fruit is rich in potassium and a good source of calcium, magnesium, sodium and phosphorus.

Table 1: The occurrence of fungi species isolated from the surface of *Vitex doniana* fresh fruits at 7 days after incubation (CFUs/g)

Isolates	Faringada market	Terminus market
Fusarium oxysporum	5.5 x 10^5	Nil
Aspergillus niger	4.9 x 10^4	1.45 x 10^5
Mucor sp.	Nil	2.77 x 10^4
Rhizopus stolonifer	3.6 x 10^5	1.43 x 10^4
Penicillium chrysogenum	1.12 x 10^5	1.44 x 10^2
Control	Nil	Nil

Table 2: Mean occurrence for fruits collected from two different locations

Time	Location	
	Farin gada market	Terminus market
After 96 hours	4.3 x 10^5	1.66 x 10^4
120 hours	4.56 x 10^4	1.87 x 10^3
168 hours	5.5 x 10^3	2.77 x 10^3

Table 3: Percentage composition of the fruits of *Vitex doniana* fresh fruits from two locations in Jos metropolis

Nutrient	Farin gada maket (%)	Terminus market (%)
Moisture	66.79	66.72
Crude protein	2.69	4.07
Crude fibre	2.60	2.70
Crude fat	0.45	0.40
Ash content	1.15	1.25
Nitrogen Free Extract (NFE)	26.32	24.86

Table 4: Elemental composition of *Vitex doniana* fresh fruits from two markets

Element	Concentration	
	FG (mg/1)	TM (mg/1)
Calcium	2.8583	2.9612
Magnesium	2.9993	3.0914
Iron	0.5155	0.4993
Zinc	0.0499	0.5121
Sodium	2.9144	2.8913
Potassium	24.7248	23.9945
Phosphorus	2.176	2.195

DISCUSSION

The mycological quality of *Vitex doniana* fresh fruits in this study revealed that certain fungi species colonizes the matured ripe fruits obtained from Jos metropolis. Fruits from Farin gada market were more prone to fungi than those obtained from Terminus market. This variation in mycological quality of the black plum fruit could be due to handing, storage and marketing conditions, this findings is in line with what has been reported by Droby (2006); Zhu (2006), that about 20-25% of harvested fruits are decayed by pathogens during post-harvest and handling even in developed countries.

The research findings revealed the relatively high occurrence of *Fusarium oxysporum* followed by *Aspergillus niger* on the surface of *Vitex doniana* fruit, *Fusarium* species is a soil borne disease which can easily gain entrance through wounds or injuries. This is in agreement with documented literature by Prescott *et al*. (2002) who observed that *Fusarium* species were observed even on the slightest bruise on tomato skin which results into rapid fungal growth that affects the quality of tomato products including juice. These propagules of pathogenic fungi are found abundantly on the fruit surface during the maturation of fruits and are responsible for postharvest spoilage of the *Vitex doniana* fruits even though some of them like *Aspergillus niger*, *Rhizopus* and *Penicillium spp* are of interest in industrial microbiology and biotechnology. *Vitex doniana* fruits which are cherished by children as snacks either fresh or dried must be well harvested to avoid bruises, properly handled, stored and marketed to avoid fungal infections, especially their toxins which are hazardous to health. Some of the potential spoilage fungi especially *Fusarium* may originate from the ground where children usually pick the fruits without washing them before consumption. This finding is in conformity with the documented records by Jay (1996), who reported that poor personal hygienic practices could result in addition of microorganisms in food. Since the plant is a forest plant, the harvesting, handling, storage and marketing conditions and environment should be checked and sanitized in order to avoid rot during storage, because some of the propagules of fungi like *Mucor* species are known to be found in all temperatures.

The result of proximate composition of *Vitex doniana* fruits revealed high moisture content from both markets (66.79 and 66.72%). This is in agreement with what is documented by Kochhar (2005) who reported that fruits are quite watery, that the water content may be as high as 96% of the total weight. Nyam (2011) reported 27% for *Canarium schweinfurthii* fruits and 15% for olive. Moisture content is an index of the fruit's stability, quality and high yield (Joslyn, 1970). High moisture content may speed up the biodeterioration process because excess water will aid microbial spore germination. *Vitex doniana* fruits if not properly handled can easily be colonised by fungi. The *Vitex doniana* fruits were also found to be rich in Nitrogen Free Extract (NFE), 24.86 - 26.32% respectively, indicating that substantial energy can be derived from the pulp of the fruits particularly by children and during food shortage in the period of crisis in Nigeria. The fruits could, therefore, be used to feed people in refugee camps as a source of energy. The NFE is lower than that of *Ziziphus jujuba* 76.42% as reported by Suberu (2014). The *Vitex doniana* fresh fruit contains appreciable percentage of protein 2.69% and 4.07% respectively. This is comparable with that of *Ziziphus jujuba* (4.89%) as reported by Suberu (2014). This is in conformity with what was reported by Kochhar (2005) that fruits are low in proteins and fats. The protein level is comparable with that of *Canarium schweinfurthii* fruit 3.59-4.39% as reported by Nyam (2011). Proteins are necessary for the biosynthesis of new cells, enzymes, hormones, antibodies and other substances required for healthy functioning and development of the body cells as well as for protection (Cheesbrough, 1987). This finding suggests that other food rich in protein must be eaten alongside with the fruits in order to supplement the protein level of this fruit. The crude fibre 2.70% was lower compared to that of *Canarium schweinfurthii* fruit 19.37% as reported by Nyam (2011), lower than (21g/100g) crude fibre content of cotton seed reported by Garrow and James (1993). Crude fibre plays an important role in the gastrointestinal tract activities. The finding, therefore suggests that other fruits rich in crude fibre must be consumed alongside the fruit. Low crude fat (0.45%) was recorded in the study which is even lower than what was reported for Raisin (3.7g/100) by Morton (1987). The findings are in agreement with the documented record by Kochhar (2005) who reported that fruits are poor in fat. The low fat value makes *Vitex doniana* fruit useful in health-related fat problems such as heart diseases and cancer. The element composition of *Vitex doniana* showed 24.7248mg/1 potassium 2.9993mg/1 magnesium, 2.9144mg/1 sodium, 2.8583mg/1 calcium, 0.5155mg/1 iron 0.0499mg/1 zinc respectively. In a similar study carried out by Robert *et al.* (1997) on black plum in Oyo state of Nigeria, the results differ, 30.27mg/100 for calcium was recorded which is higher than what was obtained in the study, 20.10mg/100 of magnesium was recorded which is also higher than what was obtained in the study, 15.70mg/100g of potassium and 10.40mg/100g of sodium were recorded which are all higher than what was recorded in this study. The element analysis of *Vitex doniana*, even though from two different parts of the country showed that the fruits are rich in potassium and calcium and contain appreciable levels of sodium, magnesium and iron respectively. This implies that the fruits are rich and could be used to feed children and people in refugee camps in the country. The variation in

element composition could be as a result of environmental factors in different locations. This study is in conformity with the traditional use of the fruit for the treatment of anaemia. The potentials of the fruit include the use as a new tree and a root stock which can be used in agro-forestry systems. There was a significant difference in the CFUs, nutrient and element compositions of the fruit samples obtained from the two markets. The variation could possibly be due to locations, handling, storage and environmental factors.

In conclusion, the contribution of the wild underutilized fruit *Vitex doniana* in a developing country such as Nigeria which is faced with enormous problems of insurgency is vital. The fruits could be a rich source of nutrients and elements for children, lactating mothers and those with health-related problem in refugee camps.

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