JHER Vol. 20, September 2014, pp. 144-153

Determination of Phytochemical and Shelf-Life Properties of *Brachystegia Eurycoma (Achi)* and *Mucuna Flagellipes (Ukpo)*

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Abstract

This study evaluated the phytochemical properties and keeping quality (shelf-life) of *Brachystegia eurycoma* (*achi*) and *Mucuna flagellipes* (*ukpo).* The samples were grown in Nigeria and bought as sold in Nsukka local market in Enugu State, Nigeria. The seeds were processed, milled into fine flours and stored in cellophane bags to avoid contamination. Qualitative analysis was used for analyzing the phytochemicals, while determination of shelf-life was after one month of storage based on physical changes, determination of percentage Total Dissolved Solute (%TDS), viscosity, spectophotometer and microbial growth on these flours. Processed *B. eurycoma* and *M. flagellipes* flours contained many phytochemical properties at varying levels: (alkaloids + & ++; tannins +++ & - ; glycosides + & + respectively). The result also revealed that the flours of *B. eurycoma and M. flagellipes* could be stored on the counter for an average of thirty nine days. After which, were changes in their absorbance levels, %TDS, viscosity and microbial growth. The study would be useful to food sellers and consumers of these products.

Key Words: *Brachystegia eurycoma, Mucuna flagellipes*, Shelf-life, Phytochemical, Properties, Evaluation.

Introduction

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Plants produce these chemicals to protect themselves but recent researches demonstrate that they can also protect humans against diseases (Tan, Konczak, Sze & Ramzan, 2010; Vasanthi & ShrishriMal, 2012). Many traditional plant based medicines are playing an important role in health care. Phytochemicals are natural bioactive compounds found in vegetables, fruits, medicinal plants, aromatic plants, leaves, flowers and roots which act as a defense system to combat against diseases (Vasanthi & ShrishriMal, 2012).

The phytochemicals from natural products cover a diverse range of chemical entities such as polyphenols, flavonoids, steroidal saponins, organosulphur compounds and vitamins. A number of bioactive compounds generally obtained from terrestrial plants such as isoflavones, diosgenin, resveratrol, quercetin, catechin, sulforaphane, tocotrienols and carotenoids are proven to reduce the risk of cardiovascular diseases and aid in cardio-protection which is the leading cause of death (Vasanthi & ShrishriMal, 2012). The cardio-protective effects of the various phytochemicals are perhaps due to their antioxidative, antihypercholesteroemic, antiangiogenic, anti-ischemic, inhibition of platelet aggregation and anti inflammatory

activities that reduce the risk of cardiovascular disorders. The multi-faceted role of the phytochemicals is mediated by its structure-function relationship and can be considered as leads for cardiovascular drug design in future (Vasanthi & ShrishriMal, 2012).

There are more than a thousand known phytochemicals. Some of the well-known phytochemicals are lycopene in tomatoes, isoflavones in soy and flavonoids in fruits. There are many phytochemicals and each works differently (Singh, Sharma & Singh, 2014).

The easiest way to get more phytochemicals is to eat more fruits (blueberries, cranberries, cherries, and apple) and vegetables (cauliflower, cabbage, carrots, and broccoli). It is recommended that one takes daily at least 5 to 9 servings of fruits and vegetables (WHO, 2006). Fruits and vegetables are also rich in minerals, vitamins and fibre and low in saturated fat.

Phytochemicals are naturally present in many foods but it is expected that through bioengineering new plants will be developed, which will contain higher levels of the chemicals. This would make it easier to incorporate enough phytochemicals into foods (Tyagi, Ringh, Sharma, & Aggarwal, 2010).

Brachystegia eurycoma and *Mucuna flagellipes* flours possess phytochemicals and hydrocolloids properties hence they are used for thickening soups (Uzomah, & Odusanya, 2011) and are good source of soluble fibres, and have the ability to replace fat in processed food and so have been shown to be essential component in low fat and fat-free products (Aman, 2006). Hydrocolloids are mainly from plant materials. Two of such plants are *Brachystegia eurycoma* and *Detarium microcarpum* known locally in south-eastern part of Nigeria as "*Achi" and "Ofor*" respectively. *Brachystegia eurycoma* belongs to the family of Fabaceae, sub-family Caesalpinopdeae and Tribus detariae (USDA, 2006). It is one of the lesser-known legumes used in Nigerian communities as soup thickener. *Mucuna flagellipes* Bak, leguminosae, is one of the popular medicinal legumes in India and its constituent of more than 200 indigenous drug formulations. These legumes are great sources of nutrition because they carry the embryonic necessities for starting a new plant. They are high in protein, fibres and phytochemicals.

In Nigeria especially, adults suffer mostly from high cholesterol level in the bloods example hypercholesterolaemia which can lead to atherosclerosis (Nwaneli, 2010). These legumes (*Brachystegia eurycoma and Mucuna flagellipes*) can help to reduce cholesterol level and glucose response in diabetes (Obun, 2013).

The lesser-known legumes like *B. eurycoma* might be toxic and contain anti-nutrients unless they are properly processed prior to use to destroy these toxicants and antinutrients (Obun, 2013). There are some locally processed legumes sold in the Nigerian markets that may have exceeded their shelf-lives. This subsequently reduces their nutritive values and encouraged the growth of micro-organisms, which make them harmful when consumed by man (Okwu, Achar & Sharma, 2010). There is no clear evidence on how preservation of these legumes can improve their shelf-life. It was on this premise this study therefore determined to evaluate the phytochemical properties and shelflives of *Brachystegia eurycoma* and *Mucuna flagellipes.* Phytochemical analysis is a procedure used to determine the

bioactive properties in *Brachystegia eurycoma and Mucuna flagellipes* flours. This was carried out by the method described by Higuchi & Brocham (1973).

Objectives of the study:

The major objective of the study is to evaluate the phytochemical properties and shelflives of *Brachystegia eurycoma* and *Mucuna flagellipes.* Specifically, the study determined:

1. the phytochemical properties of *Brachystegia eurycoma* and *Mucuna flagellipes*
2. the keeping qualities (shelflives) of *Brachystegia eurycoma* and *Mucuna flagellipes.*

Materials and Methods:

***Sample Collection and Pretreatment*:** The mature dry seeds *B. eurycoma* and *M. flagellipes* were bought as sold in Nsukka local market in Enugu state, Nigeria. The seeds were sorted to remove debris and unviable ones and stored in cellophane bags to avoid contamination.

***Pre-de-hulling Treatment*:** The traditional methods of processing as described by (Ene-Obong & Carnovalue, 1982) were adopted in the treatment of the seeds. The seeds (i.e. *Brachystegia eurycoma and Mucuna flagellipes)* were sorted and roasted for 10-15 minutes; then soaked immediately for one hour in cool clean water after which the cotyledons were soaked overnight (6hrs) in cool clean water. The water was drained off and the cotyledons were sundried, milled and sieved into fine powder.

Phytochemical determination (Qualitative Assay) Phytochemical analysis is a procedure used to determine the bioactive properties in *Brachystegia eurycoma and Mucuna flagellipes* flours. This was carried out by the method described by Higuchi & Brocham (1973) as follows:

- *Test for Saponins*: Froth test for saponins was used. One gram of the sample was weighted into a conical flask in which 10ml of sterile distilled water was added and boiled for 5minutes. The mixture was filtered and 2.5ml of the filtrates added to 10ml of sterile distilled water in a test tube. The test tube was stopped and shaken vigorously for about 30 seconds. It was then allowed to stand for half an hour. Honey comb froth indicates the presence of Saponin.
- *Test for Tannins*: Three grammes of the powered sample were boiled in 50ml distilled water for 3 minutes on a hot plate. The mixture was filtered and a portion of the filtrate diluted with sterile distilled water in a ratio of 1:4 and 3drops of 10% ferric chloride solution added. A blue or green colour indicated the presence of tannins.
- *Test for Glycosides*: A 25mls of dilute sulphuric acid was added to 5mls of extract (obtained as) in test for tannin in a test tube and boiled for 15 minutes, cooled and neutralized with 10% NaOH, then 5mls of fehling solution A and B added. A brick red precipitate of reducing sugar indicated the presence of glycosides.
- *Test for Alkaloids*: A 2.0ml extract (as obtained in test for Tannin) was measured in a test tube to which picric acid solution was added. The formation of orange colouration indicated the presence of alkaloids.
- *Test for Volatile Oils*: A 2.0mls of extract solution (obtained as in for tannin) was shaken with 0.1ml dilute sodium hydroxide and a small quantity of dilute HCl. A white precipitate would signify the presence of volatile oils.

- *Test for Flavonoids*: Ten millimeters of ethylacetate was added to about 0.2g of the extract and heated on a water bath for 3minutes. The mixture was cooled, and the filtrate used for the following test.
- *Ammonium Test*: About 4mls of filtrate was shaken with 1ml of dilute ammonia solution. The layers were allowed to separate and the yellow colour in the ammonical layers were allowed to separate and the yellow colour in the ammonical layer indicate the presence of flavonoids.
- *One percent Aluminium Chloride solution test*: Another 4ml portion of the filtrate was shaken with 1ml of 1% Aluminum chloride solution. The layers were allowed to separate. A yellow colour in the aluminum chloride layer indicates the presence of flavonoid.
- *Test for Steroids*: About 9mls of ethanol were added to 1g of the extract and the refluxing for a few minutes and filtered. The filtrate was concentrate to 2.5mls or boiling bath, about 5ml of hot water were added. The mixture was allowed to stand for 1hour and the waxy matter filtered off. The filtrate was extracted with 2.5ml of chloroform using separating funnel. To 0.5ml of the chloroform extracted in a test tube was carefully added 1ml of concentrated sulphuric acid to form a lower layer. A reddish brown interface shows the presence of steroids.
- *Test for Acidic Compounds*: About 0.1g of the extract was placed in a clear dry test tube and sufficient water added. This was warmed in a hot water bath and then cooled. A piece of water-wetted litmus paper was dipped into the filtrate and colour change on the litmus paper observed.

Determination of Shelf life properties

- Packaging, storage and preparation of samples:

The samples were packed in polythene packets weighing 10g each. All the packets were kept at room temperature and stored for a period of one month. Each of the stored samples was observed and examined daily for physical organoleptical changes, chemical changes and microbial growth examination, so appropriate data were collected on each sample for a period of one month.

- Shelf- life properties of *Brachystegia Eurycoma* (*achi*) and *Mucuna flagellipes* (*ukpo)* were determined using five parameters as follows:

i. Physical Properties/Changes (organoleptics):

Here, the determination of the flours' chemical and physical changes between 1-10 days was done. Food spoilage is a natural phenomenon. It occurs at various rates depending on the temperature, chemical reactions, kind of food, kind of microorganism present, moisture content, packaging materials used, food additives and method of preservation and so on. These can bring about undesirable changes in the colour, flavour, odour, taste and texture.

ii. Determination of percentage (%) Total Dissolved Solute (TDS):

The percentage total dissolved solute was calculated by dividing weight of the residue by weight of the filtrate and multiplied by hundred.

The weight of a container (crucible) was taken and recorded= w_1, the weight of crucible plus 5mls of the sample= w_2, $w_2 - w_1 = w_3$ (weight of filtrate).

Evaporated filtrate and weighed = w_4

$W_3 - W_4$ = weight of solid in the filtrate (residue)

$$\%TDS = \frac{\text{weight of residue}}{\text{Weight of filtrate}} \times \frac{100}{1}$$

iii. Determination of Viscosity**:** The viscosities of the samples were determined using a viscometer. The method of Sathe and Salunkhe (1981) was adopted in determining the viscosity of the flour samples. Sample dispersion 2.0% (w/v) was prepared with distilled water at room temperature (28±2°C) under continuous stirring by using British magnetic stirrer. The viscosity of the hydrated dispersion was measured at 28±2°C using the NDJ-8S digital viscosity. Measurements were on 2% (w/v) on dispersion of each flour sample of constant time intervals of 2 hours shear-rate (30/m).

iv. Spectrophotometer: One solution of each of the samples was scanned for the determination of the wavelength for which it was read at: for the *Ukpo* (*Mucuna flagellipes*) flour, the wavelength was 475nm, while that of *Brachystegia eurycoma* flour was 365nm.

Ten samples of different concentration of both preparations were made. The concentration was in specific combination ratio of the sample and distilled water, ranging for 0.1ml to 1ml, i.e. 0.1ml of water: 0.9ml of sample i.e. 1:9, 2:8, 3:7; 4:6, 5:5, 6:4, 7:3, 8:2, 9:1 and 1ml of sample.

v. Microscopic Examination: A drop of the 1% solution of the samples was dropped on a clean, grease-free microscope slide and covered with a cover-slip and viewed under microscope.

All phytochemical analysis and determination of shelf life of the processed seed flours were carried out in the Department of Pharmaceutical laboratory, University of Nigeria, Nsukka.

Results

The following findings were:

- Phytochemical Properties of *Brachystegia eurycoma* and *Mucuna flagellipes*

Table 1: Phytochemical properties of *Brachystegia eurycoma* and *Mucuna flagellipes*

Secondary Metabolite	Relative Aboundance	
	B. eurycoma	*M. flagellipes*
Alkaloids	+	++
Flavonoids	-	-
Glycosides	+	+
Steroids	+	-
Saponins	+	-
Tannins	+++	-
Terpenoids	+	-
Volatile Oils	++	++
Acidics compounds	+	++
Reducing sugar	++	+
Renins	+	+

Keys: - Absent; + Present in small quantity; ++ Moderately Present; +++ Abundantly present

Table 1 presents the phytochemical properties of *Brachystegia eurycoma* and *Mucuna flagellipes*. Alkaloids :- The level of alkaloids in *Brachystegia eurycoma* was lower in concentration (+) than the level of alkaloids in *Mucuna flagellipes* (++).

Glycosides: The level of glycosides in *Brachystegia eurycoma* was the same with *Mucuna flagellipes* though they had low concentration (+).

Steroids: The level of steroids in *Brachystegia eurycoma* was in low concentration (+) but it was absent in *Mucuna flagellipes*.

Saponins: The level of saponins in *Brachystegia eurycoma* was in low concentration (+) and absent in *Mucuna flagellipes*.

Volatile Oil: The level of volatile oil in *Brachystegia eurycoma* was the same with *Mucuna flagellipes* though it was in low concentration (+).

Acid compounds: The level of acid compounds in *Mucuna flagellipes* was moderate in concentration (++) and that of *Brachystegia eurycoma* was in low concentration (+).

Reducing sugar: The level of reducing sugar in *Brachystegia eurycoma* was moderate in concentration (++) and that of *Mucuna flagellipes* was in low concentration (+).

a. Determination of Shelf-life

a. Physical properties/changes were observed after one month of storage, between 1-10 days.

For *Brachystegia eurycoma* (1st to 10th Day):

1st day = clear particles settling at the base of the container.

2nd – 3rd day = Formation of supernant; floating particle.

4th – 5th = Suspension observed (reconstitutes after vigorous shaking)

6th – 7th day = Suspension reduced (shaking reduces suspension and reconstitutes poorly), slight change in smell; stated having odour, medium becomes cloudy.

8th - 10th day = A dense pungent odour perceived, suspended particle become cloudy, cloudy layer of precipitate disappeared.

For *Mucuna flagellipes* (1st to 10th Day):

1st day = Sample clear with particles sticking at the base and wall of container floating.

2nd – 3rd day = Particles and precipitate floating freely, forming of supernant; floating particle.

4th – 5th day = Clear separation of medium and precipitate.

6th – 7th day = Medium cloudy, particles settles fast after shaking.

8th – 10th day = Intense pungent odour perceived, suspended particle become cloudy and whitish particles developed.

b. Determination of Percentage (%) Total Dissolved Solute (TDS)

For the *Brachystegia eurycoma (Be)*(first day)

Weight of container = 60.81

Weight of container + 5mls of BE = 65.70

5mls of sample *Be* = 4.90

After heating; weight of container + Residue = 60.92

Residue = 0.11

%TDS = 2.24%

NB;- For the 2nd to 9th day, there was no significant changes.

For the *Mucuna flagellipes* (*Mf*) (first day)

Weight of container = 65.50

Weight of container + 5mls of *Mf* = 70.30

5mls of sample *Mf* = 4.80

After heating; weight of container + Residue = 66.38

Residue = 0.88

%TDS = 18.33%
NB; For the 2nd to 9th day, there were no significant changes.

On the *Brachystegia eurycoma (Be)* (tenth day)
Weight of container = 61.35
Weight of container + 5mls of *Be* = 72.70.
5mls of sample A = 11.35
%TDS = 2.0%.

On the *Mucuna flagellipes (Mf)*(tenth day)
Weight of container = 65.35
Weight of container + 5mls of *Mf* = 70.70
5mls of sample A = 5.35
After heating: weight of container + residue = 66.0
Residue = 0.65
%TDS = 12.14%

Using the %TDS: The TDS value for *Brachystegia eurycoma* on the 1st day (2.24%) and on the tenth day (2.0%), while that of *Mucuna flagellipes* on the 1st day (18.33%) and on tenth day (12.14%) showed that there was a significant decrease in its TDS for the later.

c. Determination of Viscosity
For *Brachystegia eurycoma*
1st day (when freshly prepared) = 1.06.
10th day (after it was stored) = 1.09.
For *Mucuna flagellipes*
1st day (freshly prepared) = 6.42.
10th day (after it was stored) = 9.0

d. Viscosity Determination: The result showed that the seed *Brachystegia eurycoma* had a viscosity of 1.06 and 1.09 on the 1st and 10th day respectively while that of *Mucuna flagellipes s* had a viscosity of 6.42 and 9.0 on the 1st and 10th day respectively. This showed that within these periods, the prepared samples from the seeds became thicker in its flow but it was more with *Mucuna flagellipes* predisposing them to microbial activity.

Spectrophotometer

Table 2: Absorbance of *Brachystegia eurycoma* and *Mucuna flagellipes* for different concentration at different days of analysis.

Concentration	Absorbance (1st day)		Absorbance (10th day)	
	BE	MF	BE	MF
0.1	0.222	0.063	0.796	0.38
0.2	0.393	0.066	1.124	0.522
0.3	0.501	0.075	1.552	0.727
0.4	0.679	0.085	1.57	0.808
0.5	0.772	0.098	1.738	0.941
0.6	0.804	0.133	1.882	1.176
0.7	0.946	0.139	2.165	1.255
0.8	1.098	0.157	2.194	1.310
0.9	1.232	0.166	2.221	1.466
1.0	1.456	0.176	2.450	1.648

BE- *Brachystegia eurycoma* MF- *Mucuna flagellipes*

Table 2 shows that there were changes in absorbance level for each of the samples (*Be* and *Mf*) between the 1st and the 10th day. The result showed that for seed flours, *B. eurycoma* and *M. flagellipes,* on the 1st day and the 10th day respectively, the rate of absorbance i.e. light intensity decreased based on their various concentrations.

Based on the results of the spectrophotometer analysis, it clearly stated that there was a significant degradation of seeds between the 1st day and the 10th day.

e. Microscopic examination:

For *Brachystegia eurycoma*

The first day: Nothing was seen indicating of micro-organism

The tenth day: plant – like growth was present.

For *Mucuna flagellipes*

The first day: no micro- organism present.

The tenth day: Microbial growth present.

Discussion

The result of the analysis showed that *Mucuna flagellipes* contained little more alkaloids than *Brachystegia eurycoma.* This study agreed with the work done by Uhegbu, Onuwuchekwa, Iweala, and Kanu (2009) who reported quantitatively the presence of alkaloid in dehulled seeds of *B. eurycoma* and D. *microcarpum respectively*. According to Papp *et al.* (2001), they reported that *Mucuna flagellipes* is more toxic and have more bitter taste than *Brachystegia eurycoma* because of the level of alkaloid it contained. The amazing effect of these alkaloids on humans has led to the development of powerful pain-killer medications, spiritual drugs, and serious addictions by people who are ignorant of the properties of these powerful chemicals (*Herbal powers.com, 2011*). *Mucuna flagellipes* is a reputed remedy of Ayurveda in nervous and sexual diseases. Traditionally, Mucuna *flagellipes* is commonly used as carminative, hypertensive and hypoglycemic agent. *Mucuna flagellipes* has been found to contain L-Dopa, 40 mg/g of the plant. The plant/seeds contain the bioactive alkaloids mucunine, mucunadine, mucuadinine, pruriendine and nicotine, besides B-sitosterol, glutathione, lecithin, oils, venolic and gallic acids. Studies in experimental model show L-Dopa also helps in the reduction of cholesterol and blood sugar levels.

From the result the tannin level of *Brachystegia eurycoma* was aboundantly present but absent in *Mucuna flagellipes*. The concentration may make the excessive consumption of *Brachystegia eurycoma* to inhibit the absorption of other minerals such as iron, which if prolonged may lead to anemia (Afsana, Shiga, Ishizuka & Hare, 2003); this agrees with Uhegbu *et al*. (2009) who reported that *B. eurycoma* contains tannins, with the undehulled seeds having a significantly ($P < 0.05$) higher content than the dehulled. The presence of tannin explains the darkening of soups within few days of its preparation, which mothers complain of. Tannin being complex phenolic polymer is capable of enzymatic oxidation, hence the pigmentation or browning of foods that contain tannin as seen in some yam species which browns when cut. Tannin has stringent properties, it hasten the healing of wounds and inflammation of mucous membranes (Agoha, 1979; Uhegbu *et al*., 2009).

Saponins were observed present in a small quantity in *B. eurycoma* which is in agreement with Uhegbu *et al.* (2009). However, there seems to be little or no danger with the concentrations of saponins present, as the saponins ingested are destroyed in the gastro intestinal tract, hence very little is absorbed into the system if any.

A moderate presence of reducing sugar was observed in the *B. eurycoma* than *M. flagellipes*, which could contribute to reducing **blood glucose** levels in diabetic patients. Medicinal plants are of great importance to the health of individual and

communities (Edeoga, 2005), also stated that the medicinal value of some plants lies in some chemical active substances that produce a definite physiological action on the human body.

Mucuna flagellipes has more acidic compounds than *Brachystegia eurycoma* showing that as thickeners, *Brachystegia eurycoma* could be preferred in use for preparing soups for ulcer patients because of its lower acidic content.

The Shelf Life or Sell by date of a food product is defined as the time between the production and packaging of a product and the time at which it becomes unacceptable to the customer. Shelf Life is a combined measure of both food quality and food safety of a product and therefore the microbial testing during shelf life trials must be designed to demonstrate both of these measures (www.ils-limited.co.uk/food-division/microbiology/shelf-life, 2014). The shelf-life determination revealed that for *Brachystegia eurycoma*, dense pungent odour was observed, a suspended particle became cloudy and the cloudy layer of precipitate disappeared while *Mucuna flagellipes,* between the 8th and 10th day of storage period, had intense pungent odour which was perceived, suspended particles became cloudy and whitish particles developed. Again there was microbial presence on the 10th day. This study agrees with work done by Okwu *et al*. (2010) in which samples of food thickeners obtained from Nigerian market revealed a high incidence and an alarming levels of aflatoxin; and that the presence of aflatoxin in food thickeners poses a potential health threat to consumers.

Conclusion

The study showed that the seeds, *Brachystegia eurycoma* and *Mucuna flagellipes* contain varying levels of phytochemicals. It also showed that the keeping quality of these flours do not exceed the average of one month unrefrigerated. The % Total dissolved Solute had significant changes at the 2nd to 9th day for *Brachystegia eurycoma* while *Mucuna flagellipes s* had no significant changes at those periods. Viscosity determination showed that the prepared samples became thicker in its flow but was more with *Mucuna flagellipes* predisposing them to microbial activities on the 1st and 10th day. Spectrophotometer showed changes in absorbance level for each sample between 1st and 10th day, while microscopic examination\ showed microbial growth for both flours on the 10th day.

Recommendations

1. More work should be done in prolonging the shelf-life of *Brachystegia eurycoma* and *Mucuna flagellipes* flours.
2. There is need for nutrition education to reach the rural and urban populace for promotion of the consumption of *Brachystegia eurycoma*.

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