Contributory and synthetic portrayal of *Moringa oleifera* Lam root starch as a mechanical biomaterial

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Abstract

Moringa oleifera Lam. (Moringaceae family) is a deciduous plant with tuber-like root at the earlier stage. Starch was isolated from the young tuber of the plant and examined instrumentally for its functional groups, X-ray diffractometer (XRD) profile, elemental analysis and antioxidant activities. The Fourier transform infrared spectroscopy analysis shows that peaks at 3465.23 to 3577.11 cm^{-1} represent OH stretch of alcohol; 3385.18 cm^{-1} represents O-H band of carboxylic acid; 3116.11 cm^{-1} represents =CH stretch of alkenes; 1647.26 cm^{-1} represents C=C stretch of alkenes and 1023.27 cm^{-1} represents C-O stretch. The result of elemental analysis revealed that the starch granules contained: Fe (430.19 ppm), Cu (0.055 ppm) and Zn (0.19 ppm). XRD pattern revealed that the starch granules is an amorphous material and contained iron complex ($C_{24}H_{16}FeN_{10}$). The radical scavenging activity of the plant extract against DPPH (Sigma-Aldrich) was determined by UV-visible spectrophotometer at 517 nm. The result showed higher absorbance of the reaction mixture which indicated lower free radical scavenging activity and the degree of increment in absorbance measurement is indicative of the radical scavenging power of the extract, hence the anti-oxidant capacity and scavenging activity of the starch suspension revealed that it has a very low activity and percentage inhibition when compared with the ascorbic acid standard.

Keywords: *Moringa oleifera*, phytochemical screening, antioxidant activity, X-ray diffractometer.

INTRODUCTION

Moringa oleifera Lam. (Moringaceae) is one of the fourteen species of the family Moringaceae, native to India, Africa, Arabia, Southeast Asia, South America and Caribbean Islands (Iqbal et al., 2006). It is commonly called Ben oil tree and locally known as *Zogeli* among the Hausa speaking tribe in Nigeria. Almost every part of *M. oleifera* is useful to man and as forage for livestock.

Recently, starch granules were isolated from the young *M. oleifera* root and physicochemical properties determined (Fagbohun et al., 2013). Some lesser known and unconventional native starch could be good sources of nutrients and industrial biomaterial but the lack of data on the chemical composition and properties of such plants has limited the prospects for their utilization (Viano

et al., 1995). Starch is also one of the most widely used biomaterial in the food, textile, cosmetics, plastics, adhesives, paper and pharmaceutical industries. The diverse industrial usage of starch is based on its availability at low cost, high calorific value and inherent excellent physicochemical properties (Omojola et al., 2010). The versatility of starch in industrial applications is clearly defined by its physicochemical properties; therefore, a thorough evaluation of the necessary parameters is important in elucidating its industrial uses.

As a result of the competing demands for starch as food, pharmaceutical and industrial uses coupled with the need to attain self sufficiency in starch production, there is a need to find other high yield sources different from cassava, maize and potato (Gebre-Mariam et al., 2006). Little or no work appears to have been done on the isolation and instrumental characterization of starch from *M. oleifera* Lam. root. Therefore, the objective of the present study is to isolate starch from *M. oleifera* root and characterize by Fourier Transform Infrared Spectrophotometer (FTIR), Atomic Absorption Spectrophotometer (AAS), High resolution X-ray Diffractometer (XRD), UV/Visible Spectrophotometer, and Phytochemical Screening.

MATERIALS AND METHODS

Sampling

Two years old *M. oleifera* was harvested using cutlass from medicinal and botanical gardens located in Sheda Science and Technology Complex (SHESTCO) near Kwali, Abuja (8° 21' N; 6° 25' E) in 2013 and was duly identified in Chemistry Advanced Laboratory of the same organization. Analytical grade reagents were obtained from Chemistry Advanced Laboratory, Sheda Science and Technology Complex, Abuja Nigeria.

Starch isolation

The method of Loss et al. (2012) was adapted with slight modification. Briefly, the fresh roots were peeled and washed thoroughly with water to remove dirt. Peeled tubers (0.944 kg) were chopped into small pieces and wet milled into slurry using a grater and laboratory blender. The paste was dispersed in 3 L of distilled water and filtered through muslin cloth. The filtrate was allowed to stand 24 h. The supernatant was carefully decanted and the mucilage scraped off. This process was repeated three times continuously until a pure starch granule was obtained. The resulting starch was dried in the sun and further dried at 60°C in a hot air oven, pulverized, weighed and stored in sample bottles for instrumental and chemical analysis. The percentage yield was calculated as:

$$\% \text{ Percentage yield} = \frac{\text{Weight of starch granules isolated}}{\text{Weight of peeled tuber chopped}} \times 100 \quad (1)$$

Phytochemical screening

Phytochemical screening was carried out on the starch using methods previously described by Harborne (1973), Trease and Evans (1989) and Sofowora (1993).

Mineral analysis

The following minerals were determined: calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), nickel (Ni), chromium (Cr) and cobalt (Co) using the atomic absorption spectrophotometer (AAS-Shimadzu Japan), as described by the methods of the Association of Official Analytical Chemists (AOAC) (1990). All the determinations were done in duplicates. The concentrations of the mineral content of the starch granule were reported in milligram per gram (mg/g).

Determination of antioxidant activity

The radical scavenging activities of the plant extracts against 2, 2-diphenyl-1-picrylhydrazyl (DPPH) were determined by UV-visible spectrophotometer CECIL, England at 517 nm. Radical scavenging activity was measured by a slightly modified method previously described (Ayoola et al., 2008; Brand-Williams et al., 1995). The following concentrations of the extract were prepared; 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, and 5.0 mg/ml in methanol (Analar grade). Vitamin C (ascorbic acid) was used as the antioxidant standard at concentrations of 0.05, 0.1, 0.2, 0.5, 1.0, 2.0 and 5.0 mg/ml. 1 ml of the extract was placed in a test tube and 3 ml of methanol was added, followed by 0.5 ml of 1 mM DPPH in methanol and thereafter the decrease in absorption was measured on a UV-visible double beam spectrophotometer, CECIL England, ten minutes later. A blank/control solution was prepared containing the same amount of methanol and DPPH. The actual decrease in absorption was measured against that of the control. All test and analysis were run in duplicates and the results obtained were averaged. The radical scavenging activity (RSA) was calculated as the percentage inhibition of DPPH discolouration using the equation below:

$$\% \text{ Inhibition} = \frac{A_b - A_a}{A_b} \times 100 \quad (2)$$

Where A_b is the absorption of the blank sample (without the extract) and A_a is the absorption of the extract.

FT-IR analysis

The FT-IR spectra were obtained using FT-IR-8400S Fourier transform infrared spectrophotometer Shimadzu Japan. The spectra were recorded in transmission mode from 4,000 to 500 cm^{-1} (mid-infrared region) at a resolution of 0.44 cm^{-1}. The sample was mixed with KBr and compressed (1:100, w/w) before acquisition and the background value from pure KBr was acquired before the sample was scanned.

XRD analysis

In order to further characterize *M. oleifera* root starch, the XRD profile was obtained using high resolution X-ray diffractometer X pert Pro Analytical (Holland) set at the following operating conditions: anode material-copper, original K-Alpha1 wave lenght: 1.54060 Å, used K-Alpha 1 wavelength-1.54060 Å, original K-Alpha2 wavelenght-1.54443 Å, original K-Beta wavelength-1.39225 Å, specimen length 10.00 mm K-A2/K-A1 ratio 0.500, distance

Table 1. The result of Antioxidant activity *of M.oleifera* root starch.

Concentration (mg/ml)	% Inhibition of *Moringa* starch	% Inhibition of Vit. C
5.000	33.86	85.00
3.000	26.06	91.41
2.000	26.09	91.50
1.000	13.13	91.32
0.500	14.61	90.47
0.100	2.080	90.19
0.050	41.13	90.12

Table 2. Phytochemical screening of *M.oleifera root* starch.

Parameter	Result
Carbohydrate	+
Terpenoids	-
Flavonoids	+
Resin	-
Saponin	+
Alkaloids	-
Sterols	-
Glycosides	+
Tanin	-
Cardiac glycoside	-
Tanin	-
Cardiac glycoside	-
Anthrancene	-
Plobatanin	-
Phenol	-
Volatile oil	-
Carbohydrate	+

focus-diverg. Slit-100 mm; step size°2Th -0.0040, scan step time - 8.8900 s. The samples were incubated in a chamber at 100% RH for 24 h and then packed tightly in a circular aluminum cell. The samples were exposed to the X-ray beam from an X-ray generator running continuously at 40 kV and 30 mA and scanning regions of the diffraction angle, 2θ, were 9.9991 to 40.9831°, which covered most of the significant diffraction peaks of the starch crystallites. Duplicate measurements were made at ambient temperature. Radiation was detected with a proportional detector. The XRD pattern was generated from the data acquired using Microsoft Office Excel 2007 application.

RESULTS AND DISCUSSION

Yield and nature of the starch

The starch obtained was found to be a pure white, crystalline, non-hygroscopic powder with a yield of about 53%. The yield is therefore comparable with previous work on some native starch such as *Icacina trichantha* (76.8 %) and *Anchomanes difformis* (21%) (Omojola et al., 2010).

Antioxidant analysis

The DPPH test provides information on the reactivity of the test compounds with stable free radical and it gives a strong absorption band at 517 nm in visible region. When the odd electron becomes paired off in the presence of a free radical scavenger, the absorption reduces and the DPPH solution was decolorized as the colour changed from deep violet to light yellow. The starch extract is not a good antioxidant because the colour did not change even at lower concentrations. It also showed higher absorbance of the reaction mixture indicated lower free radical scavenging activity and the degree of increment in absorbance measurement is an indication of the radical scavenging power of the extract as shown in Table 1.

Flavonoids and tannins are phenolic compounds and plant phenol is a major group of compounds that act as primary antioxidant or free radical scavengers (Polterait, 1997). The biological functions of flavonoids include protection against allergies, inflammation, free radicals scavenging platelets aggregation, microbes, ulcers, hepatoxins, viruses and tumors (Ayoola et al., 2008). It is worthy to note that these phytochemicals responsible for the mentioned activities must have been lost during the starch processing as revealed in Table 2.

Infrared analysis

FT-IR is a powerful technique for elucidation of structural changes in samples, with the ability of discovering differences not seen by certain other techniques because it has a unique region known as the finger print region where the position and intensity of bands is specific for every polysaccharide. The FT-IR evaluation of starches in four main regions helps in the successive interpretation of the key bands. These regions are as follows: below 800 cm^{-1}, 800 to 1500 cm^{-1} (the finger print region), the region between 2800 and 3000 cm^{-1} (C-H stretch region) and finally the region between 3000 and 3600 cm^{-1} (O-H stretch region). The FT-IR spectrum of the starch showed the following bands as indicated in the Table 4.

Table 3. The results of elemental analysis of *M. oleifera* root starch.

Metal	Copper (Cu)	Nickel (Ni)	Chromium (Cr)	Zinc (Zn)	Calcium (Ca)	Iron (Fe)	Manganese (Mn)
Conc. (mg/g)	0.002755	BDL	0.02	0.01	BDL	21.51	BDL

BDL: Below detection limit.

Table 4. FTIR analysis result of *M. oleifera* Lam root starch.

S/no.	Peak	Intensity	Corr. intensity	Base (H)	Base (L)	Area	Corr. area
1	621.1	30.272	9.321	678	339.48	139.468	37.891
2	751.3	28.509	5.838	831.35	678.97	76.091	5.414
3	849.67	37.358	0.112	872.82	832.31	17.295	0.029
4	1023.27	23.611	2.872	1057.99	873.78	100.319	4.323
5	1106.21	23.565	2.301	1267.27	1058.96	118.903	3.881
6	1357.93	25.504	12.302	1562.39	1268.24	143.42	27.321
7	1647.26	35.48	17.516	1856.55	1563.36	89.374	14.154
8	2143.95	56.447	3.067	2313.69	1857.51	106.344	4.298
9	2916.47	24.657	9.082	3007.12	2313.69	261.573	12.862
10	3116.11	24.583	0.615	3129.61	3008.09	69.782	1.123
11	3177.83	24.133	0.604	3231.84	3130.57	61.958	0.538
12	3277.17	24.625	0.35	3345.64	3232.8	68.3	0.359
13	3465.23	24.88	0.345	3440.16	3346.61	56.202	0.281
14	3465.23	25.3	0.404	3536.6	3441.12	56.195	0.343
15	3577.11	26.347	2.834	3897.3	3537.57	142.823	2.56
16	4002.43	49.548	0.891	4181.81	3898.27	84.896	1.092
17	4661.14	50.299	0.419	4700.68	4521.29	52.811	0.343

3116.11 to 3577.11 cm^{-1} represent (O-H stretch); 2916.11 cm^{-1} represent (C-H stretch); the absorption peak at 3465.23 to 3577.11 cm^{-1} represent OH stretch of alcohol, 3385.18 cm^{-1} represent OH broad of carboxylic acid and phenol; 3116.11 cm^{-1} represent =CH stretch of alkenes; 1647.26 cm^{-1} represent C=C of stretch of alkenes; 1023.27 cm^{-1} represent C-O stretch. Aromatic absorption is absent in the diagnostic region (1600 and 1500 cm^{-1}) but medium intensity absorption at 1644 cm^{-1} is due to water absorbed in the amorphous region of the starch. 1357.95 cm^{-1} represent CH_2OH.

The absorption peak at 1106.21 cm^{-1} represents coupling mode of C-C and C- O stretching vibrations while the band at 1106.21 cm^{-1} represent C-O-H bending vibration. The absorption at 849.67 cm^{-1} vibration is typical of the system (C-O-C), skeletal mode vibration of α-1,4-glycosidic linkage while 621.1 cm^{-1} represent the skeletal mode of pyranose ring; 1106.21 cm^{-1} represents C-O and C-C stretch. The spectra of starch show complex vibrational modes at low numbers below 800 cm^{-1} due to skeletal vibration of the glucose pyranose ring (Sekkal et al., 1995). The absorption peak at 849.67 cm^{-1} represents C-H out of place of aromatics (Brandon, 2012). The region between 800 and 1500 cm^{-1} (finger print region) is the empirical proof of identity charac-teristics of the sample identity and the pattern of vibration

and band location is unique for starch sample. Although it provides complex and overlapping spectra at this region, making the exact assignment of band difficult. But the IR spectrum of polysaccharides in this region originates from the vibrational state of its monomer glucose (Cerna et al., 2003). Therefore information obtained from glucose spectra is used in the assignments of wave numbers corresponding to the vibrational mode of starch.

Since starches exhibit very similar spectra characte-ristics with glucose in this region. Therefore in this study, the major bands below 800 cm^{-1} from 625 -581 cm^{-1} and minor bands between 560 and 400 cm^{-1} in the FTIR spectra of the starch was attributed to the skeletal modes of the glucose pyranose ring, since starch exhibit very similar spectra characteristics with glucose in this region (Kemas et al., 2012).

Elemental analysis

As shown in Table 3, it was observed that *M. olifera* starch contain the following elements in their various concentrations: Cu (0.002755 mg/g), Cr (0.02 mg/g), Zn (0.01 mg/g) and Fe (21.51 mg/g).The trace metals like copper, iron and zinc are essential cofactors for a number of biological processes, including mitochondrial oxidative

Table 5. Peak list.

Pos.[°2Th]	Height [cm]	FWHM [°2Th]	d-spacing [Å]	Rel.Int.[%]
11.3175	52.61	0.4408	7.81860	7.26
15.1251	272.06	0.6298	5.85781	37.55
17.1776	724.55	0.2204	5.16223	100.00
19.6726	46.92	0.5038	4.51279	6.48
22.2002	197.08	0.6298	4.00437	27.20
24.3275	189.65	0.3149	3.65883	26.18
26.2960	69.56	0.7557	3.38922	9.60
38.2726	28.72	0.6298	2.35173	3.96
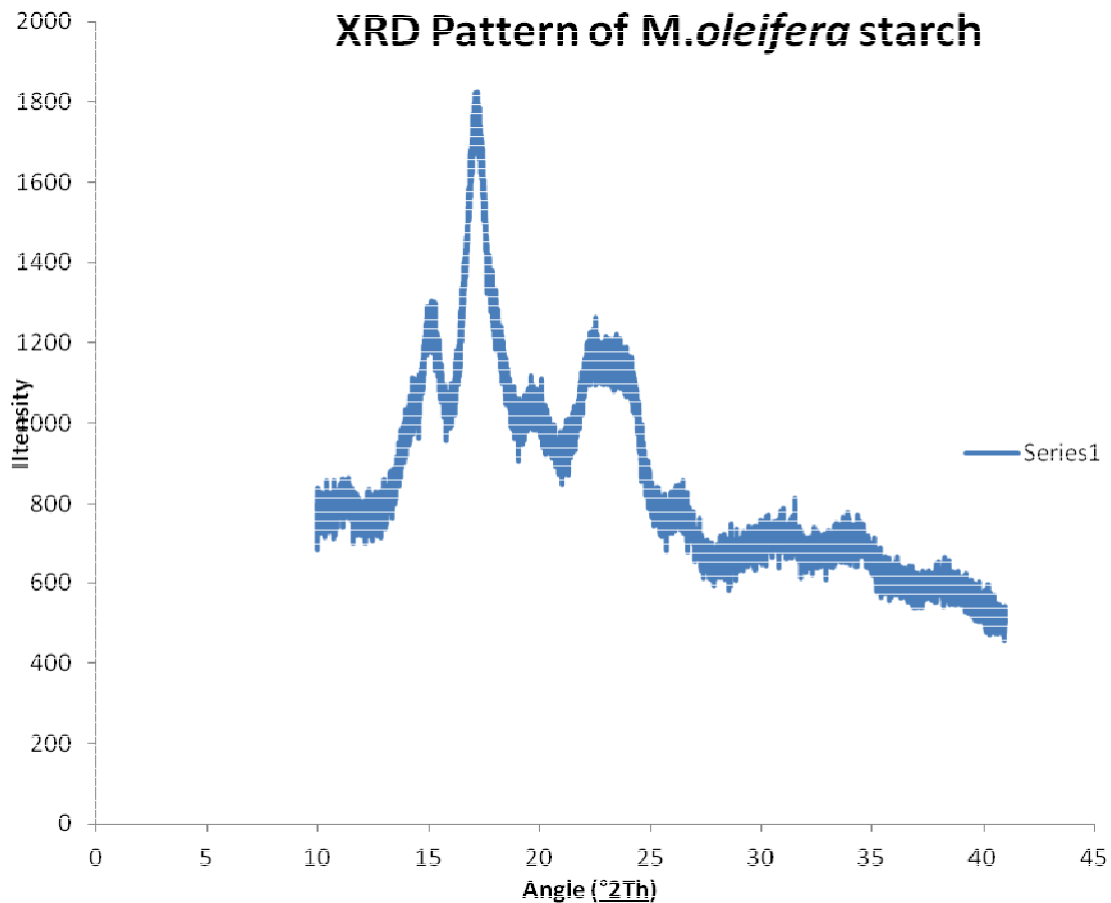
Figure 1. XRD Pattern of *M. oleifera* starch.

phosphorylation, free radical detoxification, neurotransmitter synthesis and maturation and iron metabolism.

X-ray diffractometer analysis

From the XRD pattern shown in the Figure 1 and Table 5, eight peaks were identified; six of these peaks can be seen as major peaks. The reflections at 17.18°, 15.13° and 22.2° correspond to the reflections in yam starch (generated from library) at 17.04°, 14.9° and 22.2°, respectively. Within experimental error, all the major peaks in the yam starch match with the major peaks in the sample. The high background is due to the amorphous nature of the starch. It was also observed that iron complex ($C_{24}H_{16}FeN_{10}$) was recorded as one of the compounds detected. X-ray diffraction has offered academia a potent tool for elucidating molecular structures

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of crystalline or semi-crystalline materials; however, its use as an analytical tool in the food industry is likely limited somewhat due to the expense associated with the instrumentation. Nevertheless, research has developed methods for measuring the relative percentages of the crystalline and amorphous phases in starches (Nara et al., 1978).

Conclusion

Some instrumental and chemical analyses of *M. oleifera* starch have been examined and the results presented. The percentage yield as indicated previously showed that *M. oleifera* starch is comparable with other native starch isolated. Thus, the starch can be derivatized and characterized with scanning electron microscope (SEM), Rapid visco-Analyzer (RVA) and differential scanning colorimeter (DSC) to really demonstrate its potential as industrial biomaterials.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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