A Novel Extraction Method and Some Physicochemical Properties of Extractives of *Irvingia Gabonensis* Seeds

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ABSTRACT

The plant *Irvingia gabonensis* contains lipids and polymer extractives that can be a good source of excipients for oral pharmaceutical formulations. These constituents are usually extracted from the seeds with the aid of either organic solvents or enzymes. The purpose of this paper was to describe a novel, simpler, cheaper, and safer method for the simultaneous extraction of lipids and gum from *I. gabonensis*. A 100.0 g of seeds of *I. gabonensis* in 200 mL deionized water was heated at 78°C in the presence of 2.0 g sodium chloride for 1 h, and the mixture was allowed to stand for the lipids to be separated and removed by filtration. The samples were air dried at 25–30°C. The liquid fraction was centrifuged at 3445 rpm for 30 min, and the supernatant portion containing the gum was freeze dried. The samples were investigated for their true, bulk, and tapped densities as well as particle size and particle size distributions. The rheological, and near infrared absorption spectra as well as thermal behavior of the samples were also studied. The lipid and the polymeric components of *I. gabonensis* seeds were successfully extracted simultaneously. The true densities of the fatty and gum components were, respectively, 1.000 and 1.544 g/cm³. The melting point of the fat was 40°C and T_g of the gum was 232°C. These values were similar to those described in the literature for the components using the traditional method, demonstrating the effectiveness and efficiency of this simple novel method.

Key words: Gum and fat fractions, *Irvingia gabonensis*, physicochemical properties, simultaneous extraction

INTRODUCTION

Irvingia gabonensis (Aubry Lecomte ex. O'Rorke) Baill. commonly known as 'African mango' or 'bush mango' is a tree of 15–40 m, with a bole slightly buttressed.[1-4] The plant is a wild forest tree[3,5] with dark green foliage and yellow fragrant flowers and occurs in the wild lowland forest; two to three trees occur together and in some areas, it is reported to be widespread. *I. gabonensis* is largely distributed in Africa.[6-9] The fruit is spherical with smooth yellow fibrous mesocarp and hard endocarp when ripe. The seed from the plant has been of interest in the food,[5,10-12] beverages,[13-15] medical[4,16-21] and pharmaceutical[22-32] as well as cosmetic circles.[33] The seed of the plant contains lipids and polymeric constituents[6,15,34-38] and are usually harvested during the dry season when ripe. The seeds are sold round the year in all markets where food stuffs are sold in Nigeria. It is a common soup ingredient freely

prepared in any part of Nigeria and many African countries. Both the lipids (also referred to as dika fat or *I. gabonensis* wax) and the polymeric (gum) components have been used by pharmaceutical scientists as excipients in various formulations. The mucilage from the kernel has been used as a binding agent in tablet formulation,[22] and as a emulsifying and suspending agent.[38, 39] On the other hand, the lipid has been employed in tableting as lubricant,[32,40] sustained release ingredient,[23,24,41] microencapsulation,[42] as suppository base[28,29,43] and as a component of film coating operation.[27]

The lipids component of *I. gabonensis* seeds have been traditionally extracted using *n*-hexane[40] or other organic solvents;[43] and more recently an enzymatic method for extracting the lipid components was developed.[44] On the other hand, the polymeric component of the seed has been extracted from aqueous dispersion using petroleum ether[22] or diethyl ether.[45] None of the extraction processes described in the literature provided for simultaneous extraction of the lipid and polymer component, which are of interest to pharmaceutical scientists. In addition, organic solvents have many disadvantages such as adverse environmental consequences of their disposal, worker safety concerns, and cost. Thus, the objective of this study was to develop a novel, simple and cost effect method for simultaneous extraction of the lipids and polymeric components and at the same time overcome the disadvantage the use of organic solvents.

MATERIALS AND METHODS

Irvingia gabonensis seeds were obtained from a main market, Jos, Nigeria; sodium chloride (lot B27630, J. T. Baker, USA).

Methods

A 100 g of ground seed of *I. gabonensis* was accurately weighed (Metler PC 4400) and transferred to a 4 L beaker containing 2 L of 1% w/v sodium chloride which was pre-heated to about 78°C. The mixture was heated on a hot plate to about 78–85°C. Gentle mixing for an hour was achieved with the aid of a magnetic stirrer. The mixture was left to stand at room temperature~21°C for 24 h. Solidification of the lipids component took place at the top and the bottom of the mixture and the lipids were subsequently removed by filtration through a 105 μm stainless sieve. The lipids were air dried and stored in air tight container until ready for use. The remaining sample containing the gum was centrifuged (Allegra 6R centrifuge, Beckman Coulter™, USA) at 3440 rpm for 5 min to remove proteins and other impurities. The resultant clear supernatant mucilage was dried in a lyophilizer and stored in an air tight container until required for further investigations.

Evaluation of physicochemical properties of extractives from *Irvingia gabonensis* seeds

Photomicrograph
Photomicrographs of the samples were obtained on a Nikon eclipse microscope (Model ME 600, Nikon Instruments Inc., Melville, NY, USA) fitted with a camera interfaced with Spot basic software version 2.41 (Linkam Scientific System, England, UK). The photomicrograph of the sample is displayed on the computer (HP Corporation, USA) and is saved as images in jpeg format until required.

Differential scanning calorimetry
About 2–10 mg of the sample was transferred to a DSC pan of known weight and was subjected to differential scanning calorimetry studies (model 2950, TA instrument, Delaware USA) at a heating rate of 10°C/min with the aid of ultra pure nitrogen as the purge gas. Thermal information was acquired and analyzed using Universal analysis 2000 software (TA instrument, Delaware USA).

True density
The samples to be analyzed were dried to about 1% w/w moisture content before the experiment. Prior to running samples on the gas pycnometer, the volume of the sample cell and the expansion volume were taken. Simply, the cup of the gas pycnometer (Model Acupyc 1330, S/N 3902; Micromeritics Instrument Corporation, Norcross, GA, USA) was first cleaned and its weight together with the two bulbs was taken. The main gas supply was turned on and the pressure was set at 20–22.0 psig. The pycnometer chamber cap was removed and greased with Dow Corning vacuum grease with the aid of the greasing disk. The transducer was zeroed. The calibration balls were placed in the cell chamber cup securely. The calibration was accomplished with the balls in the cup as the standard. The sample identification, analysis parameters and report, purge fill pressure, number of runs, run fill pressure and equilibration rate were sequentially imputed. The sample of known weight was introduced, and the readings were taken with the analysis terminating when five consecutive runs were within the specified tolerance or full scale of ten runs were executed. The results were printed on a Hewlett Parker printer (HP, USA) and analyzed. The results displayed were the average of ten determinations.

Bulk and tapped densities
The United States pharmacopoeia method II for determination of bulk and tapped density was used. Briefly,

a quantity of the material sufficient enough to complete the test was passed through a 1.00 mm (size 18) top screen mounted of the Scott Volumeter (ASTM-329-90) apparatus. The powder was allowed to slide and bounce through the glass baffles of the instrument. The sample then passes through the bottom funnel into a cylindrical cup of about 25 mL until it overflows. The excess powder was gently scrapped off the cylindrical cup by smoothly moving the edge of the blade of a spatula perpendicular to and in contact with the top surface of the cup. Care was taken to keep the spatula perpendicular to prevent packing and removal of the powder from the cup. The bulk density, g per mL, was calculated by the formula:

$$\text{Bulk density} = M/V_\text{o}$$

where M is the mass of the powder and V_o is the volume of the cup. Five determinations were made and the average of such determinations was used.

United States pharmacopoeia method II for tapped density determination was also used in the determination of the tapped density of the samples. The mechanical tapped density test was used to mechanically tap the powder at a fixed drop of 3 mm and the nominal rate of 250 drops per min. The tapped density is given by the formula:

$$\text{Tapped density} = M/V_\text{f}$$

where M is the mass of the powder used and V_f is the tapped volume.

Particle size and size distribution
The lipid fraction was dispersed in water with the aid of sodium lauryl sulfate to produce a suspension. The particle size and particle size by volume determinations were performed on Malvern Mastersizer-S® (version v2.18, Malvern Instruments, UK) set to 3$$D (Fraunhofer) presentation and the 300 RF range lens was used. A sample of the suspension was introduced into the liquid dispersion chamber connected to a small volume disperser that keeps the particles uniformly dispersed in the continuous medium. Sufficient quantity of the sample was introduced with the aid of a micropipette to achieve at least an obscuration of about 10%. The data which were acquired with the aid of the Malvern software was then transferred to Microsoft Excel (Microsoft Corporation, USA) for analysis.

In the case of the polymeric portion, Zeta Sizer® (High Performance Particle Sizer, Malvern Instruments, UK) utilizing the dynamic light scattering method was employed to estimate the particle size distribution by volume. The instrument was turned on and allowed for its temperature to stabilize for 30 min. The HPPS software was switched on, and the sample was transferred to a cuvette, filling to a height of 10 mm with the aid of Fin pipette. The cuvette was inserted and the temperature was allowed to stabilize. The programmed measurement from the software was selected and used for the measurements. The results were saved to the already open measurement database.

Rheological property of the gum fraction
The rheological characteristics of the gum extracted from *I. gabonensis* were determined using a Brookfield digital rheometer (model LVDV-III+CP, Brookfield Engineering Laboratories, Inc. Middleboro, MA, USA) using a CPE 40 cup and plate. Prior to the determination of viscosity, the rheometer was zeroed and the gap between the cup and plate was set to 0.013 mm using the rheometer programmable features. A 1 mL sample of 3.6% w/v mucilage was transferred to the sample cup and the cup was fixed in position. The viscosity of the sample was determined at spindle speed from 2 to 100 rpm and when subsequently decreased from 100 to 2 rpm at 0.5, 1 and 2 min ramping intervals. The temperature in the system was maintained at 25°C with the aid of regulated water bath (Fisher Scientific, USA).

Near infrared spectroscopy
The near infrared (NIR) absorption spectroscopic evaluation was carried out on both fractions using the rapid content analyzer (Vision® 3.20, FOSS NIR Systems, Maryland, USA). The performance and linearization checks were carried out according to the procedure in the instrument manual. The powdered sample was transferred to a transparent 20 ml glass vial. The sample was placed on the sample holder and scanned after the reference scan was made. The absorption spectra data obtained were stored in the computer till when required.

RESULTS AND DISCUSSION

General description of fractions

Two fractions were obtained from an aqueous dispersion of *I. gabonensis* using the novel method described; the lipids solidify and were removed by filtration while the gum portion remained in the aqueous dispersion. The lipid fraction was off white to yellowish in color with the yellowish fraction settling at the bottom of the container and the whitish finer portion suspended. On the other hand, the gum fraction was whitish to brownish in color [Figure 1].

Figure 1: *Irvingia* fractions (L-R): White lipid fraction (float), aqueous (middle) and yellow lipid (sediment)

Bulk and tapped densities of *I. gabonensis* gum

The bulk and tapped densities of the gum were 0.368 and 0.613 g/cm³, respectively. Bulk and tapped densities of material affect the handling and process method to be adopted when included in a formulation. A material with low tapped density would be difficult to compress while a highly compressible material should have a high tapped density. In the case of Irvingia gum, the tapped density was almost twice that of the bulk suggesting it is highly compressible, a good quality attribute for direct compression operations.

True density

Table 1 shows that the skeletal density of the lipid fractions was 1.005 g/cm³ irrespective of the appearance. The true density of the gum was 1.544 g/cm³. True density values are indicative of the physicochemical properties of the materials, and these values suggest differences between the two fractions from the plant seeds. At the same time, the lipid fraction exhibited a density of about 1.0 g/cm³ as water and this may explain why the fraction was partially afloat and partially submerged.

Photomicrograph

The photomicrograph of the gum of *I. gabonensis* is shown in Figure 2. The particles did not have any definite shape.

Differential scanning calorimetry

Figure 3 shows the thermograph of the two fractions obtained from *I. gabonensis* seeds. The melting point and the T_g of the lipid fraction were, respectively, 40.26 and 22.72°C. The melting point of the fatty component was consistent with those in the literature in which the workers
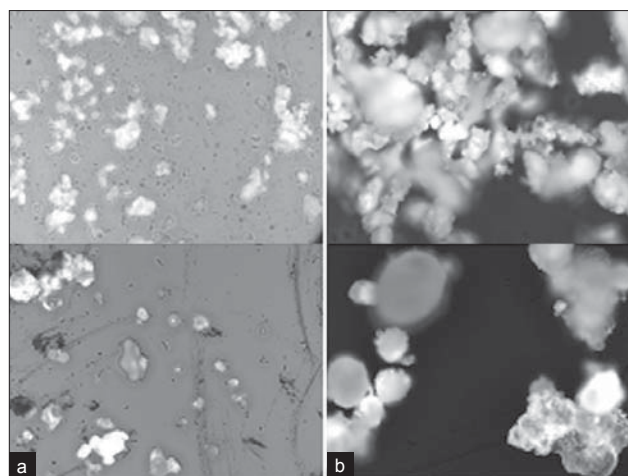

Figure 2: Photomicrographs of *Irvingia* gabonensis

Table 1: Physicochemical properties of *Irvingia gabonensis* extractives

Description	Water insoluble fractions		Water soluble fraction
	White	**Yellow**	
True density (g/cm³)	1.0055 ± 0.000	1.0050 ± 0.000	1.544 ± 0.000
$_pH$ (3.6% w/v)	-	-	6.76 ± 0.21
z-average (nm)	-	-	1309.5 ± 18.6
polydispersity index			0.483 ± 0.07
Bulk density (g/cm³)	-	-	0.368 ± 0.028
Tapped density (g/cm³)	-	-	0.613 ± 0.030
Distribution moments			
Mean volume	63 ± 7.4		
Standard deviation	101 ± 13.51		
Skewness	2.51 ± 0.11		
Kurtosis	6.73 ± 0.78		
Distribution percentile (µm)-Volume %			
10	1.76 ± 0.08		
20	3.74 ± 0.19		
50	17.02 ± 1.95		
80	97.77 ± 10.94		
90	196.72 ± 24.48		
Distribution modal sizes (µm)			
1	7.10 ± 0.16		
2	57.6 ± 5.91		
3	0.33 ± 0.00		

used *n*-hexane as the extractive solvent.[40] On the other hand, the T_g of the polymeric fraction was 232.5°C, similar to the value obtained by some other workers.[46]

Near-infrared spectroscopy

Near-infrared spectroscopy has great potential in providing real-time chemical information.[47] The most prominent absorption bands occurring in the NIR region are related to overtone and absorption bands of the fundamental molecular vibrations of –CH, –NH, and –OH functional groups observed in the mid-spectra region.[48] Figure 4 shows

the NIR spectra of the gum and the lipids from *I. gabonensis* seeds. Each of these samples exhibited the –CH$_3$ functional group at 2300 nm in the combination band region. It seemed that –ArCH is present in the gum fraction as shown in the first overtone peaks at 1700 nm while –CH$_2$ and –CH$_3$ were predominant at this wavelength in the lipid fractions. The –CH group was stronger in the gum than in the lipid in the second overtone. The spectra of the gum extract from the conventional method was different from those of the gum by this novel method at 1100 nm but showed the same pattern at 2300 nm, which may be due to higher quality of the sample from the novel extraction method. This difference in peaks at 110 nm between the two samples may be related to impurities in the conventional fraction, as that peak was similarly present in water insoluble fractions. NIR measurements are performed without dilution or the requirements of short optical path lengths or dispersion in nonabsorbing matrices that are necessary with traditional spectroscopic methods of analysis such as UV-VIS or mid-NIR spectroscopies. NIR spectra can be collected in either transmittance or reflectance mode. In general, transmittance (log *I/T*) measurement

is performed on translucent samples. Diffuse reflectance (log *I/R*) measurements are performed on opaque or light scattering matrices such as slurries, suspensions, paste, or solids and in this study, diffuse reflectance was used.

In either measurement mode, it is difficult to identify unique spectra features related to individual component within a given matrix. To enhance spectra features and compensate for baseline offsets, it is common practice in NIR spectroscopy to apply a mathematical pre-treatment to the spectra. Frequently, the second derivative of the absorbance data, with respect to wavelength, is calculated. In the second derivative data, absorbance maxima are converted to minima with positive side-lobes. The apparent spectra bandwidth is also sharply reduced, allowing for resolution of overlapping peaks and the baseline difference between spectra are largely eliminated. The presentation of the data as the second derivative and not raw spectra was based on this understanding.

Particle size and particle size distribution by volume

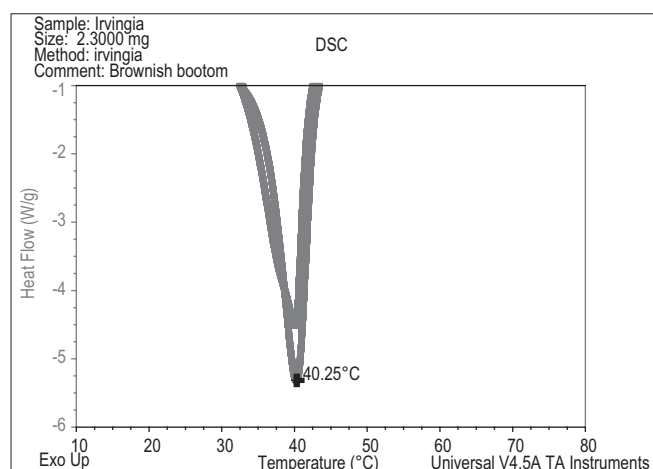The particle size distribution by volume is shown in

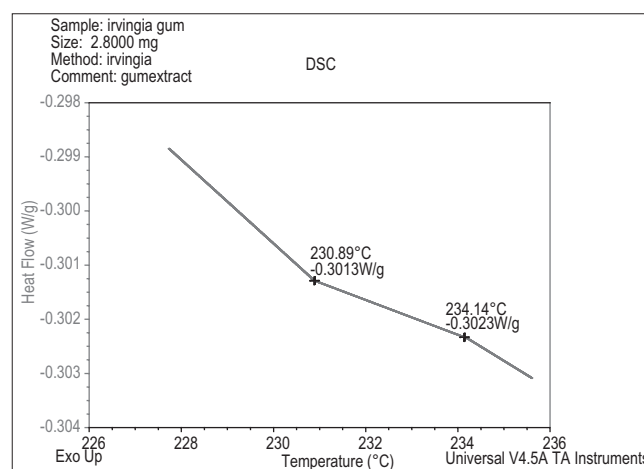**Figure 3a:** DSC thermogram of *Irvingia gabonensis* (dika fat)

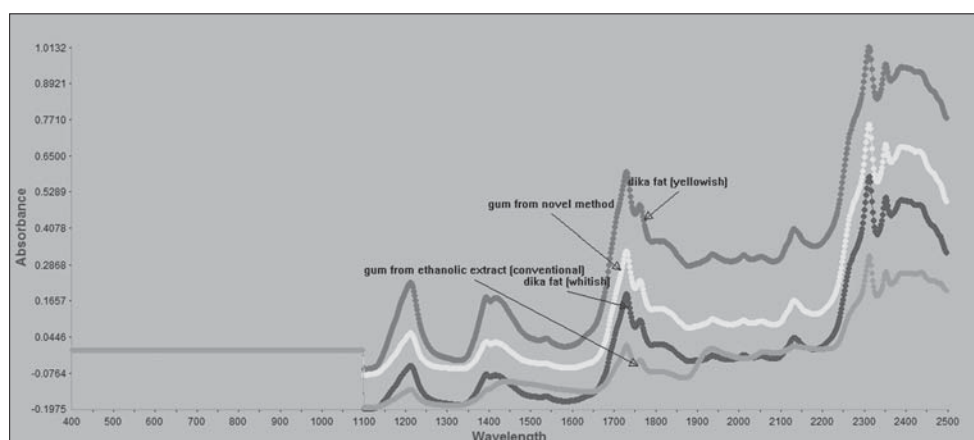**Figure 3b:** DSC thermogram of *Irvingia* gum fraction

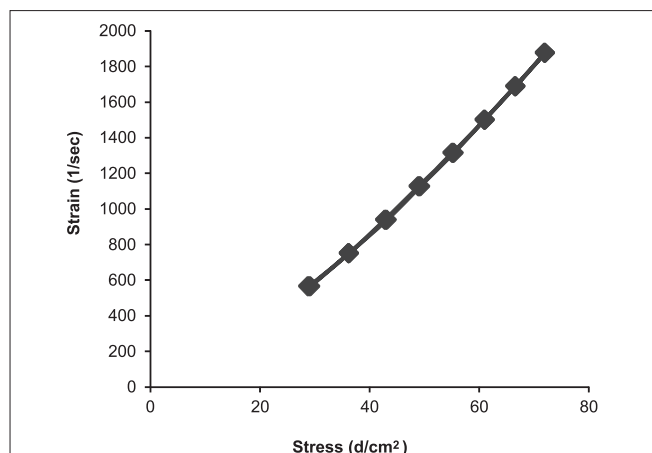**Figure 4:** NIR spectra of *Irvingia* extractives

Figure 5: Relationship between the strain and stress of *Irvingia* gum (3.6% w/v)

Figure 6: Relationship between spindle speed and the viscosity of *Irvingia* gum (3.6% w/v)

Table 1 for the fractions. The particle size of the lipid mass ranged from 0.1 to about 500 μm, and the mean volume in the distribution moments was 63.15 ± 7.40 μm while the corresponding values of the particle surface and length were, respectively, 3.45 and 0.45 μm. Distribution percentile by volume and distribution modal sizes are also shown in Table 1.

On the other hand, the z-average of the gum was 1309 nm with a polydispersity of 0.483. In dynamic light scattering, this z-average size is the most important and stable number produced by the technique and can be used for quality control purposes. Using the same instrument, it is possible to compare samples of materials with respect to the particle size by either volume or intensity. In this study, the low solubility of the extract using the conventional extraction method, for example, in the case of the gum limited such a comparison. No measurement of the z-average of the gum has been reported in the literature.

Rheological properties

Figure 5 shows the relationship between stress and strain while Figure 6 shows the relationship between speed and apparent viscosity of the gum. The viscosity of *I. gabonensis* gum (3.6% w/v) ranged between 3.85 and 5.19 cP with speed variation between 75 and 250 rpm. The rheological profile of the gum was pseudoplastic. This was similar to the results obtained previously.[12]

CONCLUSION

In conclusion, simultaneous extraction of lipids and polymeric portions of *I. gabonensis* was successfully carried out simultaneously using a method that was simple, safer, and
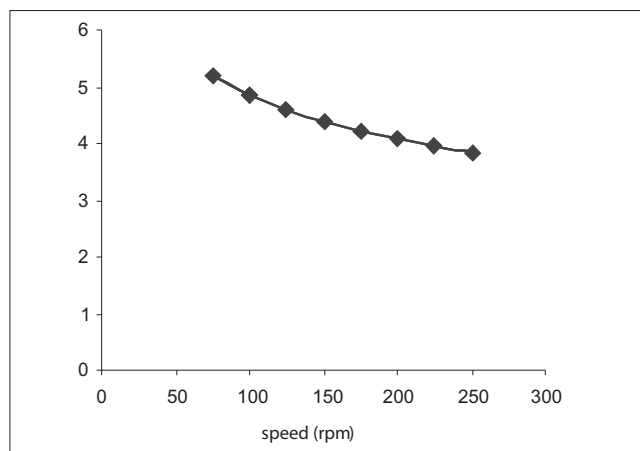
less expensive than the traditional use of *n*-hexane to extract the lipids. The method combined simplicity and safety with efficient removal of impurities from the gum fraction. The physicochemical properties of the extractives were evaluated, and the results showed similarities in the extractives obtained by this method and those obtained by conventional methods.

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