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Extraction and Characterization of Mucilage from Irvingia Gabonesis and Irvingia Wobolum Seeds

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Abstract— *This study is aimed at the extraction and characterization of mucilage from Ogbono seeds. In this research work, two species of Irvingia was used (Irvingia gabonensis and Irvingia wombolu). Proximate analysis was carried out on the samples to determine crude protein, moisture, crude fiber, ash, oil, carbohydrate and energy value. The mucilage was extracted using different methods and at different process parameters such as seed to liquid ratio (1:10, 1:20, 1:30, 1:40), temperature (40ºC, 50ºC, 60ºC, 80ºC), time (30mins, 60mins, 90mins, 120mins) and pH (3, 5, 7, 9). The results obtained from the effect of seed to liquid ratio showed that the best seed to liquid ratio for Irvingia gabonensis is from 1:20 to 1:30 for the three solvent. These gave the highest yield of averagely above 17%. Similarly ratio range of 1:30 to 1:40 gave the highest yield of averagely of above 12%. Also for effect of temperature, for both ogbono seeds evidence has shown that increase in temperature increases the yield of mucilage for the three solvents (Ethanol, Petroleum ether and Acetone) but Irvingia wombolu shows higher yield than Irvingia gabonensis with increase in temperature. This is an indication that high temperature promotes precipitation. The results of pH on mucilage yield for Irvingia gabonensis and Irvingia wombolu shows that increase in pH for both seed and the three precipitated solvents increases the mucilage yield with highest yield at pH of 9 (alkaline medium). The result has shown that precipitation with ethanol exhibited yield followed acetone on both seed but Irvingia gabonensis gave the highest yield. The results as the boiling time increases from 30 to 120 minutes, the mucilage yield also increases for both seed samples in the three precipitated solvents. Higher yield was obtained in Irvingia wombolu and the best solvent was ethanol followed by acetone. This proved that higher time create more surface area for the isolation of the mucilage. The summary of the results showed that the two sample of Ogbono seeds (Irvingia gabonensis and Irvingia wombolu) contain high yield mucilage and the mucilage also contain high carbohydrate, protein, fibre, energy, ash, fat, and moisture content which proved that both ogbono seeds mucilage have high nutritional value. With these it can be concluded that Ogbono mucilage is good excipient for food and pharmaceutical products. The mucilage was characterized to examine the solubility, bulk density and the pH. Ogbono seeds mucilage is highly recommended for use as good excipient for food and pharmaceutical products due to its high nutritional value.*

Keywords— *Mucilage, Irvingia gabonesis, Irvingia wombolu seeds.*

I. INTRODUCTION

Recent research has shown that plant seed contains water-soluble gum and mucilage which are also called hydrocolloid that are being used in foods (Arash et al., 2008). Chemically they are polysaccharides (gum Arabic, guar gum, carboxyl methyl cellulose, carrageen an, stanch and pectins) or proteins (such as gelatin). Hydrocolloid are broadly used in food system for various purposes example as thickener, gelling, texture modifiers and stabilizers (Arash et al., 2008). There has been an increase in demand of these hydrocolloid (Williams and Philips, 2000) and those from plant such as Ogbono, Okra, Achi, and Ukpo seed have advantage over those from animals because of their friendly image toward consumers (Arash et al.,2008). These create a substantial market for seeds mucilage to substitute for some of these hydrocolloids. Natural mucilages and gums can either dissolve in water or absorb water to create a viscous solution. Natural mucilage and gum are less cost-effective but readily available; they have been employed extensively as tablet binder, emulsifying agents, and thickeners in cosmetics, as well as suspension as film-forming agents and transitional colloids. In pharmacological preparations, polysaccharides (such as gum and mucilage) are most frequently utilised as adjuvants. They are most useful specifically when creating suspensions and emulsions. Polysaccharides have a long history of use as binding, suspending, and emulsifying agents (kolne, et al, 2011). Gums and mucilages are complexes of polysaccharides made of units of sugar and uronic acid. While they dissolve or swell in water, they are insoluble in alcohol. They are usually formed from the cell wall (e.g. tragacnth) or deposited on it in successive layers. Gums and mucilage are obtained mainly from seeds or other plant parts. Some are obtained from various marine algae, and some from selected animal. Mucilage is a complex polymers substance composed mainly of carbohydrate with simply branched structure which include l-arabinose, d-GA lactose, l-ramose, d-xylose and gelatos acid in various proportions. It also contains glycol proteins and other substances such as tannish, alkaloid and steroids (Monroy et al.,2017)

The rheological studies of these polysaccharides have drawn the attention of many researchers. Aqueous extractions are the most common methods used for the extraction of the seed mucilaginous material (Bummer et al., 2003). Because of difference in gum structure and extrinsic conditions within the fluid food system, the rheological behavior is quite different from one gum to another. Due to the diversity of gums and their modified derivate, food companies may have a difficult time making decisions regarding the choice of gums or mucilage for addition to their fluid food formulation (Mohammed et al., 2014). Similarly, the pharmaceutical industries also need to understand rheological properties of these gums and mucilage to be guarded on the right choice as thickeners or stabilizers.

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Thus understanding of the rheological properties of mucilage extracted from the seeds are essential for evaluating its potential application and uses as food and drug thickeners and stabilizers.

Several investigations have reported different functional properties of the mucilage from Ogbono, Okra, Achi and Ukpo seeds, for example Odeku and patent reported the binding in tablet formulation concluded that, incorporation of ogbono mucilage increased disintegration and dissolution times and as such could be used to modify drug release (Isimi and Olobayo 2000). Thus, the rheological studies of the mucilage extracted from Ogbono, Okra, Achi and Ukpo seeds have not gain much attention. So there is need to research toward the direction in order to explore all the rheological behavior of this mucilage for its essential in both food and pharmaceutical industries.

Irvingia gabonnensis genus of African and Southeast Asia trees in the family irvingiacea, sometimes known as by names, wild mango, African mango, bush mango, dika or ogbono. They bear mango like fruit which are especially valued for their fat and protein rich nut. They are grown for its fruits and kernels popularly known as ugiri and ogbono (Igbo language) respectively in Nigeria (Onaeri 2015). It has both edible (irvingia gabonnensis) and non-edible fruits (irvingia wombolu). The edible is eaten fresh or used to make juice and the kernel when ground is used to make ogbono soup but the non-edible is likely grown for the production of ogbono from its kernel. Research by Ehiem and Simonnyan, (2012) shows that the kernel powder can be used as ingredient in other sauces like tomatoes and groundnut for a sticky effect and taste. The seed of the plant contains lipids and polymeric constituents (Ogaji, et al., 2012). The lipids component of ogbono seed have been traditionally extracted using n- hexane (Megwa 1987) or solvent such as ethanol and enzymatic method (Ogaji, et al., 2012). The polymeric component of the seed also has been extracted from aqueous dispersion using petroleum ether or diethyl ether (Dudu ital.1998).

Fat extracted from the kernels can be used for food applications, such as cooking oil or margarine and is also suitable for pharmaceutical, cosmetics, and soap (Sheltu and Njinga,2018). It has been also reviewed that the potentials applications of wild mango kernel fat include margarine, cooking oil, perfume, soap and pharmaceutical (Joseph, 1995). The residual kernel cake had also been proven to be used as a thicker for food and pharmaceutical products (Shittu and Njinga, 2018).

The polymeric (gum) components have been used by pharmaceutical scientists as excipients in various formulation (Ogaji et al.2012). The mucilage from the kernel has been used as binding agent in tablet formulation and as emulsifying and suspending agent.

Irvingia gabonnensis (ogbono) seed consists approximately 62.8% of facts and 19.79% of carbohydrates. Protein is about 8.9% dietary fiber 5.3% and ash 3.2% (Asori, et al., 2020). Similarly, Oluwaseun et al., (2015) showed that the nutritional composition of dika seed contains 8.65% protein,14.6% carbohydrate,2.1% moisture,1.4% crudefiber,16.8% ash and38.9% dietary fiber

II. Materials and Methods

3.1 Materials

The two species of Irvingia gabnonensis and Irvingia wombolu seeds were purchased from Orba international market in Udenu Local Government Area of Enugu State Nigeria with geographical coordinates of $6^0 51^i 0^o$North and $7^0 27^1 0^1$ East.

The seeds were manually cleaned and packaged in plastic containers and stored at room temperature for further use. All chemical used in this study were of analytical grade and purchased from local shop in Ogbete main market Enugu, Nigeria.

2.2 Methods

2.2.1 Sample Preparations and Treatments

The seeds of the Irvingia gabonnensis and Irvingia wombolu were dried at room temperature for one week, pulverized and stored in airtight plastic container prior to the commencement of analysis and extraction processes.

2.2.2 Proximate Analysis

Proximate analysis was carried out to determine crude protein, moisture, crude fiber, ash, oil, carbohydrate and energy value. All parameters were analyzed according to AOAC, 1990.

- *Crude Protein*

Crude protein was determined using the kjeldahl digestion method. 2g of sample was weighed into a 50ml kjeldahl digestion flask. 8g of catalyst mixture was added to the flask. The catalyst mixture consist of 7.2g of sodium sulphate and 0.8g of copper (II) sulphate. 20ml of concentrated sulphuric acid was added to the whole mixture in the kjeldahl flask. Sulphuric acid serves as the oxidizing agent, sodium sulphate increases the boiling point of the whole mixture, while copper(II)sulphate catalyses the reaction. The mixture was heated on a Bunsen burner under fume cupboard until it turns from black to light greenish-blue coloration.

The digest was allowed to cool overnight so that it solidifies. 400ml of distilled and de-ionized water was used to dissolve out the digest into a 1000ml distillation flask. The solution was made alkaline by adding 50ml of 50% NaOH solution. The distillation flask was connected to its condenser. At the receiving end of the condenser was placed a 250ml conical flask containing 100ml of 4% boric acid solution. Three drops of screened methyl red solution was added to the boric acid solution. The distillation flask was heated at 100^0C using a heating mantle to distil ammonia over into the boric acid solution. Distillation was discontinued when about 300ml of the digest solution has distilled over. The distillate was titrated with 0.05M sulphuric acid solution until solution returns to the colour before distillation.

% Protein = Titre Value × 0.0014 × Conversion Factor(6.25)×0.05×100/ Sample Weight 2.0

- *Moisture Content*

Moisture was determined using the dry oven method. A clean glass Petri dish was weighed (w_1). 2g of the test sample was weighed into the weighed Petri dish (w_2). The Petri dish containing the sample was introduced into a hot air oven and left for an hour at 105^0C. After this, the sample was immediately transferred into a desiccator and allowed to cool. It was weighed again to obtain the final weight (w_3).

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%Moisture=W_2–W_3/W_2×100/1 ……2.1

- *Crude Fibre*

2g of the test sample was weighed into a 500ml flat bottom flask. 200ml of 0.1275M sulphuric acid (H_2SO_4) i.e 2.50g sulphuric acid in 200ml solution was added to the flask containing the sample. The flask was connected to a reflux condenser and the whole set up was heated using a laboratory hot plate. Water was allowed to circulate through the outer jacket of the condenser and the mixture was allowed to boil gently for 30 minutes. The hot sample/acid mixture was carefully filtered after boiling, using the whatman No.1 filter paper. The residue was washed with distilled water. The washed residue in the filter paper was quantitatively transferred back to the round bottled flask. 200ml of 0.313M sodium hydroxide solution was introduced to the acid treated sample in the round bottomed flask, that is 2.50g of carbon free sodium hydroxide in 200ml solution. The flask was returned under condenser and boil again for 30 mins. The mixture was filtered through a weighed ash-less whatman filter paper. The residue was washed thoroughly with alcohol (ethanol). The residue and ash-less filter paper were placed in a hot air oven (for drying) at 105^{o}C to constant weight. The moisture free residue and the filter paper was weighed. The fiber and ashless filter paper were transferred into a weighed platinum crucible and transfer into a muffle furnace at 600^{o}c for 3hrs. At 3 hours, the furnace was switched off and crucible transfered (using thongs) as quickly as possible to a desiccator. It was allowed to cool and weighed.

Calculation for crude fibre
Weight of sample …….. X
Weight of filter paper …..… y
Weight of residue + filter paper after oven drying …… z
Weight of residue …… z – y
Weight of crucible …… w
Weight of crucible +ash after ashing ……... U
Weight of ash …… u – w
Weight of fibre =weight of reside – weight of ash
% crude fibre = weight of fibre/weight of sample × 100 /1……2.2

- *Total Ash*

The total weight loss that occurs during the burning of the sample in the presence of oxygen at a temperature high enough to burn up all organic matter without allowing for considerable breakdown of the ash content or loss by volatilization is used to calculate the ash content. After the organic substance has been burned away, it is the inorganic residue that is still present.

An empty clean porcelain crucible (w_1) was weighed. 2g of sample (w_2) was weighed into the crucible and heated in a Bunsen burner at 600^{o}c for three hours. It was allowed to cool in desiccator and weighed (w_3).

Calculation

$$\% \text{ Ash} = \frac{W_1 - W_3}{W_2} \times 100 \quad \ldots\ldots\ldots\ldots 2.3$$

- *Oil (Soxhlet Method)*

50g of sieved sample was weighed into cotton material and inserted into the thimble of a 250ml soxhlet extractor. 250ml n-hexane was measured into a 500ml flat bottom round flask. The condenser was attached to the flat-bottom round flask holding n-hexane, and the soxhlet with the extraction thimble and sample contained in a semi-permeable membrane was linked to it. Water was permitted to circulate at the condenser's outer jacket while being heated using a heating mantle in the soxhlet extraction system. When all of the oil had been removed from the sample, the extraction was stopped. While the oil and n-hexane combination in the flat bottom flask was separated by distillation, the de-fatted sample in the semi-permeable membrane was discarded. When the oil was still in the flask, the n-hexane distilled over.

% Oil = weight of oil × 100 / weight of sample……2.4

- *Carbohydrate*

Total carbohydrate was determined by difference.

% Carbohydrate = 100 – (protein + ash + lipid + crude fibre + moisture) ……2.5

- *Energy Content (Calorific Value)*

Energy content of the products was calculated by Atwater's method (AOAC, 1990).

$(protein \times 4 + carboydrate \times 4 + fat \times 9)$ ……2.6

2.2.3 Extraction of mucilage from Ogbono seeds flour samples

The extraction of mucilage from the Ogbono seeds was carried out using different methods.

Step 1: Maceration method of extraction

Ogbono powder was soaked in cold distilled water for 30 minutes. The soaked seeds was transferred to hot boiling water and boiled for 2 hours to make the solution more concentrated. The mucilage was then filtered with the help of muslin cloth and the material was squeezed to separate mark from the filtrate. Solid to liquid ratio adopted is 1:10, at 35^{0}C, this procedure is repeated at different solid: liquid ratio of 1:20, 1:30, and 1:40 and at different temperatures, of 40^{0}C, 50^0, 60^0 and 80^{0}C. The pH can be adjusted using acid or alkaline, Effect of pH was also investigated (Sandy, et al, 2015). Effect of time, solid to liquid ratio, temperature and pH were also investigated.

Step 2: Precipitation method (with solvents).

The filtrate from maceration method was centrifuged at 500 rpm for 20 minutes using centrifugation apparatus. The supernatant was collected, cooled and the product was treated using suitable solvent (i.e ethanol, petroleum ether or Acetone) for the precipitation of mucilage and the precipitate was washed so many times using same solvent for removing unwanted impurities. The precipitate was then separated from solvent and kept for drying in hot air oven at 40^{0}C. The mucilage product was crushed and uniform particle size was obtained by sieving the mucilage powder. The percentage yield of mucilage is calculated using equation by Sandeep et al, (2015).

$$\% \text{ yield} = \frac{practical\ yield}{Thorictical} \times \frac{100}{1} \quad \ldots\ldots..2.7$$

Step 3: Other methods of mucilage extraction

(a) Precipitation of mucilage in Ethanol.

1. Boiled extract of seeds was precipitated in ethanol.

100g of irvingiaga bonenssis and lrvingia wombolu seeds were taken in a beaker ad 1 litre of distilled water was added to it. The beaker content was boiled for 15min.and later filtered using Buchner Funnel without filter paper or cloth. The residue was again boiled with 500ml of distilled water and filtered. The eight-folded muslin cloth was then used to filter the combined

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filtrate. After being precipitated in ethanol, the filtrate was oven dried.

2. Boiled extract of soaked and crushed seeds were precipitated in ethanol

In Balagon et al., (2015) 100g of both Ogbono seeds were soaked in the distilled water for 12 hours. The soaked seeds were then blended for the 20 minutes. The boiled seed mass was filtered by the muslin cloth and its filtrate was then precipitated with ethanol. The mucilage was separated and dried in the oven.

(b) Precipitation of mucilage in Acetone

1. Boiled extract of soaked and crushed seeds were precipitated in acetone

100g of Ogbono seeds samples were put in one litre of distilled water and boiled for 30 minutes. The boiled seed mass was crushed and filtered by the muslin cloth with 8 folds. The filtrate was then precipitated using 1000 ml of acetone and the precipitated mucilage was separated from the acetone and dried in the oven at 105^0C. (Balagani, et al, 2013).

2. Free precipitation of soaked and blended seeds in acetone

100g of Ogbono seeds samples were soaked in the distilled water and blended for 30 minutes. The blended seeds were then passed through filter cloth 8 folds and the filtrate was precipitated with equal amount (100g) of the acetone and precipitated mucilage was separated from acetone and oven dried.

(c) By defatting with Petroleum Ether

1. Defating by 12 hours shakings with petroleum ether.

The Ogbono seeds were blended and kept in contact with petroleum ether in a conical flask for 12 hours. The flask was shaked continuous with an electric shaker. The material was then filtered out and dried at room temperature for complete removal of petroleum ether. The powder seed was then soaked in distilled water. The swollen wet mass then spread on a glass tray and dried at 60^0C. The dried material was then passed through mesh30$\mu m.$ The material was winnowed and again passed through mesh 60µm. The weight of mucilage obtained has recorded (Balagani, et al. 2013).

2. Defatting by soxhlet apparatus with petroleum ether.

The Ogbono seeds were blended and kept in contact with petroleum ether in soxhelt apparatus. The cycles of petroleum ether were run fill and completely defatted was obtained. The defatted material was then dried at room temperature for compete removal of petroleum ether. The dried defatted seed powder was then soaked in distilled water. The swollen wet mass was then passed through mesh 30$\mu m.$ The material was winnowed and again passed through mesh 60 $\mu m.$ weight of mucilage obtained was noted.

The method that gave maximum yield of mucilage was utilized for further studies such as effect of solid – liquid ratio, time, temperature and pH on the yield of mucilage (Balagani, et al 2013).

2.2.4 Characterization of the Mucilage

(a) Physical properties characterization

The dried extracted mucilage was evaluated for percentage yield, organoleptic evolution, solubility, pH and particle size

I. Percentage yield.

The percentage yield of each extraction method was determined using the method described in section above.

II. Organoleptic properties.

The extracted mucilage were analyzed for colour, odour, taste, texture and fractions using the standard procedure (Sharuia, et al., 2008). Organoleptic testing involves the assessment of flavour, odour, appearance and mouth filled of a food product. The organoleptic testing of food products is essentials in ensuring products comply with organizational and customer requirements.

III. Solubility studies of the extracted mucilage.

The solubility of the extracted mucilage were tested in different solvent. One part of the mucilage was shaken with the solvent and analysis was carried out using uv-spectrophotometer (Sandeep, et al, 2016). The solubility determination was carried out by dissolving 450mg of mucilage in 5ml of solvent and stir gently with a glass rod. Record the sample as soluble or insoluble. If the unknown is water-soluble, test the solution, test the solution with pH paper. The solubility was tested in water ethanol, Benzene, Acetone and petroleum ether.

IV. pH of solution

The pH of the 1% $^{w}/_{v}$ aqueous mucilage solution was measured with a pH meter (Equip. Tronces, EQ-610) (Balagani, et al, 2013).

V. Particle size of extracted mucilage

According to Sandeep, et al (2013), particle size of mucilage were determined by using compound microscope at 10 x lens. Magnification value was calculated by calibrating stage micrometer and eye piece 50 readings of the sample were taken and their mean value were calculated. The particle size of the mucilage extracted from the two species of irvingia seeds were calculated by using the formula given below.

Size of each particle = number of individual in eye x calibration factor.

Calibration factor

$$= = \frac{Reading\ of\ stage\ micrometer}{Reading\ of\ accular\ micrometer} \times \frac{100}{1} \quad 2.8$$

III. RESULTS

3.1: Effect of process parameters on the mucilage yield

Irvingia gabonensis and Irvingia wombolu seeds mucilage were extracted with different methods and at different process parameters. The result of the effect of process parameters are shown below.

(1) Effect of solid to liquid ratio for Irvingia gabonensis

TABLE 3.1: Effect of sample to water ratio (w/w); temperature 40 ^{0}C, pH 3, boiling time 1 hr.

S/N	Sample: Water (w/w)	Ethanol ppt. (yield %)	Petroleum ether ppt. (yield %)	Acetone ppt. (yield %)
1	1:10	4.63	3.74	6.38
2	1:20	19.89	15.61	19.59
3	1:30	19.77	12.26	15.80
4	1:40	13.10	11.45	12.87

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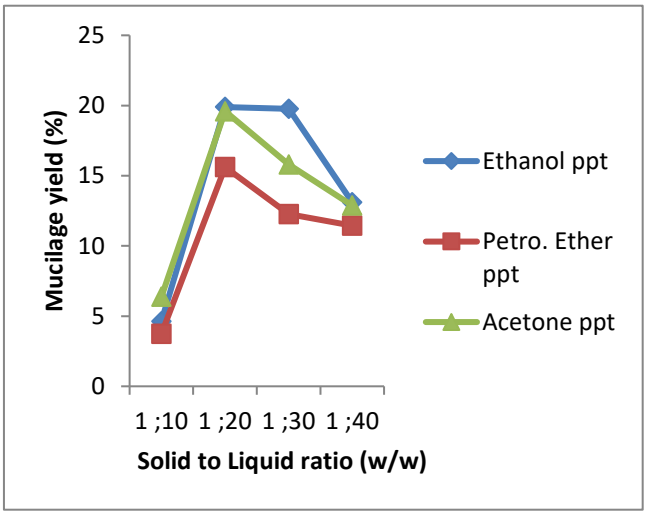

Figure 3.1 Effect of solid to liquid ratio on mucilage yield

2) Effect of solid to liquid ratio for Irvingia wombolu

TABLE 3.2: Effect of sample to water ratio (w/w); temperature 40 ^{0}C, pH 3, boiling time 1 hr.

S/N	Sample: water (w/w)	Ethanol ppt. (yield %)	Petroleum ether ppt. (yield %)	Acetone ppt. (yield %)
1	1:10	3.04	2.88	3.96
2	1:20	6.84	5.22	9.13
3	1:30	9.27	5.50	9.16
4	1:40	14.03	10.50	10.76

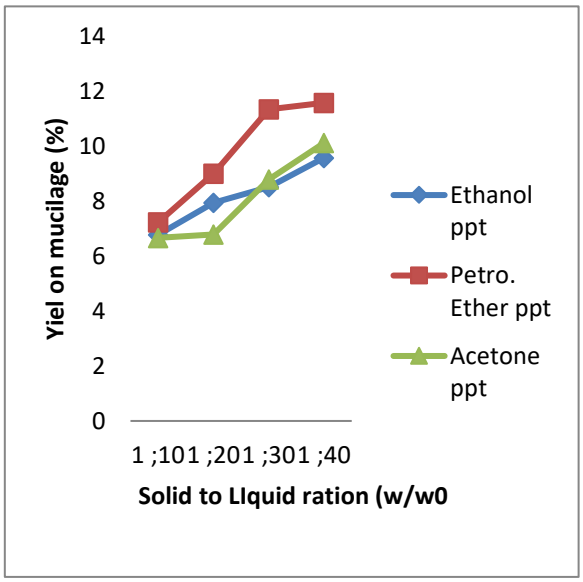

Figure 3.2: Effect of solid to liquid ration on mucilage yield for Irvingia wombolu

The results above shows that the best seed to liquid ratio for irvingia gabonensis is 1 : 20 to 1 : 30 for the three solvent. These gave the highest yield of averagely above 17%. Similarly ratio range of 1:30 to 1:40 gave the highest yield of averagely of above 12%. It is an evidence that high solid to liquid ratio result to low mucilage yield which is in agreement with the results of other researchers.

(3) Effect of temperature on mucilage yield for Irvingia gabonensis

TABLE 3.3: Effect of temperature (^{0}C); pH 3, boiling time 1 hr, sample to water ratio 1:10 w/w (irvingia gabonensis)

S/N	Temperature (^{0}C)	Ethanol ppt. (yield %)	Petroleum ether ppt. (yield %)	Acetone ppt. (yield %)
1	40	13.34	8.86	11.36
2	50	14.50	9.30	13.04
3	60	14.57	9.62	15.46
4	80	14.64	9.67	15.54

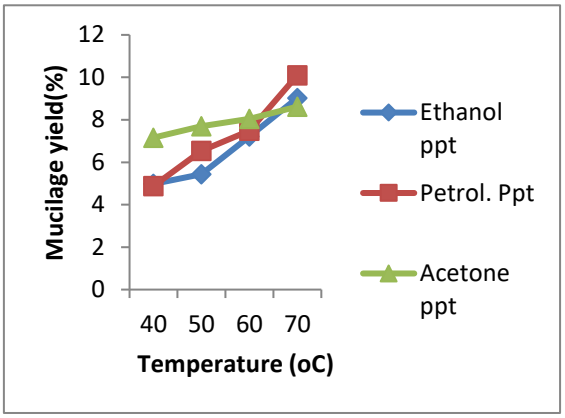

Figure 3.3: Effect of temperature on mucilage yield for Irvingia gabonensis

(4) Effect of temperature on mucilage yield for Irvingia wombolu

TABLE 3.4: Effect of temperature (^{0}C); pH 3, boiling time 1 hr, sample to water ratio 1:10 w/w (irvingia wombolu)

S/N	Temperature (^{0}C)	Ethanol ppt. (yield %)	Petroleum ether ppt. (yield %)	Acetone ppt. (yield %)
1	40	13.34	8.86	11.36
2	50	14.50	9.30	13.04
3	60	14.57	9.62	15.46
4	80	14.64	9.67	15.54

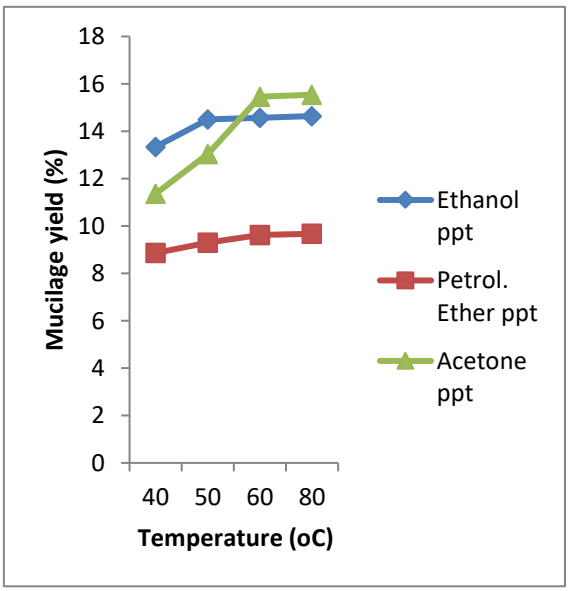

Figure 3.4: Effect of temperature on mucilage for Irvingia wombolu

From figures 3.3 and 3.4 for both ogbono seeds evidence has shown that increase in temperature increases the yield of mucilage for the three solvents (Ethanol,Petroleum ether and

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Acetone)but Irvingia wombolu show higher yield than Irvingia gabonensis with increase in temperature. The is an indication that high temperature promote precipitation and it is accordance with previous works of other researchers.

(5) Effect of pH on mucilage yield for the two Ogbono seeds

TABLE 3.5; Effect of pH; boiling time 1 hr, sample to water ratio 1:10 w/w, temperature 40 ^{0}C.(Irvingia gabonensis)

S/N	pH	Ethanol ppt. (yield %)	Petroleum ether ppt. (yield %)	Acetone ppt. (yield %)
1	3	7.55	5.47	5.40
2	5	7.64	5.58	5.42
3	7	9.69	6.99	9.73
4	9	9.89	9.13	11.51

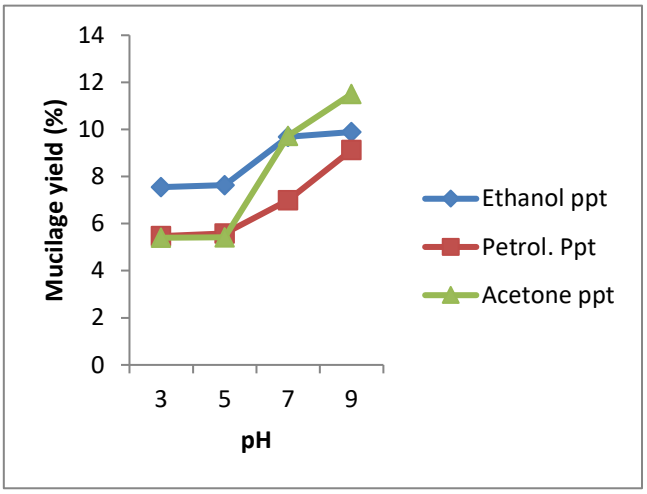

Figure 3.5: Effect of pH on mucilage yield for Irvingia gabonensis

TABLE 3.6: Effect of pH; boiling time 1 hr, sample to water ratio 1:10 w/w,temperature 40 ^{0}C (Irvingia wombolu).

S/N	pH	Ethanol ppt. (yield %)	Petroleum ether ppt. (yield %)	Acetone ppt. (yield %)
1	3	10.03	5.58	9.03
2	5	11.78	6.89	9.09
3	7	11.85	7.08	9.52
4	9	13.14	7.89	11.79

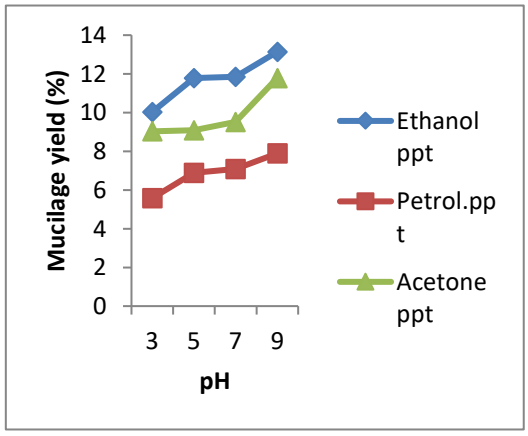

Figure 3.6: Effect of pH on mucilage yield for Irvingia wombolu

Figure 3.5 and 3.6 above show the results of pH on mucilage yield for Irvingia gabonenesis and Irvingia wombolu respectively. From the results it has shown that increase in pH for both seed and the three precipitated solvents increases the mucilage yield with highest yield at pH of 9 (alkaline medium). The results has shown that precipitation with ethanol exhibited yield followed acetone on both seed but Irvingia gabonenesis gave the highest yield.

(6) Effect of boiling time on mucilage yield for the two Ogbono seeds

TABLE 3.7: Effect of boiling time (hr); sample to water ratio 1:10 w/w, temperature 40 ^{0}C, pH 3 (Irvingia gabonensis)

S/N	Sample: water (minute)	Ethanol ppt. (yield %)	Petroleum ether ppt. (yield %)	Acetone ppt. (yield %)
1	30	6.77	7.22	6.66
2	60	7.93	8.99	6.78
3	90	8.51	11.33	8.76
4	120	9.57	11.57	10.11

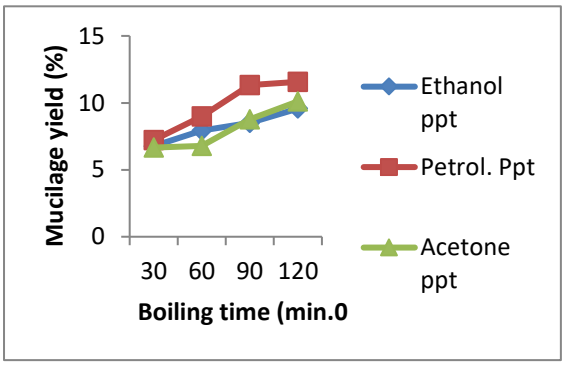

Figure 3.7: Effect of boiling time on mucilage yield for Irvingia gabonensis

TABLE 3.8: Effect of boiling time (hr); sample to water ratio 1:10 w/w, temperature 40 ^{0}C, pH 3 (Irvingia wombolu)

S/N	Sample: water (minute)	Ethanol ppt. (yield %)	Petroleum ether ppt. (yield %)	Acetone ppt. (yield %
1	30	11.87	7.11	12.02
2	60	14.68	8.88	12.08
3	90	16.04	9.09	13.02
4	120	17.80	9.45	15.22

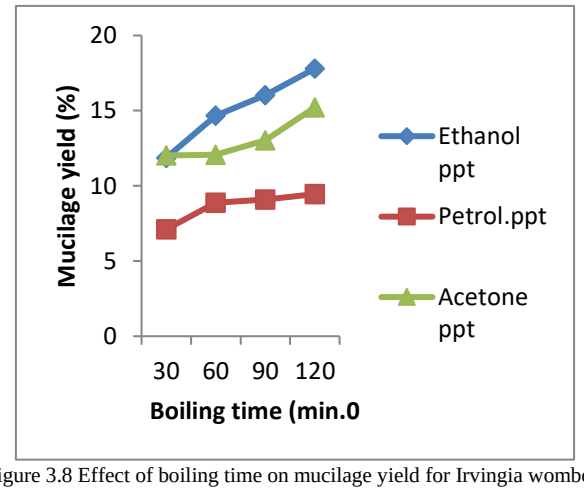

Figure 3.8 Effect of boiling time on mucilage yield for Irvingia wombolu

Evidence of effect of boiling time on the mucilage yield for the two species of Ogbono are shown in figure 3.7 and 3.8 for Irvingia gabonensis and Irvingia wombolu respectively. From the results as the boiling time increases from 30 to 120 minutes

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the mucilage yield also increases for both seed samples in the three precipitated solvents. Higher yield was obtained in Irvingia wombolu and the best solvent was ethanol followed by acetone. This proved that higher time create more surface area for the isolation of the mucilage.

3.2 Effect of extraction method on the mucilage yield for the two Ogbono species

TABLE 3.9: Effect of extraction methods on mucilage yield

S/N	Method	Sample	Mucilage yield (%)
1	Boiled extract precipitated in ethanol	*Irvingia wombolu*	17.87
		Irvingia gabonensis	22.15
2	Boiled extracted of soaked and blended seed precipitated in ethanol	*Irvingia wombolu*	19.74
		Irvingia gabonensis	14.82
3	Boiled extract precipitated in acetone	*Irvingia wombolu*	16.05
		Irvingia gabonensis	15.85
4	Boiled extracted of soaked and blended seed precipitated in acetone	*Irvingia wombolu*	19.66
		Irvingia gabonensis	10.56
5	Defatting by 12 hours shaking with petroleum ether and precipitated in ethanol	*Irvingia wombolu*	28.19
		Irvingia gabonensis	29.01
6	Defatting by 12 hours shaking with petroleum ether and precipitated in acetone	*Irvingia wombolu*	31.28
		Irvingia gabonensis	32.36

From the table 3.9 above, different mucilage isolation methods were investigated with Irvingia gabonensis and Irvingia wombolu and their mucilage yield calculated. The result shows different yield for different method for the two species. For Irvingia gabonensis the highest yield (32.36%) was obtained with defatted by 12hours shaking with petroleum ether and precipitated in acetone. Followed defatted by 12hours shaking with petroleum ether and precipitated in ethanol with yield of 29.10%. Similar results was obtained for Irvingia wombolu where defatted by 12hours shaking with petroleum ether and precipitated with acetone and ethanol produced higher yield of 31.28 and 28.19% respectively. It is an evidence that defatting by shaking with petroleum ether and precipitated in ethanol or acetone is the most promising method for extraction of mucilage from Ogbono seeds.

3.3; Results of Proximate Analysis for the extracted mucilage

Quantitative Analysis on Two Different Species Of Ogbono Seed

Proximate Analysis Results

S/N	Parameters	Sample A	Sample B
1	% Ash	2.375	2.603
2	% Moisture	7.062	7.051
3	% Crude Fiber	3.726	4.084
4	% Protein	8.651	6.924
5	% Lipid	21.984	20.163
6	Bulk Density	0.424	0.428
7	Tapped Density	0.458	0.476
8	True Density	2.5	2
9	Porosity %	83	78.6
10	% Carbohydrate	34.242	32.011

TABLE 3.10: Proximate Analysis Result

S/N	Method	Sample	Moisture (%)	Ash (%)
1	Boiled extract precipitated in ethanol	*Irvingia wombolu*	5.75	5.42
		Irvingia gabonensis	5.58	2.96
2	Boiled extracted of soaked and blended seed precipitated in ethanol	*Irvingia wombolu*	9.52	1.46
		Irvingia gabonensis	7.14	1.98
3	Boiled extract precipitated in acetone	*Irvingia wombolu*	7.75	6.34
		Irvingia gabonensis	10.56	1.47
4	Boiled extracted of soaked and blended seed precipitated in acetone	*Irvingia wombolu*	7.58	8.96
		Irvingia gabonensis	7.52	9.85
5	Defatting by 12 hours shaking with petroleum ether and precipitated in ethanol	*Irvingia wombolu*	6.15	14.63
		Irvingia gabonensis	10.12	40.29
6	Defatting by 12 hours shaking with petroleum ether and precipitated in acetone	*Irvingia wombolu*	5.00	4.98
		Irvingia gabonensis	11.00	21.78

The results of proximate analysis shown in table 3.10 above proved that the two Ogbono seeds contain substantial amount of moisture, ash, crude fiber, protein, oil, carbohydrate and energy. This is in agreement with the results of other researchers which means it high nutritional and excipient for food and pharmaceutical products. From the results there is no much difference in the proximate analysis on the two Ogbono seeds and the different methods which conclude that the both Ogbono seeds contain nutritional value.

3.4: Results of Solubility Studies for the extracted mucilage

TABLE 3.11: Solubility Studies Result

S/N	Method	Sample	Benzene	Acetone	Water
1	Boiled extract precipitated in ethanol	*Irvingia gabonensis*	Soluble	Soluble	Soluble
		Irvingia wombolu	Insoluble	Insoluble	Soluble
2	Boiled extracted of soaked and blended seed precipitated in ethanol	*Irvingia gabonensis*	Insoluble	Insoluble	Soluble
		Irvingia wombolu	Insoluble	Insoluble	Soluble
3	Boiled extract precipitated in acetone	*Irvingia gabonensis*	Insoluble	Insoluble	Soluble
		Irvingia wombolu	Insoluble	Insoluble	Soluble
4	Boiled extracted of soaked and blended seed precipitated in acetone	*Irvingia gabonensis*	Insoluble	Insoluble	Soluble
		Irvingia wombolu	Insoluble	Insoluble	Soluble
5	Defatting by 12 hours shaking with petroleum ether and	*Irvingia gabonensis*	Insoluble	Insoluble	Soluble
		Irvingia wombolu	Insoluble	Insoluble	Soluble

	precipitated in ethanol				
6	Defatting by 12 hours shaking with petroleum ether and precipitated in acetone	*Irvingia gabonensis*	Insoluble	Insoluble	Soluble
		Irvingia wombolu	Insoluble	Insoluble	Soluble

S/N	Method	Sample	Ethanol	Petroleum ether
1	Boiled extract precipitated in ethanol	*Irvingia gabonensis*	Soluble	Soluble
		Irvingia wombolu	Partially soluble	Insoluble
2	Boiled extracted of soaked and blended seed precipitated in ethanol	*Irvingia gabonensis*	Insoluble	Insoluble
		Irvingia wombolu	Insoluble	Insoluble
3	Boiled extract precipitated in acetone	*Irvingia gabonensis*	Insoluble	Insoluble
		Irvingia wombolu	Insoluble	Insoluble
4	Boiled extracted of soaked and blended seed precipitated in acetone	*Irvingia gabonensis*	Insoluble	Insoluble
		Irvingia wombolu	Insoluble	Partially soluble
5	Defatting by 12 hours shaking with petroleum ether and precipitated in ethanol	*Irvingia gabonensis*	Insoluble	Insoluble
		Irvingia wombolu	Insoluble	Insoluble
6	Defatting by 12 hours shaking with petroleum ether and precipitated in acetone	*Irvingia gabonensis*	Insoluble	Insoluble
		Irvingia wombolu	Insoluble	Insoluble

From the results above it shows that mucilage are soluble in water and insoluble in organic solvents.

3.5 Results of Bulk Density and pH for the extracted mucilage

TABLE 3.12: Bulk Density and pH Result

S/N	Method	Sample	Bulk density (g/cm^3)	pH
1	Boiled extract precipitated in ethanol	*Irvingia gabonensis*	0.770	5.75
		Irvingia wombolu	1.146	5.81
2	Boiled extracted of soaked and blended seed precipitated in ethanol	*Irvingia gabonensis*	0.874	5.73
		Irvingia wombolu	1.086	5.78
3	Boiled extract precipitated in acetone	*Irvingia gabonensis*	1.124	5.72
		Irvingia wombolu	1.018	5.96
4	Boiled extracted of soaked and blended seed precipitated in acetone	*Irvingia gabonensis*	0.714	5.68
		Irvingia wombolu	1.000	5.62
5	Defatting by 12 hours shaking with petroleum ether and precipitated in ethanol	*Irvingia gabonensis*	0.765	5.73
		Irvingia wombolu	0.769	5.79
6	Defatting by 12 hours shaking with petroleum ether and precipitated in acetone	*Irvingia gabonensis*	0.966	5.82
		Irvingia wombolu	0.896	5.78

The results of bulk density and pH shown in table 3.12 above proved that the mucilage from the two Ogbono seeds is acidic. The result also shows the concentration of the mucilage of the two Ogbono seeds from the different method of extraction.

IV. Conclusion and Recommendations

4.1: Conclusion

It has been shown above that the two Ogbono seeds (Irvingia gabonensis and Irvingia wombolu) contain high yield mucilage and the mucilage also contain high carbohydrate, protein, fibre, energy, ash, fat, and moisture content which proved that both ogbono seeds mucilage have high nutritional value. With these, it can be concluded that Ogbono mucilage is good excipient for food and pharmaceutical products.

The results also shown that the three solvents ethanol, petroleum ether and acetone has the potential of precipitating the mucilage with a reasonable yield. It is concluded that the three solvents can be used for isolation of Ogbono mucilage with ethanol given the highest yield followed by acetone and petroleum ether. Defatting the seed with 12 hours shaking with petroleum ether and precipitated with either ethanol or acetone proved to be the promising extraction method according to the results above.

Effect of process parameters such as seed to liquid ratio, temperature, pH and boiling time investigated above, the increase in temperature (30-80 ^oC) , pH (3-9) and boiling time (30-120 minutes) resulted to increase in mucilage yield with the highest at temperature of 80^oC,pH of 9 and boiling time of 120minutes for both seeds. Similarly, seed to liquid ratio of 1; 20 for Irvingia gabonensis and 1; 30 for Irvingia wombolu are the best solid to liquid ratio to be adopted for the extraction of mucilage from Ogbono seeds. All these are in agreement with the results of other researchers.

4.2: Recommendations

The following recommendations were made

i. Ogbono seeds mucilage is highly recommended for use as good excipients for food and pharmaceutical products due to its high nutritional value
ii. Also recommended is the use of ethanol, acetone and petroleum ether as precipitating solvents in increasing order of mucilage yield.
iii. The method of defatting by 12 hours shaking in petroleum ether with precipitating in either ethanol or acetone is also recommended for a better yield of mucilage.
iv. In extraction of Ogbono mucilage, low seed to water ratio (1: 20 and 1: 30) is required, temperature of 80 ^oC, 120 minutes and pH of 9 are also recommended for higher yield of mucilage.
v. Further studies are also recommended to investigate the rheological studies to ascertain how this mucilage can be applied in food drug excipients.

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