Extraction and Characterization of Mucilage from Okra Fruit and Ogbono seed

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Abstract

The study involved extraction and characterization of mucilage from okra fruit *(Abelmoschus esculentus)* and ogbono seed *(Irvingia gabonensis)*. Both samples were purchased from Ekwulobia market, Aguata L.G.A., Anambra State. Mucilage extraction was carried out using conventional method following standard procedures of maceration followed by precipitation using ethanol. The extracted mucilages were characterized using standard methods. The percentage yield of mucilage from okra fruit and ogbono seeds were 11. 65 and 14.62 % respectively. The properties of the extracted okra fruit and ogbono seed mucilages evaluated were respectively: pH (5.7 and 5.9), viscosity (1.5 and 22.8 mPa·s), true density (1.06 and 2.85 gm/ml), water holding capacity (3.41 and 4.35 gH_2O/g dry mucilage), oil-holding capacity (2.16 and 2.42 g/g). Both mucilages were soluble in hot water but insoluble in ethanol and acetone. The results of the phytochemical analysis for okra fruit and ogbono seed mucilages were respectively: carbohydrate (+ and +), protein (- and -), fat and oil (- and -), phenolic compounds (- and -), alkaloids (- and -), saponins (- and -), and mucilage (+ and +). The analyzed minerals and their concentration (mg/L) for okra fruit and ogbono seed mucilages were respectively calcium (249.38 and 235.14), magnesium (302.15 and 276.09), and potassium (210.60 and 227.41). High yield of mucilages were obtained and the extracted mucilages were considered to be pure. The properties of the extracted mucilages were comparable to other plant mucilages and the extracted mucilages could be good excipient for food and pharmaceutical products based on their properties.

Keywords: Okra, Ogbono, mucilage, extraction, characterization

1. INTRODUCTION

Research has shown that plant parts namely seeds, fruits, leave, and roots, contain water soluble gum and mucilage also called hydrocolloid that are being used in foods (Arash *et al*., 2010). Chemically, they are polysaccharides (gum Arabic, guar gum, carboxyl methyl cellulose, carrageen, starch and pectins) or proteins (such as gelatin). Mucilage is a complex polymer substance composed mainly of carbohydrate with simple branched structures which include l-arabinose, d-GA lactose, lramose, d-xylose and gelatos acid in various proportions. It also contains glycol proteins and other substances such as tannins, alkaloid and steroids (Tosif, *et al*., 2021). They are usually formed from the cell wall (e.g. tragacanth) or deposited on it in successive layers. While they dissolve or swell in water, they are insoluble in alcohol. Hydrocolloids are broadly used in food system for various purposes; example as thickener, gelling, texture modifiers and stabilizers (Arash *et al*., 2010). Hydrocolloids from plants such as okra *(Abelmoschus esculentus L.)*, achi *(Brachystegia eurycoma)*, ogbono *(Irvingia gabonensis)*, and ukpo *(Mucuna flagellipes)* seeds have advantage over those from animals because of their friendly image toward consumers (Arash *et al*., 2010).

Irvingia gabonensis is a species of African trees in the genus Irvingia in the family irvingiacea, sometimes known by the common names, wild mango, African mango, bush mango, dika nut (English) or ogbono (Igbo), Oro, Oyin (Yoruba), and Ogwe, Ohere (Edo). It grows to a height of 15-40 m bole slightly buttressed. It has a dense, compact crown, branchlets ending in a narrow curved, stipular sheath covering the leaf bud. Ohaeri (2015) emphasized that they are grown for their fruits and kernels popularly known as ugiri and ogbono (Igbo language) respectively in Nigeria. The fruit is yellowish when ripe, broadly ellipsoid and variable in size between varieties, 5-7.5cm with a yellow, fibrous pulp surrounding a large seed (Orwa *et al*., 22009). The fruit pulp is palatable and can be used for a fruit drink and for jam production. The kernel can be processed into flour by extraction, drying and grinding. The pounded seeds can be used to prepare sauce. Equally, Oil obtained from the kernel can be used in cooking, cosmetics, and pharmaceuticals. The kernel contains wax and mucilage useful for drug production (Orwa *et al*., 22009).

The okra *(Abelmoschus esculentus)* is an annual or perennial, growing to 2 meters tall. The leaves are 10-20 cm long and broad, palmately lobed with 5-7 lobes. The flowers are 4-6cm in diameter, with 5 white to yellow petals, often with a red or purple spot as the base of each petal. The fruit is a capsule up to 18cm long, containing numerous seeds (Wikipedia –Okra, 2010). Generally, okra is a high-value crop because it represents a source of nutrients that are important to human health, e.g., vitamins, potassium, calcium, carbohydrates, dietary fiber, and unsaturated fatty acids such as linolenic and oleic acids, and also of bioactive chemicals (Habtamu, 2014). Its immature, fresh, green seed pods are eaten as vegetable, while the extract obtained from the fruit is used in different recipes thicken stews, soup, and sauces to increase their consistency. It offers, in fact, a mucilaginous consistency after cooking. The immature pods are also used in making pickle. Often water-soluble polysaccharides from okra are also used in ice-cream, potato chips, and baked goods, providing a healthy option and more stable shelf-life. Beside its nutritional role, it is suitable for certain medical and industrial applications *(*Benchasr, 2012).

Though, natural mucilage and gum are expensive (less cost effective), they are 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 (Ravi *et al*. (2009)). In pharmacological preparations, polysaccharides (such as gum and mucilage) are most frequently utilized as adjuvants (Amiri *et al*., 2021). They are most useful specifically when creating suspensions and emulsions. Polysaccharides have a long history of use as binding, suspending, and emulsifying agents (Mousumi, and Amalesh, 2023**).** Isimi *et al*. (2000) reported the binding effect of mucilage in tablet formulation and concluded that, incorporation of ogbono mucilage increased disintegration and dissolution times and as such could be used to modify drug release. Okra mucilage is frequently employed as drug-delivery carrier in various ways (Rajalakshmi and Sangeetha, 2023). Furthermore, they promote tissue permeability to improve medicine oral bioavailability and can substitute synthetic polymers for improved mucoadhesive nature (Newton *et al*., 2015).

Though synthetic polymer used as drug-delivery carrier, thickeners, stabilizers, or emulsifying agent, may be less expensive than plant mucilage, they are not always available and most of them may not be compatible with most people's health (Amiri *et al*., 2021). Also, extraction and determination of some functional properties of mucilage from these plants parts for subsequent application in pharmaceutical and food industries have not gained enough attention; limiting utilization of these plant products mainly to soup making; thereby, depriving the farmers and the government the income and gains that would arise from increased demand of these plant part for mucilage production for industrial uses. Thus, this work focused on extraction and characterization of mucilage from Okra fruit and ogbono seed in South East, Nigeria.

2. MATERIALS AND METHODS

2.1 Sample collection and Preparation

Okra (*Abelmoschus esculentus*) fruits and ogbono *(Irvingia gabonesis)* seeds were purchased from Ekwulobia market, Aguata Local Government Area, Anambra State, Nigeria and were identified at the Biology Laboratory, Science Laboratory Technology Department, Federal Polytechnic, Oko, Anambra State. The identified okra fruits were carefully washed and dried under shade for 24 h, and further dried at 30-40^{o}C in an oven until constant weight was obtained. The dried fruit was ground to reduce the size using electric blender and the powdered fruit was passed through sieve no. 22 to obtain uniform size and then stored in an air tight container for further use (mucilage extraction and characterization).Equally, the identified ogbono *(Irvingia gabonesis)* seeds were manually cleaned, washed with clean water, and dried at room temperature for one week, pulverized using electric blender, sieved with (sieve no. 22) and stored in an airtight plastic container prior to mucilage extraction and analysis. All chemicals used in this study were of analytical grade and purchased from Bridge head market, Onitsha, Anambra State, Nigeria.

3. METHODS

3.1 Extraction of Mucilage

Extraction of mucilage from okra fruit and ogbono seed flour samples was achieved in two steps using the conventional method of mucilage extraction from plant parts.

Step 1: Maceration

The powdered sample was soaked in cold distilled water (500ml) for 30 minutes. The soaked sample was transferred to hot boiling water at 60 ^{o}C and left to boil for 3 hours to make the solution more concentrated. The concentrated solution 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 1:20.

Step 2: Precipitation (with solvents)

The filtrate from maceration step was centrifuged at 500 rpm for 20 minutes using centrifugation apparatus. The supernatant was collected, cooled and the product was treated using ethanol for the precipitation of mucilage and the precipitate was washed 4-5 times using same solvent ethanol to remove impurities. The precipitate was then separated from solvent using rotary evaporator (Stuart R3300) and then dried in a hot air oven at 40^{o}C. The dried mucilage was crushed using electric blender and the mucilage powder was passed through an 80-mesh sieve to obtain a uniform particle size. The sieved mucilage was then stored in a desiccator until further use (characterization of the mucilage). The percentage yield of mucilage was calculated using Equation 1.

$$Percentage\ yield\ of\ mucilage = \frac{Weigh\ of\ mucilage\ obtained\ (g)}{Weight\ of\ plant\ sample\ (g)} \times \frac{100}{1} \qquad (1)$$

3.2 Characterization of the Extracted Mucilage

The following properties of each mucilage were characterized;

3.2.1 pH of mucilage

The pH of solution was determined using digital pH meter.

3.2.2 Viscosity

Viscosity of each mucilage was determined using Oswald viscometer and calculated using Equation 2.

$$S = w \times \frac{t_s \rho_s}{t_w \rho_s} \quad (2)$$

Where: s = viscosity of solution, w = viscosity of water, t = time, and ρ = density.

3.2.3 True density

The true density of each powdered mucilage was determined using simple method of liquid displacement following the procedures described by Phanikumar *et al*. (2011), and Malviya *et al*. (2009).

3.2.4 Water holding capacity (WHC) and oil-holding capacity (OHC)

The water-holding capacity (WHC) and oil-holding capacity (OHC) were determined according to the methods of Thanatcha and Pranee (2011) and (Oh and Kim, 2022).

3.2.5 Solubility studies of the extracted mucilage

The solubility of the extracted mucilages was tested in water, ethanol, and acetone. The solubility of the mucilages was determined by dissolving 450mg of mucilage in 5ml of solvent and stirred gently with a glass rod and analysis was carried out using UV spectrophotometer. The result of each sample was recorded either as soluble or insoluble.

3.2.6 Test Purity

Phytochemical screenings were done according to the procedures described by Trease and Evans (2009), and Raman (2006).

3.2.7 Elemental Analysis of the Mucilage

One gram (1g) of the mucilage was first dissolved in 5ml of hot water (100 ^{o}C) and then in 10ml of concentrated nitric acid.Then the elemental analysis of the mucilage was evaluated using Atomic Absorption spectrophotometer 969 Unicam AAS.

4. RESULTS AND DISCUSSION

The percentage yields and the evaluated properties of the mucilage from okra fruit and Ogbono seed are presented in Table 1. From the table, it can be seen that higher yield of mucilage was obtained from ogbono (14.62%) seed than okra fruits (11. 65%). Powder compaction behaviour is influenced by particle density, as dense, hard materials may require greater compression pressure to form cohesive and less friable tablets, whereas materials with low particle density would produce more cohesive compacts than those with higher values (Bamiro *et al*., 2012). The pH of an excipient is important because the stability and physiological activity of most preparation are determined by their pH (Zeng *et al*., 2017). The pH of the mucilage from the two samples showed that the mucilages would be suitable to use. This is because, pH of mucilage nearer to neutral pH indicates that the mucilage would be less irritating to mucosal membrane and formulations formed by the mucilage at this pH would be comparatively more stable (Haile *et al*., 2020). The pH of 1% w/v aqueous solution of ogbono mucilage (6.62) is slightly acidic indicating the presence of cations (Murray *et al*., 2000).

Table 1: Evaluated Properties of Mucilage from Okra Fruit and Ogbono Seed

S/N	Parameters	Values	
		Okra	Ogbono
1	Percentage yield (%)	11. 65	14.62
2	pH	5.7	5.9
3	Viscosity (mPa·s)	1.5	2.8
4	True density (gm/ml)	1.06	2.85
5	Water holding capacity (g water/g dry mucilage	3.41	4.35
6	Oil-holding capacity (g/g)	2.16	2.42

7	Solubility	Soluble in hot water, insoluble in ethanol & acetone	Soluble in hot water, insoluble in ethanol & acetone

From table 1, it can be seen that the viscosities of the mucilage from okra fruit (1.5 mPa·s) and ogbono seed (2.8 mPa·s) are. The low viscosity solutions at low concentrations should enable it to form solutions in a wide range of concentrations with excellent properties as an emulsifier and stabilizer. The mucilages were found to be insoluble in ethanol and acetone. This reveals that the structural network of the mucilage is composed of hydrophilic chains leading to low affinity for these organic solvents. These results are in agreement with the study done by Ogaji *et al*. (2013) on the *Grewia mollis* mucilage.

Ogbono mucilage presents a better WHC (4.35g water/g dry mucilage) than the okra mucilage. (3.41 g oil/g dry mucilage). The WHC influences the formation of viscous solutions that can facilitate industrial processes (Del-Valle *et al*., 2005). Mucilage forms a three-dimensional network in contact with water, trapping it and resulting in highly viscous solutions. Mucilage is primarily composed of galactose, mannose, xylose, and other sugars, and, thus, has a high capacity to bind or retain water, similarly to pectins, gums, and some algal polysaccharides. Because of this large water absorption capacity, mucilage may find applications in foods, cosmetics, and pharmaceuticals, in which it can dissolve, be dispersed, and form colloids (Del-Valle *et al*., 2005). The oil holding capacity of both mucilage extracted from okra (2.16) fruit and ogbono seed (2.42) are appreciably high. Oil holding capacity is the most important functional property of hydrocolloids, which indicates oil absorption capacity (Kalegowda *et al*. 2017). Good oil holding values indicate that mucilage could improve the texture of food products. Fruit or seed extracted mucilage has good oil binding capacity due to presence of monopolar molecules, therefore, it can trap higher amounts of oil particles and also prevent loss of oil and flavour from food systems (Kalegowda *et al*. 2017). So, fruit or seed-derived mucilage can be used as a good functional ingredient in formulated foods (Kalegowda *et al*. 2017).

Table 2 presents the results of the phytochemical analysis/test for purity of extracted mucilage from okra fruits and ogbono seed. The test for purity of the extracted mucilage gave positive value for carbohydrate and mucilage and negative value for protein, alkaloid, saponin, and phenolic compounds. The presence of mucilage and absence of protein, alkaloid, saponin, and phenolic compound can be considered as proof for purity of the isolated mucilage (Farooq *et al*., 2013).

Table 2: Result of the Test for purity of Mucilage from Okra Fruits and Ogbono Seed

S/N	**Parameters tested**	**Results**	
		Okra	**Ogbono**
1	Carbohydrate	+	+
2	Protein	-	-
3	Fat and oil	-	-
4	Phenolic compounds	-	-
5	Alkaloid	-	-
6	Saponin	-	-
7	Mucilage	+	+

The elemental composition of the extracted mucilage from okra fruit and ogbono seed is presented in Table 3. From the table, it can be seen that the concentration of magnesium (302.15mg/L and 276.09 mg/L for okra and ognono mucilage respectively) is higher than the other two elements namely, potassium (302.15 mg/L and 227.41 mg/L) and calcium (249.38 mg/L and 235.14 mg/L) respectively for okra and ogbono mucilages.

Table 3: Elemental Composition of the Extracted Mucilage from Okra fruit and Ogbono Seed

S/N	**Elements**	**Concentration (mg/L**	
		Okra	**Ogbono**

1	Calcium	249.38	235.14
2	Magnesium	302.15	276.09
3	Potassium	210.60	227.41

5. CONCLUSION

High yield of mucilage was obtained from okra fruit and ogbono seed using conventional method of mucilage extraction with water for maceration and ethanol as precipitating solvent. The extraction parameters selected were suitable for the mucilage extraction, and the ethanol was effective in precipitating the mucilage leading to the high yield obtained. The extracted muclages were considered to be pure and the elemental compositions and functional properties of the mucilage were comparable to other plant mucilage. These properties of the extracted mucilage showed that the mucilage could be good excipient for food and pharmaceutical products.

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