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Anti-diabetic potential of water-soluble polysaccharide from okra pods mucilage diabetes

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***Abstract*---**Plant derived mucilage, due its role in promoting human health, has been extensively used as active constituent for the preparation of different pharmaceuticals, functional and nutraceutical products. Mucilage mainly consists of complex carbohydrates with extremely branched structure. *Abelmoschus esculentus L.* (Moench), belongs to family *Malvaceae*, is an annual herb commonly known as okra. The purpose of this study was the identification of bioactive polysaccharides present in the mucilage of okra pods and evaluation of their biological activities. Mucilage was extracted by soaking okra pods in water. The dried mucilage was fractionated in 75% ethanol, which resulted in soluble and insoluble fractions. Each fraction was screened which confirmed the presence of galacturonic acid, saturated and unsaturated polysaccharides. Crude, soluble and insoluble fractions were processed for investigating their biological activities. The results indicated that insoluble fraction was able to inhibit the growth of five human pathogenic bacterial strains i.e. *Staphylococcus aureus* (1.9 mm ± 0.27mm), *Escherichia coli* (2.8mm ± 0.44mm) and *Shigella* (2.9mm ± 0.85mm). However, no significant inhibition was observed against *Klebsiella pneumonia* and *Salmonella typhi*. Antioxidant capacity was determined through DPPH assay where the soluble fraction showed IC_{50} value 785.5 µg/mL while the insoluble fraction showed IC_{50} value 987.21 µg/mL. Antidiabetic assay results of the extract showed that soluble fraction has IC_{50} value 451.63 µg/mL, followed by insoluble and crude fraction having IC_{50} value

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767.13 µg/mL and 1152.61 µg/mL respectively. *In vivo* anti-inflammatory effect of the fractions was determined by carrageenan induced paw edema in mice model. The result revealed that soluble fraction at 800 mg/kg was able to reduce the paw edema volume from 4.81mm ± 0.18mm to 2.82 mm ± 0.12mm which was comparable to standard drug diclofenac sodium (2.12 mm ± 0.24mm). Our results conclude that okra pods exude contains potential bioactive polysaccharides. However, further molecular identification and characterization of these bioactive polysaccharides will further broaden our knowledge of the important roles played by these compounds in human health and nutrition.

***Keywords*---**Okra, *abelmoschus esculentus,* Polysaccharides, antidiabetic.

1. Introduction

A chronic metabolic condition with many pathogeneses that is characterised through impaired glucose digestion, and instability in the metabolism of protein, lipids, and carbohydrates that results in problems with insulin secretion or action, or possibly both, is called diabetes. Diabetic people are vulnerable to many other diseases like hypertension, cardiovascular disease, myocardial disease, and chronic kidney diseases.(Selvarasu and Maiyappan).(Kaul, Tarr et al.). In Pakistan, the epidemiology and risk factors for diabetes are curiously combined. The main causes of Pakistan's high prevalence of type 2 diabetes are Gestational diabetes, low birth weight, in utero programming, and a strong gene and environment interaction. The true cost of diabetes is its long-term complications, which increase morbidity and mortality. Due to the unique combination of many risk factors, research investigations are necessary to provide the appropriate risk assessment techniques. A better understanding of aetio-pathological genetic and environmental factors recommends prevention should begin much earlier than the start of the disease process because treatments in high-risk individuals alone won't be sufficient. launching population-based diabetes prevention programmes with a variety of initiatives aimed at people of all ages, from young children to the elderly. Public and private sectors must work together in synchronised and coordinated ways to address Synchronized and coordinated efforts from public and private sectors are needed to combat this enormous health and economic problem.(Hakeem and Fawwad 2010) Natural gums and mucilage's have been modified to overcome certain drawbacks such as uncontrolled rate of hydration, thickening, drop in viscosity on storage, and microbial contamination. The presence of anti-oxidative enzymes in natural resources (plants) reduces the fungal attack to some extent as secondary metabolites are produced from fungal filaments. As gums and mucilage's are polymeric materials so they are numerously used in pharmaceutical technology. The binding ability, thickening nature, and stabilizing and humidifying properties of mucilage's from different sources make them able to use in dosage forms of drugs and medicines. Different gums and mucilage's including guar gum, gum acacia, ghatti gum, and khaya gum have been used as binding agents in pharmaceutical formulations. Mucilage's have good binding properties compared to many synthetic

compounds.(Pasha, Bukhari et al. 2022). The aim of the study is to extraction and purification of okra pods mucilage polysaccharides to evaluate its anti-diabetic and anti-cancer properties.

2. Methodology

2.1 Extraction Methods

There are a several methods for extracting okra mucilage, most of them depend on the use of distilled water or organic solvents. Some processes also involve the application of heat. Farooq, Malviya, and Sharma removed the mucilage from the okra by continuously stirring it in distilled water at 60 °C for around 4 hours. The use of acetone allowed for the sequential (Farooq, Malviya et al. 2013). Due to the high solubility of its polysaccharides, okra mucilage is easily extracted in an aqueous media; one benefit of this process is the high extraction yield. Cahyana and Kam examined the effects of various variables, including time, temperature, and the proportion of water to okra fruits, on the extraction yield and antioxidant production. (Gemede, Ratta et al. 2015)

2.2 Plant Collection

Fresh okra (*A. esculentus*) pods were manually cleaned after being purchased from a local market in Mardan. The okra pods were free of all extraneous material.

2.3 Disinfection of Okra Pods

In order to render the okra pods free of external germs, disinfection of the okra pods was done. To maintain a sterile environment, the studies were conducted in a Laminar Flow Hood (LFH). Okra pods were cleaned using the following procedures using sodium hypochlorite to disinfect (NaOCl). The okra pods were cleaned by soaking them in sodium hypochlorite (NaOCl) for 15 minutes before washing them with distilled water. Mesh filters were used to filter the okra mucilage, which was then centrifuged at 3000 rpm for 30 minutes. A collection and drying of the supernatant was done.

2.3 Extraction of Okra Pods' Mucilage

Ameena and colleagues separated okra pod mucilage using the suggested procedure. To get rid of the external dirt, the okra pods were rinsed in tap water. Okra seeds weighed out at 15g per 80grams of okra pods were removed, measured, and incubated for 24 hours at room temperature. The solution was purified using muslin cloth. After that, transfer the mucus to a new tube. Put it through Whatman filter paper to clean it. For later usage, the removed mucilage was dried in a water bath (Ameena, Dilip et al. 2010).

2.4 Fractionation of the extracted mucilage

With the help of a microbalance, the pods were weighed. Pods and 1.5 mL of dH2O The pre-extraction mass was recorded and added to a 2 mL micro

centrifuge tube. Tubes were vortexed for a brief period of time to lower surface tension. To obtain the initial mucilage fraction, tubes were incubated for 1.5 hours at 25 °C with agitation at 1300 rpm. Following this, the tubes were centrifuged for 2 minutes at 13,000 rpm. To preserve the adherent mucilage and pods that had been pelleted, the tubes were centrifuged for 30 minutes at 13,000 rpm. A 1000 L laboratory pipette was then used to transfer the supernatant, or intense agitation extractable (IAE) mucilage fraction, to a clean, pre-labeled micro centrifuge tube.

2.5 Powdered Mucilage Polysaccharide

The extracted mucilage of okra *abelmoschus esculentus* were then dried in hot air water bath. The mucilage was poured in 4 to 5 petri dishes and put it on the water bath for 24hours.The mucilage when completely dried and powdered form, then add it to falcon tubes as stock for future purposes.

3. Results

3.1 Invitro Antidiabetic Assay

The *Invitro* Antidiabetic, Assay were carried out to determine the anti-diabetic potential of okra pod mucilage. A stock solution of plant extract was prepared by dissolving pancreatic amylase in 1 mL of 0.1M phosphate buffer saline (PBS) at 37 ºC. The crude precipitated and non-precipitated fractions were treated with 1 mL of 50% acetic acid. The contents were heated in a boiling water bath for five minutes. The reaction mixture was cooled at room temperature, diluted to a 1:5 ratios with water, and absorbance was measured in a spectrophotometer at 540nm.The percentage of enzyme inhibition activity was calculated as; %inhibition =control-sample*100/control (Haque, Hossain et al. 2022).

The outcome showed that at their greatest concentrations, all of the fractions could block alpha amylase activity. Each fraction's alpha amylase inhibition was compared to acarbose, a reference medication, which demonstrated 100% inhibition under the circumstances of our particular assay. The outcomes showed that at 1000 g/mL, crude mucilage and ethanol precipitated fractions both suppress alpha amylase activity by 70.93 percent and 77.66 percent, respectively (53.65 percent). However, at a concentration of 250 g/mL, the ethanol precipitated fraction shows the strongest alpha amylase inhibition at 42.95 percent, followed by the ethanol precipitated fraction at 37.31 percent and crude mucilage at 9.47 percent. The lowest 50 percent inhibitory concentration (IC50) for ethanol precipitated fraction was the most effective one while inhibiting alpha amylase activity.

Table 3.1 Percent alpha amylase inhibition or antidiabetic potential and IC50 in µg/mL of crude, ethanol precipitated and non-precipitated fractions of okra pod mucilage

Antidaibetic potential and IC_{50} IN µG/ML of okra mucilage and fractions.				
	250 µg/mL	**500 µg/mL**	**1000 µg/mL**	**IC_{50} µg/mL**
CRUDE MUCILAGE	9.47	35.86	53.65	852.57
ETHANOL PRECIPITATED	42.95	58.35	70.93	346.37
ETHANOL NON PRECIPITATED	37.31	59.87	77.66	376.20

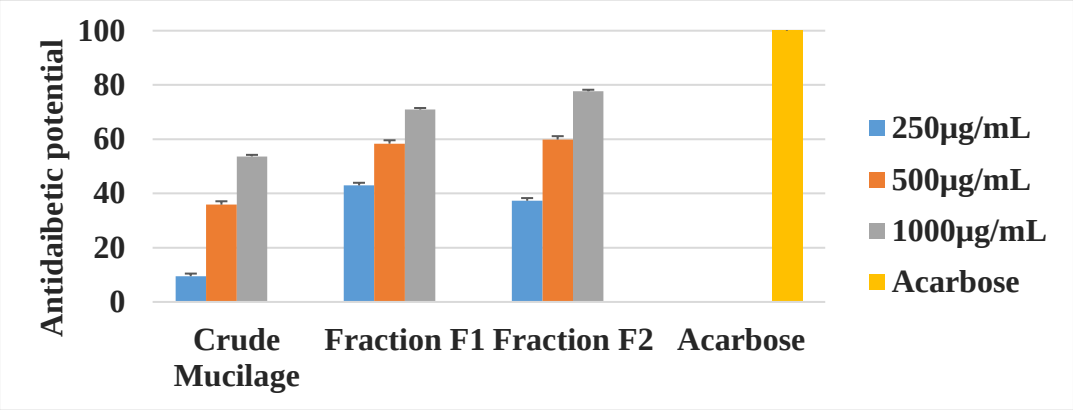

Figure 4.1 Percent antidiabetic potential of Okra pod mucilage where crude fraction, F1; ethanol precipitated and F2; ethanol non-precipitated fractions at three different concentrations where Acarbose was used as reference drug

4. Discussion

Polysaccharides obtained from okra has been found to have diverse application in a range of fields from functional food to industrial products, medicine, coating and fabrication of advanced materials (Dantas, Alonso Buriti et al. 2021). Okra mucilage contain a large number hydroxyl group which help to allow it to several types of surface chemical modifications that tends to improve mechanical characteristics and possibly could be used for enhancement of antibacterial properties of okra polysaccharides (Tosif, Najda et al. 2021). Pharmacological properties of okra have been widely studied including anticancer, antibacterial, anticoagulant. etc. but still further studies are needed to be carried out to determine the possible bioactive component and mechanism of action as limited studies are available on grafting of okra polysaccharides. The properties of okra polysaccharides isolated by microwave-assisted, hot-water, and pressurized-water extraction were compared in terms of their antioxidant and in vitro binding activities on α-amylase and α-glucosidase. The results indicated considerable antioxidant activities for all the extracts considered. Meanwhile, the binding capacities and inhibitory potency of hot- and pressurized-water extracts (for α-amylase and α-glucosidase) were similar but slightly higher than those of

microwave-assisted okra polysaccharide extracts (Raj, Shim et al. 2020). The outcomes suggested that okra polysaccharides prolonged the swimming time, increased muscle glycogen and hepatic glycogen levels, and decreased blood lactic acid and serum urea nitrogen. All these outcomes prove the effectiveness of okra polysaccharides as anti-fatigue agents (Zhao, Dai et al. 2020). In another study, it was found that okra extract reduced the hyperlipidemia and hyperglycemia in diabetic mice (streptozocin- and alloxan-induced diabetes), Recently, okra polymers were reported to exhibit a hypoglycemic effect, which may be attributed to the inclusion of rhamnogalacturonan units in their repeating structure. The *in vivo* effect of isolated polysaccharides was evaluated in streptozocin-induced diabetic mice and it was found that high doses of rhamnogalacturonan okra polysaccharides reduced blood glucose levels in the tested mice (Raj, Shim et al. 2020). Ultrasonically extracted okra polysaccharides were found to exhibit considerable *in vitro* prebiotic and antioxidant activities. It was found that okra polysaccharides assisted in *in vitro* α-glucosidase and α-amylase inhibition in a dose-dependent manner. Therefore, it may be inferred that okra extracts may be used to great advantage in treating type-2 diabetes (Liu, Qi et al. 2021). Compared with the fresh okra polysaccharide, the content of galactose and rhamnose in lignified okra polysaccharide was greatly decreased. However, the content of glucose in lignified okra polysaccharide increased, which may be due to the degradation of cellulose into glucose. In the fresh okra fruits, pectin is the main component of the middle lamella and plays an important role in cell adhesion mechanism. After the lignification of okra, the modifications of cell wall include hydrolysis of neutral sugars especially galactose and rhamnose, depolymerization and increased solubilisation of pectins and in cellulose. On the other hand, the degradation and dissolution of polyuronic acid were observed in many fruits during ripening. Compared with fresh okra polysaccharide, the content of rhamnogalacturonan in the lignified okra polysaccharide decreased greatly, and the molecular weight also decreased significantly. This may be due to the dissolution of the polymers closely bound to the cell wall during metabolism. The anti-inflammatory activity of the okra polysaccharides is helpful to elucidate the molecular mechanism and develop the novel applications of polysaccharides from the lignified okra. Macrophages play an important role in the inflammatory response and can protect the host from external stimuli infection and invasion. Interestingly, AP1-b exhibits more potent proliferation activities against RAW264.7 macrophages than the polysaccharide OFPS11 from okra flowers which only composed of galactose and rhamnose with molar ratio of 2.23: (Aloysius, Felekkis et al. 2022). AP1-b was mainly composed of galactose, rhamnose, glucose, arabinose and galacturonic acid in the molar ratio of 1.98:1.00:0.15:0.32:0.29. Therefore, proliferation inhibition was related to monosaccharides composition. LPS is effective activator of macrophages and can be considered as one of the main virulence factors in the inflammatory response, which induce the release of inflammatory mediators by secreting inflammatory cytokines (Agregán, Pateiro et al. 2022). LPS-induced RAW264.7 cells were used to evaluate the anti-inflammatory activity of AP1-b. Consistent with the anti-inflammatory suppression effect on NO production, a dose-dependent suppression of AP1-b on IL-1β, TNF-α and iNOS in LPS-induced RAW264.7 cells were also observed. AP1-b could suppress the mRNA expression of IL-1β, TNF-α and iNOS, indicating that it may affect the transcription process or the mRNA stability of inflammatory cytokines. Therefore, the results can provide a basis for

the structural characteristics and anti-inflammatory effect of the lignified okra polysaccharide.

5. Conclusion

Okra has been widely used as promising low-cost functional food. Plant based mucilage are biocompatible natural resources and are low cost and having important nutrients. Okra pods when soaked in water release a white gelatinous product called as mucilage. Mucilage is mainly composed of carbohydrates. Besides its composition, mucilage has been well studied for its functional food properties along with its application in pharmaceutical industries. Our results revealed that okra crude mucilage and fractionated extract showed significant biological potential with notable activity against Alpha glucosidase. Also prominent antimicrobial potential was observed against selected human pathogenic bacterial strain. Aqueous okra mucilage was found to be non-toxic as no significant percent haemolysis was observed when subjected to cytotoxicity assay. Further study is needed to evaluate the proper mechanism involved in biological potential of mucilage and to establish functional and pharmaceutical properties of okra mucilage. Also structural and quantification studies are needed to identify and isolate the bioactive component in okra mucilage that is responsible for inducing *in vivo* anti-inflammatory responses.

References

Agregán, R., M. Pateiro, et al. (2022). "Biological activity and development of functional foods fortified with okra (Abelmoschus esculentus)." Critical Reviews in Food Science and Nutrition: 1-16.

Aloysius, M., K. N. Felekkis, et al. (2022). "Chitosan Nanogel with Mixed Food Plants and Its Relation to Blood Glucose in Type 2 Diabetes: A Systematic and Meta-Analysis Review of Observational Studies." Nutrients 14(22): 4710.

Ameena, K., C. Dilip, et al. (2010). "Isolation of the mucilages from Hibiscus rosasinensis linn. and Okra (Abelmoschus esculentus linn.) and studies of the binding effects of the mucilages." 3(7): 539-543.

Dantas, T. L., F. C. Alonso Buriti, et al. (2021). "Okra (Abelmoschus esculentus L.) as a potential functional food source of mucilage and bioactive compounds with technological applications and health benefits." Plants 10(8): 1683.

Farooq, U., R. Malviya, et al. (2013). "Extraction and characterization of okra mucilage as pharmaceutical excipient." 6(4): 168-172.

Gemede, H. F., N. Ratta, et al. (2015). "Nutritional quality and health benefits of okra (Abelmoschus esculentus): A review." 6(458): 2.

Hakeem, R. and A. J. J. o. d. Fawwad (2010). "Diabetes in Pakistan: epidemiology, determinants and prevention." 1(3): 3.

Haque, M. A., M. S. Hossain, et al. (2022). "Abelmoschus esculentus (L.) Moench Pod Extract Revealed Antagonistic Effect against the Synergistic Antidiabetic Activity of Metformin and Acarbose upon Concomitant Administration in Glucose-Induced Hyperglycemic Mice." 2(2): 128-138.

Kaul, K., J. Tarr, et al. Introduction to diabetes mellitus. InDiabetes 2013 (pp. 1-11), Springer, New York, NY.

Liu, Y., J. Qi, et al. (2021). "Okra in food field: Nutritional value, health benefits and effects of processing methods on quality." Food Reviews International 37(1): 67-90.

Pasha, A. Z., S. A. Bukhari, et al. (2022). "Evaluation of Modified Date Palm (Phoenix dactylifera L.) Mucilage as a Potential Pharmaceutical Excipient." 2022.

Raj, V., J.-J. Shim, et al. (2020). "Grafting modification of okra mucilage: Recent findings, applications, and future directions." Carbohydrate polymers 246: 116653.

Selvarasu, K. and A. Maiyappan "DIABETES AND CHRONIC DISEASES: A CORRELATIONAL STUDY."

Tosif, M. M., A. Najda, et al. (2021). "A comprehensive review on plant-derived mucilage: Characterization, functional properties, applications, and its utilization for nanocarrier fabrication." Polymers 13(7): 1066.

Zhao, Z., X. Dai, et al. (2020). "Pyrazolone structural motif in medicinal chemistry: Retrospect and prospect." European Journal of Medicinal Chemistry 186: 111893.