**Original Research Article**

**Qualitative phytochemical analysis and mucilage characterization of *Opuntia ficus-indica* and *Opuntia robusta* (motoroko) seeds from Botswana**

**ABSTRACT**

The aim of the study was to perform a qualitative phytochemical analysis and mucilage characterization of seeds from two *Opuntia* species: *Opuntia Ficus-indica* and *Opuntia robusta* from Botswana. Phytochemical extractions were conducted using ethanol, methanol, and n-hexane. The results revealed that both species contained saponins, terpenoids, and flavonoids, with *O. ficus-indica* showing a stronger presence of saponins in polar solvents compared to *O. robusta*. Both species tested negative for tannins, phenols, and alkaloids across all solvents. The mucilage yield was slightly higher in *O. ficus-indica* (3.19%) compared to O. robusta (3.03%). Ash content was recorded at 2.44% for *O. ficus-indica* and 2.28% for *O. robusta*. Moisture was 4.97% for *O. ficus-indica* and 7.33% for *O. robusta*. The water holding capacity (WHC) was higher for O. ficus-indica (5.20 ml/g) compared to that of *O. robusta* (4.80 ml/g). These findings highlight the potential applications of *Opuntia* seeds in the food and pharmaceutical industries due to their phytochemical contents and functional properties of the mucilage. The study underscores the importance of promoting the utilization of these underutilized seeds in Botswana.

Keywords: *Opuntia Ficus-indica*, *Opuntia robusta*, phytochemical extraction, mucilage

**INTRODUCTION**

It has long been acknowledged that the genus *Opuntia*, which includes species like *O. ficus-indica* and *O. robusta* known as Motoroko in Botswana, is important as the different parts of the plant (fruits, seeds, flowers) are used in various health problems which include wound healing, anti-inflammatory and urinary tract infection from ancient times1 and they have served as an inspiration for scientific research. *O. ficus-indica*, often known as prickly pear, cactus pear and barbary fig are a dicotyledonous angiosperm belonging to the *Cactaceae* family2. It is grown in many parts of the world for its fruit, which has a nice flavor and a high quantity of minerals, vitamins, dietary fiber, and phytochemicals3. This shrub plant has a maximum height of five meters with a highly branched root system which grows laterally and horizontally up to 10 to 15 meters from the base of the plant. Fruits typically have a thick skin with few prickles, and the pulp has a fixed number of seeds, which are often consumed whole and left in the pulp4. Fruits, in general, have a thick peel with little prickles, and the pulp has a consistent number of seeds, which are usually not removed from the pulp and are also eaten.

Wheel cactus (*O. robusta*) is a branching upright shrub native to northern and central Mexico that grows 1-2 m high, sometimes up to 4 m high5. Yellow flowers, 5-8 cm wide, are borne singly along the upper borders of stem segments. Flowering takes place primarily in late spring and summer. The pads are enormous, 20-25 cm wide, with some reaching 40 cm, hefty, and securely linked to one another. Up to 12 whites to pale brown or yellow sharp spines up to 5 cm long can be found in each pad. The green meaty fruit can grow to be up to 8 cm long and 6 cm wide, becoming pink-purple as they develop. The seeds are spherical and light or dark brown. It reproduces by stem fragments as well as seeds. Stem pieces spread via becoming attached to animals, footwear, and vehicles, and they are also found in discarded horticultural waste. Various animals, including birds and foxes, consume the fruit and spread it in their droppings. Wheel cactus favors arid and semi-arid environments, as well as milder temperate zones. It can invade pastures, rocky outcrops, open forests, and ranges.

Motoroko (*Opuntia)* offers both fruits and seeds with substantial nutritional value. However, in Botswana, while the fruits are consumed, the nutrient-rich seeds are largely overlooked. These seeds contain essential nutrients critical for plant-growth, presenting an untapped resource to addresses nutritional deficiencies. There is lack of information that study emphasizes the potential health benefit of the seeds. This work explores the qualitative phytochemical examination of two *Opuntia* species seeds, revealing a variety of bioactive chemicals and providing insight into the field of mucilage characterization. Normally, after the pulp is extracted, the prickly pear seeds are typically thrown away, leaving a significant volume of seeds as waste. Analysing the ingredients of these seeds may assist in identifying potential applications for them in the pharmaceutical and cosmetic sectors, as well as in animal feed and human nutrition as a novel source of meal and oil.

**MATERIALS AND METHODS**

**Description of the study area**

*O. robusta* seeds were collected from Letlhakeng village (24.0973° S, 25.0288° E) and *Opuntia Ficus-indica* from Gakuto village (24.4402° S, 25.8082° E) in Botswana

**Sample collection**

*O. robusta* and *O. ficus-indica* fruits were harvested using gloves to protect the hands from the spines and avoid contamination. Pruning shears were employed to carefully cut the fruits from the plants, and a plastic bag was utilized to gather them safely. Mature and healthy fruits were selected to ensure the seeds were fully developed and unharmed. To remove the seeds from the fruits, the harvested fruits were gently sliced open with a sharp knife. The seeds were then carefully extracted from the pulp using a spoon or tweezers, ensuring not to damage them during the process. Once all the seeds were extracted, they were thoroughly washed with water to remove any remaining pulp residue and spines. After washing, the seeds were spread out in a single layer on a clean surface to air dry. Placing them in a well-ventilated area allowed for efficient drying. Once completely dry, the seeds were stored in labelled sealable plastic bags.

**Sample preparation**

Following storage, the dried seeds were crushed into a fine powder using a mortar and pestle in the laboratory. This step was essential to increase the surface area for subsequent analysis. The powdered seeds were then transferred to sealed plastic bags for storage until further use for the qualitative phytochemical analysis and mucilage characterization. All analysis were done triplicate.

**Mucilage extraction**

The mucilage extracted from *O. ficus-indica* and *O. robusta* seeds was conducted using a method adapted from previous studies6. Samples were crushed using mortar and pestle to increase the surface area. Each sample was first weighed into a different beaker containing 200 grams. Subsequently, each beaker was filled with 600 mL of distilled water, covering the crushed seeds. It was heated to 60oC in a water bath while being constantly stirred for one hour. After filtering, the concentrated solution was allowed to cool to ambient temperature. After adding 40 mL of acetone to the concentrated solution, the sample was centrifuged at 250 rpm for 15m to isolate the mucilage. In a drying oven, the sediment was then dried to constant weight at 45oC. Using a mortar and pestle, the hard mucilage cake was pounded into a fine powder.

Mucilage yield (%) = (weight of dried mucilage)/(weight of sample) X 100

**Determination of moisture content**

The moisture content of the mucilage was determined according to the Association of Official Analytical Chemists (AOAC) method7. To ensure even moisture distribution, 5 grams of each sample were weighed. A glass dish was then precisely weighed and recorded as W1. The ground seed sample was evenly distributed across the plate. The sample dish was placed in an oven preheated to 105°C for a predetermined time, typically two to three hours, to effectively eliminate moisture. After the drying process, the dish was allowed to cool in a desiccator and then weighed again, recording this weight as W2. The moisture content was calculated using the formula:

[(W1 - W2) / W1] x 100 and expressed as a percentage.

**Determination of ash content**

The ash content of the mucilage was determined using methods outlined in AOAC7. Three grams of each sample were used for carbonization and ashing in a muffle furnace. Initially, the crucible was cleaned and dried at 120oC for one hour, then cooled in a desiccator. The mass of the crucible was weighed on an analytical balance and recorded as M1. About 3 grams of the sample, recorded as M2, were then dried in an oven at 120oC for one hour. Following drying, the sample was heated on a Bunsen burner until the content turned black. The dish with its content was then transferred into a muffle furnace and ignited at 550oC for about 8 hours until it was free from carbon. After ashing, the sample was removed from the furnace, allowed to cool in a desiccator until reaching ambient temperature, and then weighed. This final mass was recorded as M3. Ash on dry matter base was expressed as follows:

Ash (%) = M3-M1 x 100 M2

**Determination of water holding capacity**

The procedure outlined by Aremu et al8 was followed to determine the water holding capacity. In a centrifuge tube, one gram of each sample was mixed with 10 mL of distilled water. The combination was then allowed to sit at room temperature for an hour. Following that, the mixture was centrifuged at 300 rpm for 20 minutes, and the supernatant was then poured into a graduated cylinder measuring 10 mL.

**Qualitative analysis of phytochemicals**

* **Phytochemical Extraction**

For the phytochemical extraction, 5 grams of each sample were weighed into three separate beakers. For the first sample, 100 mLof ethanol was added to the first beaker, 100 mL of methanol to the second beaker, and 100 mL of n-hexane to the third beaker. The second sample followed the same procedure with the same solvents. All the six beakers were labelled and covered with aluminium foil. They were left at room temperature for 2 hours, then placed in a water bath at 60 degrees Celsius for 30 minutes. After cooling, the mixtures were filtered using filter paper, and the resulting solutions were transferred into labelled centrifuge tubes. These tubes were centrifuged for 15 m at 250 rpm. After centrifugation, the extracts were placed in a refrigerator ready for analysis9.

* **Test for Saponins**

To test for saponins, 6 mL of distilled water were added to 2 mL of each extract. The mixture was then vigorously shaken. The formation of persistent froth or bubbles indicated the presence of saponins in the extracts10.

* **Test for Tannins**

To test for tannins, 2 mL of each extract were mixed with 10% alcoholic ferric chloride. The appearance of a brownish-blue or black color indicated the presence of tannins in the extracts10.

* **Test for Phenols**

To test for phenols, 2 mL of 5% aqueous ferric chloride were added to 2 mL of each extract. The formation of a blue color indicated the presence of phenols in the sample extracts11.

* **Test for Flavonoids**

To test for flavonoids, 2 mL of each extract were taken. After adding 2-3 drops of 20% sodium hydroxide, a bright yellow color appeared. When a few drops of 70% diluted hydrochloric acid were subsequently added, the yellow color disappeared. The appearance and subsequent disappearance of the yellow hue indicated the presence of flavonoids in the extract samples10.

* **Test for Terpenoids**

To test for terpenoids, 1 milliliter of each extract was taken, and 0.5 milliliters of chloroform was added. This was followed by the addition of a few drops of concentrated sulfuric acid. The formation of a reddish-brown precipitate indicated the presence of terpenoids in the extracts10.

* **Test for Alkaloids**

For the alkaloid test, 1mL of each extract was mixed with 1mL of Marquis reagent, 2mL of concentrated sulfuric acid, and a few drops of 40% formaldehyde. The mixture was observed for a color change. The presence of a dark orange or purple color indicated the presence of alkaloids in the extracts12.

**RESULTS**

**Phytochemicals Profile**

The qualitative analysis of phytochemicals was tested using three solvents (ethanol, methanol, and n-hexane). Tannins, phenols, and alkaloids in *O. ficus-indica* and *O. robusta* produced negative results in all the solvents (Table 1). This suggests that these phytochemicals are either entirely absent or present in very small amounts/below detection limits in the examined samples. *O. ficus-indica* demonstrated a stronger presence of saponins in polar solvents compared to *O. robusta*, as it showed less intense saponin related foam formation across all solvents tested. Terpenoids were detected in both the species while alkaloids are not.

**Mucilage Characterization**

The Mucilage yield from *O. ficus-indica* and *O. robusta* ranged from 3.03 to 3.19 g/100g (Table 2). There is a significant different between the mucilage yield of the two samples. The mucilage yield of *O. ficus-indica* was significantly (p<0.05) high compared to that of O*. robusta*. The amount of ash in mucilage serves as a proxy for inorganic minerals in the sample. In this study *O. robusta* seeds (2.28% ± 0.043) had a slightly lower ash content than *O. ficus-indica* seeds (2.44%± 0.043). *O. robusta* exhibited a higher moisture content (7.33% ±0.125) compared to *O. ficus-indica*, which had a moisture content of 4.97% ± 0.125) and there is a signaficant diffrence between the two values (p<0.005). *O. robusta* had a slightly lower water holding capacity of 4.80 ± 0.082ml/g in than *O. ficus-indica,* which showed a capacity of 5.20 ± 0.082. This indicates that the mucilage of *O. ficus-indica* is somewhat more capable of holding water than that of *O. Robusta* (Table 2).

Table 1: Qualitative phytochemical analysis for *O.ficus indica* and *O.robusta* seeds

|  |  |  |  |
| --- | --- | --- | --- |
| **Phytochemicals** | **Solvent** | ***O. ficus-indica*** | ***O. robusta*** |
| Saponins | Ethanol | ++ | + |
|  | Methanol | ++ | + |
|  | n-Hexane | + | + |
|  |  |  |  |
| Tannis | Ethanol | - | - |
|  | Methanol | - | - |
|  | n-Hexane | - | - |
|  |  |  |  |
| Phenols | Ethanol | - | - |
|  | Methanol | - | - |
|  | n-Hexane | - | - |
|  |  |  |  |
| Flavonoids | Ethanol | + | + |
|  | n-Hexane | + | - |
|  | Methanol | + | - |
|  |  |  |  |
| Terpernoids | Ethanol | + | + |
|  | Methanol | + | + |
|  | n-hexane | + | + |
|  |  |  |  |
| Alkaloids | Ethanol | - | - |
|  | Methanol | - | - |
|  | n-Hexane | - | - |

Key: ++), strong positive, +, positive, - negative

Table 2: Chemical Composition of mucilage extracted from *O.ficus-indica* and *O.robusta*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Samples | Mucilage yield (%) | Ash Content (%) | Moisture Content (%) | WHC (mL/g) |
| *O. ficus-indica* seeds | 3.19±0.013a | 2.44 ± 0.043 | 4.97 ±0.125a | 5.20 ±0.082 |
| *O. robusta seeds* | 3.03 ± 0.013b | 2.28 ±0.043 | 7.33 ±0.125b | 4.80 ±0.082 |

WHC: water holding capacity. Reported values were mean ±SE (n=3). Means with different letters in the same column are significantly different (P<0.05).

**DISCUSSION**

**Phytochemicals profile**

*O. ficus-indica* contained more saponins than *O. Robusta.* Methanol and ethanol, both polar solvents, are effective at extracting saponins due to their ability to dissolve these compounds more readily compared to non-polar solvents like n-hexane13. The stronger presence of saponins in *O. ficus-indica* suggests that it may have greater potential for applications that benefit from the surfactant properties of saponins, such as emulsifying agents in food and cosmetics, and as natural detergents or foaming agents in various industrial processes. The lower saponin content in *O. robusta* may make it more suitable for uses where high saponin levels could be undesirable, such as in certain food products where excessive foaming or bitterness needs to be minimized. These findings indicate that while both species have valuable applications, *O. ficus-indica* may be preferable for industries seeking strong surfactant properties, whereas *O. robusta* could be better suited for applications requiring lower saponin content.

The absence or minimal presence of tannins, phenols, and alkaloids in both species suggests that these *Opuntia* seeds may not be suitable sources for these specific phytochemicals, which are often sought for their antioxidant, anti-inflammatory, and antimicrobial properties14. However, it is important to note that the phytochemical composition of *Opuntia* species has been found to vary depending on factors such as the plant part, age, and environmental conditions14.

This shows that terpenoids are present in substantial amounts in a variety of solvent polarities, indicating that they constitute an important part of the phytochemical profile of *O. ficus-indica* seeds. Terpenoids are an extensive and varied group of organic substances that are made by many different types of plants. Terpenes and terpenoids as important chemical mediators of these abiotic and biotic interactions15.

**Mucilage characterization**

Gums and mucilage's are complex carbohydrates made up of polysaccharides that comprise one or more monosaccharides or their derivatives bound together in a dizzying array of connections and structures16. The food industry uses mucilage, a naturally occurring biopolymer derived from plants, extensively as a binder, emulsifier, film coating, and fat substitute because of its availability, safety, and low cost17. By serving as an emulsifier, thickening, gelling agent, or texture altering agent, it also improves food quality6. The Mucilage yield for *O. ficus-indica* and *O. Robusta*, in our study ranged from 3.03 to 3.19 g/100g (Table 2). Reports indicated that many factors such as environmental circumstances, plant age, and the precise portion of the plant used, can affect the variation in mucilage yield18. Mature plant generally give more mucilage than younger ones do, and environmental variables like temperature, availability of water, and type of soil can have a big impact on how much and how well mucilage is produced. The weight of dry seeds of *Opuntia ficus-indica* seeds can have a mucilage yield of up to 4-6%19.

Ash in mucilage serves as a proxy for inorganic minerals in the sample. The ash content of *O. robusta* seeds (2.28% ± 0.043) and *O. ficus-indica* seeds (2.44%± 0.043) are relatively low, but still indicate that both samples contain minerals contributing to their overall composition. Moisture content in *O. robusta* (7.33% ±0.125) and *O. ficus-indica*, (4.97% ± 0.125) are different and this suggests that *Opuntia ficus-indica* either underwent more extensive drying or inherently contains less water. Previous research has reported moiture content for *O.ficus-indica* sample as 5.39%20.

The mucilage's ability to hold onto water is indicated by its water holding capacity (WHC), which is crucial to its functional qualities.  *O. robusta* had a slightly lower water holding capacity of 4.80 ± 0.082ml/g while *O. ficus-indica,* showed a capacity of 5.20 ± 0.082 ml/g. This difference in mucilage composition or structure may influence the results. According to study by García-Barradas et al.,(2023)21 mucilage showed a WHC range from 5.59 to 13.45 ml/g and is almost similar to this study.

Both species, *O. Ficus-indica* and *O. robusta* demonstrated the presence of saponins, terpenoids, and flavonoids, with *O. ficus-indica* showing a stronger presence of saponins in polar solvents, highlighting its potential for industrial applications that benefit from the surfactant properties of saponins. The absence of tannins, phenols, and alkaloids in both species suggests limited use in applications requiring these compounds. The mucilage characterization revealed that *O. ficus-indica* seeds had slightly higher ash and moisture contents, and a better water holding capacity compared to *O. robusta*, indicating its superior functional properties for potential use in food and pharmaceutical industries. It is recommended that further research be conducted to explore the full potential of *O. ficus-indica* and *O. robusta* seeds native to Botswana. Reports indicate that Zinc Oxide Nanoparticles Synthesized Using *Opuntia ficus* indica Fruit Aqueous Extract exhibit good Antibacterial Activity22.

Botswana's underutilization of motoroko seeds indicates a crucial knowledge gap and a missed opportunity. These seeds are a useful nutritional source since they contain critical nutrients required for plant growth, making them suitable for human consumption. This study revealed the seeds' potential as a sustainable and locally available dietary supplement by concentrating on their quantitative phytochemical composition and nutritional richness. Addressing this gap is critical not just for nutritional enhancement, but also for supporting food security and boosting overall population well-being. Furthermore, investigating the mucilage qualities offers a comprehensive strategy to employing these seeds in a variety of industries, potentially boosting socioeconomic growth and encouraging sustainable agricultural practices. Bridging this knowledge gap has the potential to make Botswana a more resilient and health-conscious society.

**Conclusion**

*Opuntia* seeds can potentially be used in the food and pharmaceutical industries since they contain useful bioactive compounds and mucilage with good functional properties. The utilization of these underutilized seeds in Botswana must be promoted. Quantitative determination of the phytochemicals is recommended.

**Disclaimer (Artificial intelligence)**

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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