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Evaluation of the acid-neutralizing and cytotoxicity properties of novel plant mucilage used as an alternative treatment for peptic ulcers and as antacids in Namibia

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ABSTRACT. Hypersecretion of gastric acid damages the stomach lining, causing the formation of peptic ulcers. Mucilage from medicinal plants offers a relaxing and soothing effect to the endodermal lining of the gut and has antacid properties, which can protect the mucosal lining from gastric acidity. This is the first report aimed to evaluate the physicochemical characteristics, acid-neutralizing, and cytotoxicity properties of traditionally used aqueous mucilage from *Asparagus exuvialis* and *Sesamum capense*. The physicochemical properties were determined by biochemical methods. Acid neutralizing and buffering capacities were determined by titration methods. Normal mouse embryonic fibroblast cells were used for cytotoxicity evaluation by MTT assay. The physicochemical characterization confirmed the presence of carbohydrates, alkaloids, saponins, proteins, tannins, flavonoids, and glycosides. *Sesamum capense* mucilage exhibited the most potent artificial gastric juice neutralizing capacity pH of 4.62±0.01, 8.0±0.00 acid neutralization capacity per gram of acid, and 30 minutes duration of acid neutralization. The aqueous mucilage from *S. capense* did not cause any significant cytotoxicity to 3T3 cell lines showing an IC50 value of 91.5 ± 0.06 µg mL^{-1}, confirming the safe nature of the mucilage. These findings revealed that *S. capense* has the potential to neutralize gastric acid responsible for ulceration and can be safely consumed.

Keywords: buffering capacity; mucilage; therapeutic compounds; lethality.

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Introduction

Stomach is an organ of the digestive system, responsible for the mechanical and chemical digestion of food. Chemical digestion is achieved through the secretion of gastric juice, which contains hydrochloric acid, mucus, intrinsic factor, and enzymes. However, hypersecretion of gastric juice, mainly hydrochloric acid, damages the stomach lining and causes the formation of ulcers (Yafout, Elhorr, El Otmani, & Khayati, 2022). In many cases, *Helicobacter pylori* are also reportedly responsible for causing peptic ulcers together with the prolonged use of non-steroidal anti-inflammatory medicines, such as aspirin and ibuprofen, and other factors, such as stress and consumption of spicy foods (Houshia et al., 2012). In 2018, a global mortality rate of over 15,000 due to peptic ulcer diseases was reported, with 50 cases (0.33%) reported from Namibia (Lirazan, Cua, Alvarez, 2018; World Health Organization, 2018).

Current treatments for gastric ulcers and gastroesophageal reflux work by neutralizing excess gastric acid or by reducing the amount of acid secreted by oxyntic cells also called parietal cells. Moreover, other treatments work by protecting the gastric mucosa from auto-digestion as well as mucosal erosion (Wu, Chen, & Chen, 2010). The available chemosynthetic drugs and pharmaceutical products for ulcers are not only costly but have also demonstrated an increase in side effects and drug interaction complications (Henry & Langman, 1981; Mehta, 2016). Patients suffering from ulceration usually receive prescribed medication, such as H_2 receptor antagonists, proton pump inhibitors, and cryoprotectants. However, some patients have shown side effects such as diarrhea, constipation, chronic renal failure, cardiac arrhythmia, and hematological disorders. Furthermore, these therapeutics alter the biochemical mechanisms of the body upon prolonged use (Sharma, Bhot, & Chandra, 2014; Chaudhary, Saxena, Sharma, & Mohseen, 2020). Hence, there is a need for an alternative treatment that will provide the same benefits to patients with peptic ulcers, however, with less to no antagonistic effects.

Medicinal plant decoctions have been used worldwide to treat chronic peptic ulcers since they are considered safer with little to no side effects (Wu et al., 2010; Ardalan & Rafieian-Kopaei, 2013). Mucilage specifically offers a relaxing and soothing effect to the endodermal lining of the gut, and also acts as a relaxant and antispasmodic to the lungs and the urinary tract through the spinal reflex. Moreover, mucilage protects the mucosal lining from gastric acidity while regulating intestinal flora and protecting the body against ingested toxins or bacteria, such as *H. pylori* that are associated with peptic ulcers (Marciano, 2020). This is due to the presence of polysaccharides that cause slippery viscosity and swelling properties in the water, resulting in a production of a gel-like mass that can help protect irritated tissues, such as inflamed mucous membranes (Marciano, 2020). Among biological secondary metabolites, alkaloids, flavonoids, anthraquinone, and tannins are reported to have antiulcer activity as well as gastro-duodenal protective effects (Nascimento et al., 2015; Goel, Das Gupta, Ram, & Pandey, 1991).

Asparagus exuvialis and *S. capense* produce mucilage that has been reported worldwide to have numerous medicinal properties. Tuber of *A. exuvalis* is used in the traditional setting as alternative medicine as antacids, to offer relief from stomach cramps, and stomach ulcers and to detoxify the blood. *S. capense*, on the other hand, is used to treat stomach pain, stomach cramps, and bloating, and as an antacid, in Namibia. Despite the long history of using *A. exuvialis* and *S. capense,* there is limited data regarding the cytotoxicity and biological properties of these plants. Hence, this study aimed at evaluating the physicochemical characteristics, acid-neutralizing and buffering capacities, and cytotoxicity properties of aqueous mucilage from *A. exuvialis* and *S. capense.*

Material and methods

Plant collection and preparation of mucilage crude extracts

Fresh tubers of *A. exuvialis* Burch.ex DC (Voucher number BRL 38) and the whole plant of *S. capense* Burm. f. (Voucher number AI 05) were collected in March 2017 (permit number: 2221/2017). The plants were collected based on their ethnomedicinal uses by local people from Northern Namibia in treating stomach ulcers, stomach pain, and gastric reflexes relieving properties, and the voucher specimens collected were authenticated. The collected fresh tubers of *A. exuvialis* and leaves of *S. capense* were washed to remove sand and shade dried at room temperature for 30 days and then ground to powder. Briefly, 20 g powdered plant material for each plant was macerated in 100 mL distilled water, respectively, at room temperature for 24 hours. Macerations were further placed in a shaker water bath with the temperate set at 60°C for 1 hour. Mucilage extracts were filtered through Whatman 1 filter papers with pore sizes of 110 mm to collect the filtrates. Filtrates were further dried into powder by rotary evaporation and freeze-drying. Dry crude extracts were stored at 4°C until further used.

Preparation of mucilage suspension for physicochemical characterization

Briefly, 1.5 g each mucilage dry extract and 5 g magnesium carbonate were triturated in a motor pestle containing 50 mL distilled water to form a paste (Chatterjee, Auddy, & Chaudhuri, 2016). This was used as suspension for different physiochemical characterization assays such as flow rate, redispersion, sedimentation, pH, swelling index, and foaming index.

Determination of mucilage flow rate

The flow rate of each mucilage suspension was determined by recording the time required for the suspension solution to flow through a 10 mL pipette and the flow rate was calculated by the formula:

Flow rate = Volume of solution in pipette (milliliters)/ Flow time (seconds)

Redispersion

Each suspension solution was poured into calibrated tubes and kept at room temperature for complete 20 days. At an interval of 5 days, each tube was taken out and shaken vigorously to redistribute the sediments. The presence of deposits, if any, was recorded.

Determination of sedimentation volume

The sedimentation volume of each mucilage suspension was measured by taking 25 mL suspension in 45 mL measuring tubes, allowed to stay at room temperature for 10 days, and observed at regular intervals of 24 hours, for five days. The F% (percentage) was calculated by the formula:

$F\% = (V_u/V_0) \times 100$

where, V_u= ultimate volume of the suspension (mucilage + magnesium carbonate) after a certain interval, V_0= original volume of the suspension.

Determination of pH for different mucilage suspension

The pH of each suspension was observed by taking 1mL the suspension solution and diluting with 99 mL distilled water and shaking for about 5 minutes, and then measuring the solution pH using a pH meter.

Swelling index

The swelling and water holding capacity of *A. exuvialis* and *S. capense* mucilage were determined using the modified method reported by Poosarla and Muralikrishna (2017). Briefly, 1 g *A. exuvialis* and *S. capense* mucilage powders were accurately weighed and transferred to 250 mL stoppered measuring cylinders. The initial volume occupied by the powder was noted as 10 mL and the volume was made up to 120 mL with distilled water, 0.1N hydrochloric acid (HCL), or phosphate buffer saline (PBS) (6.8 pH). Cylinders were stoppered, shaken gently, and set aside for 24 hours. The swelling index (SI) was expressed in percentage and was calculated by the following equation:

$SI\% = (Vt - V0)/V0 \times 100$

where SI% = swelling index, V0 = volume occupied by the mucilage powder before hydration and Vt = volume occupied by the mucilage powder after hydration.

Determination of the foaming index of mucilage extracts in different solvents

Exactly 1 g mucilage was added to a 250 mL conical flask containing 100 mL boiled water. Conical flasks with macerations were maintained at 90°C for 30 minutes under magnetic stirring. The mixtures were allowed to cool at room temperature, filtered into a volumetric flask and topped up to 100 mL using distilled water. Ten stopper test tubes were labeled 1 to 9. Exact amounts of 1 mL to 9 mL of the extracts were added to respective test tubes and topped up to 10 mL in each tube using either distilled water, 0.1N hydrochloric acid, or phosphate buffer saline (6.8 pH). If the foam produced in a tube is less than 1 cm, then the foaming index is less than 100 and was considered non-significant. Tubes were shaken and allowed to stand for 15 minutes, the height of the foam was then measured.

Foaming index = 1000/a

where a = Volume of decoction used for preparing the dilution in the tube where exactly 1 cm foam or more is observed.

Screening for therapeutic compounds in aqueous mucilage extracts

The therapeutic compound screening was carried out to detect compounds associated with gastric benefits using the methods summarized in Table 1. The analysis was performed using 2g mL^{-1} mucilage powder in distilled water.

Table 1. Screening for therapeutic compounds in aqueous mucilage.

Active constituents	Test	Reference
Carbohydrates	Molish's test	Ramamurthy and Sathiyadevi (2017)
Alkaloids	Dragodroff's test	Ukoha, Cemaluk, Nnamdi and Madus (2011)
Fat and oil	Saponification test:	-
Acid compounds	Acid compound test	Ukoha et al. (2011)
Saponins	Foam test	Ismail et al. (2017)
Proteins	Biuret test	Ukoha et al. (2011)
Tannins	Ferric chloride test	Ukoha et al. (2011)
Flavonoids	Braymer's test	Ismail et al. (2017)
Coumarins	Sodium hydroxide test	Ismail et al. (2017)
Glycosides	Modified Bontrager's test	BaoDuy, Trang, & Trang (2015)

Antacid assays

Preparation of stock solutions for antacid assays

The antacid test was performed based on the method by Lirazan et al. (2018) with a few modifications. The aqueous mucilage extracts of *A. exuvialis* and *S. capense* were used to prepare stock solutions of 100 mg mL^{-1} concentrations in distilled water. Another extract was prepared to contain equal quantities of *A. exuvialis* and *S. capense* since these plants are also used in combination.

In-vitro antacid screening

A concentration of 1 mg mL^{-1} was prepared by adding 1mL stock solution to 99 dm^3 distilled water. Calcium carbonate 1 mg mL^{-1} in distilled water was used as a positive control; while distilled water was used as a negative control. For this experiment, the setups were maintained at 37°C. Briefly, 40 mL each test solution was continuously stirred on a hot plate magnetic stirrer at 300 rpm for 1 minute. Exactly 10 mL 0.5 N hydrochloric acid was added to the test solution while stirring on a magnetic stirrer at 300 rpm for 10 minutes after the addition of acid. The pH was read and recorded with a standardized pH meter.

Determination of the neutralizing effect of extracts on artificial gastric juice

The artificial gastric juice was prepared by dissolving 2 g sodium chloride and 3.2 g pepsin in 500 dm^3 deionized water. Briefly, 7 mL ice-cold hydrochloric acid was added. The volume was made up to 1,000 mL by adding deionized water. The pH of the solution was adjusted to 1.2. Exactly 50 mL each test solution (i.e. 1 mg mL^{-1} mucilage extract solution, 1 mg mL^{-1} calcium carbonate solution positive control or distilled water negative control) was added to 55 mL artificial gastric juice at pH 1.2, and the resulting pH value was determined at 37°C.

Acid neutralization capacity

The titration method was used to determine the acid-neutralizing capacity of the aqueous mucilage extracts, which showed antacid activity with a pH of 3.5 or greater during the preliminary antacid screening. Briefly, 5 mL antacid suspension was measured in 50 mL flat-bottom flasks and weighed. The suspension was then poured into a 250 mL beaker and made up to 70 mL with carbon dioxide-free distilled water and stirred for 1 minute. An exact volume of 30 mL 1.0 N hydrochloric acid was pipetted into the suspension while stirring for 15 minutes and the pH was measured. The excess hydrochloric acid was titrated with 0.5 N sodium hydroxide (NaOH) until a pH of 3.5 was obtained. The experiment was carried out at 37°C on a magnetic stirrer. The number of milliequivalents (mEq) of acid consumed per gram of antacid was calculated using the Equation:

$$\text{Total mEq} = (30 \times \text{NHCl}) - (\text{VNaOH} \times \text{NNaOH})$$

where NHCl and NNaOH are the normalities of HCl and NaOH, respectively, and VNaOH is the volume of NaOH used for the back titration (Ayensu, Bekoe, Adu, Brobbey, & Appiah, 2020).

Determination of the buffering capacity

Briefly, 5 mL each of the antacid mucilage and positive control were measured and transferred into 500 mL beakers and 50 mL distilled water was added and heated to 37°C. Suspensions were stirred for 1 minute and the initial pH was recorded with a standardized pH meter. An exact volume of 100 mL 0.1 N HCl previously heated to 37°C was added to each suspension under continuous stirring. The rate of pH change of the resulting solution was measured at an interval of 5 minutes. During this process, a quantity of 20 mL suspension was taken with a pipette and replaced with 20 mL fresh 0.1 N HCl. This process was repeated at 5 minute intervals until a pH below 2.75 was observed for the different treatments in triplicates (Ayensu et al., 2020). Data are shown as the mean ± SEM.

Cell culture and cytotoxicity test

The normal mouse embryonic fibroblast cells (NIH/3T3) were used for cytotoxicity screening of the *S. capense* and *A. exuvialis* mucilage by MTT assay, as described by Das and Devi (2015) with a few adjustments. Cell viability was evaluated by the MTT assay method. NIH/3T3 cells were seeded in 96-well plates at 5 x 10^3 density. Following a 24 hours incubation and attachment, cells were treated with different concentrations of

extract from 1.60 to 100 µg mL^{-1}. The 96 well plates were incubated at 37ºC with 5% CO_2 for 24 hours. Then, after an additional 2 hours incubation period with (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide), the culture media was carefully aspirated without disturbing the crystals of MTT formed at the bottom of the wells and 100 µL solubilization solution of Dimethyl sulfoxide (DMSO) was added. The plate was gently shaken in a shaker to solubilize the formazan. The microplate reader was used to measure the absorbance at the wavelength of 570 nm. The following formula was used to calculate the % viability after subtracting the mean mass of the blank.

% Cell viability = (Mean mass absorbance of treated cells/Mean absorbance of control) x 100

Statistical analysis

The results reported in this study are the mean values of at least three analytical determinations. All experiments were done in triplicates and statistical analysis was performed employing Graph Pad Prisms software version 7.0. Comparison between groups was made by Two-way ANOVA, followed by Bonferroni's post-tests. All data were presented as mean ± Standard deviation, $p < 0.005$.

Results and discussion

Mucilage suspension physiochemical characterization

Physicochemical characterization of mucilage isolated from *A. exuvialis* and *S. capense* showed between green and brown color, respectively, mucilaginous taste and characteristic odor. They are soluble in water but insoluble in organic solvents. The pH of the mucilage suspensions was ⩾ 5.0 in distilled water at 25°C. This indicates that this mucilage would be less irritating to the gastrointestinal tract. The swelling index provides essential information regarding pharmaceutical disintegration. The swelling index of the mucilage was ⩾ 2.0 in hydrochloric acid, ⩾ 4.0 in phosphate buffer saline, and ⩾ 5. 0 in distilled water. This suggests that the mucilage swells excessively in water. The physicochemical properties are listed in Table 2. The application of plant mucilage has been linked with protective properties of the gastrointestinal mucosal tract (Sharma et al., 2014). The phytochemical tests revealed that mucilage was present in the novel extracts. Hence, understanding the physiochemical physicochemical of mucilage will offer a clear understanding of the behavior of the mucilage formulation in the gastrointestinal tract (Sayyad & Sakhare, 2018).

Table 2. Physiochemical characterization of mucilage suspension.

Parameters	*Asparagus exuvialis*	*Sesamum capense*
Colour	Brown	Green
Smell	Odor	Odor
Flowrate in (mL sec^{-1})	1.25 ± 0.12	0.833 ± 0.13
Redispersion property (mL)	0.4 ± 0.11	0.8 ± 0.12
Foaming index: Water	⩾ 111	No foaming
0.1 N HCL	⩾ 111	No foaming
PBS (pH 6.8)	⩾ 111	No foaming
Sedimentation volume (%)	40	48
Swelling index: Water	5.53 ± 0.12	6.52 ± 0.12
0.2N HCL	2.3 ± 0.10	2.4 ± 0.11
PBS (pH 6.8)	4.2 ± 0.00	4.6 ± 0.12
pH	4.56	5.54
Solubility: Cold water (25ºC)	slightly soluble	soluble
Warm water (60ºC)	soluble	soluble
Methanol	insoluble	insoluble
Ethanol	insoluble	insoluble
Dichloromethane	insoluble	insoluble
n-hexane	insoluble	insoluble

Values presented as mean ± SD of triplicate determinations.

The ability of mucilage to swell in aqueous medium as observed in this study is crucial for mucoadhesion; excessive swelling due to overhydration allows the formation of a slippery surface, which could be favorable in cases of antiulcer medication. Studies have also shown that mucilage with foaming ability appears to be effective in therapy for acute postprandial heartburn (Lanza, Smith, Page-Castell, & Castell, 1986). Moreover, foaming properties of some antacids, such as that reported in this study could be due to the presence of

alginate in the mucilage. Foaming properties of an antacid may reduce the amount of acid that comes in contact with the esophagus and this may help prevent gastroesophageal reflux (Antacids for Gastroesophageal Reflux Disease (GERD), 2014). The higher percentage swelling index of oral medication demonstrates the hydrophilic properties of the decoction.

Analysis of bioactive compounds, micro-molecules, and macromolecules

In the present study, the mucilage extracted from *S. capense* showed the presence of all screened compounds, except for coumarins (Table 3). *A. exuvialis*, on the other hand, did not show the presence of alkaloids, fat oils, and coumarins. Moreover, *A. exuvialis* showed the presence of acid compounds that were absent in *S. capense*. While the traditional usage of active natural products was done without an understanding of basic chemistry, modern research strives to understand the chemical composition of different natural products used to treat common ailments (Ismai et al., 2017). Studies have linked the presence of phytochemical compounds, such as saponins, alkaloids, tannins, flavonoids, and carbohydrates to the antacid and antiulcer activities of plants (Boruah & Nath, 2016). Swelling properties of the novel mucilage could be due to the hydration of the macromolecule present in the decoction (Kaleemullah et al., 2016). Other in vivo studies have related the presence of these secondary metabolites to the antiulcer activity of Diodiasarmentosa, Cassia nigricans, Ficus exasperate, and Synclisiascabrida commonly used in Nigeria (Zewdu & Aragaw, 2020).

Table 3. Therapeutic compounds with gastroprotective effects present in the aqueous mucilage of *Asparagus exuvialis* and *Sesamum capense.*

Active constituents	Test	Interference	
		A. exuvialis	*S. capense*
Carbohydrates	Molish's test	+	+
Alkaloids	Dragodroff's test	-	+
Fat and oil	Saponification test:	-	+
Acid compounds	Acid compound test	+	-
Saponins	Foam test	+	+
Proteins	Biuret test	+	+
Tannins	Ferric chloride test	+	+
Flavonoids	Braymer's test	+	+
Coumarins	Sodium hydroxide test	-	-
Glycosides	Modified Bontrager's test	+	+

+: the presence of a therapeutic compound; -: absence of a therapeutic compound

In-vitro antacid screening

This test showed that for 10 minutes the various samples tested, except for *A. exuvialis* mucilage extract, were capable of maintaining pH values above 3.5 to completely neutralize the acid solution. Calcium carbonate that was used as a positive control followed by *S. capense* showed the highest antacid activity. Although *A. exuvialis* mucilage did not show antacid activity, the combination formulation of *A. exuvialis* and *S. capense,* showed antacid activity (Table 4), with a pH above 3. For the acid-neutralizing capacity of the gastric acid, the pH values of calcium carbonate, *S. capense*, and a combination formulation of *A. exuvialis*+ *S. capense* were found to be significantly higher than water and *A. exuvialis*, indicating a significantly better neutralizing effect of calcium carbonate, *S. capense* compared to water (Table 5).

Table 4. The in-vitro antacid activity of different suspensions.

Samples	Initial pH	Preliminary Antacid Test (pH)
Asparagus exuvialis	4.68	1.50 ± 0.01*
Sesamum capense	6.17	5.04 ± 0.00
Negative control	5.77	1.38 ± 0.23*
Positive control (Calcium carbonate)	9.08	7.96 ± 0.00
Asparagus exuvialis + *Sesamum capense*	5.26	4.02 ± 0.01

Values presented as mean ± SD of triplicate determinations; *p < 0.05 compared to Calcium carbonate (positive control).

Acid neutralizing capacity

The acid-neutralizing capacity of all the samples was determined to be in a range of 7.6 ± 0.00 to 12.5 ± 0.01. The combination formulation of *A. exuvialis*+ *S. capense* had the lowest acid-neutralizing capacity

(Table 6), while the positive control calcium carbonate showed the highest acid-neutralizing capacity of 12.5 ± 0.01 mEq g^{-1}. *S. capense* also showed a good acid-neutralizing capacity. The Food and Drug Administration (FDA) recommends that a good antacid should have a neutralizing capacity ⩾ 3.5 (Fokunang et al., 2019). The acid-neutralizing capacity of this study clearly shows that mucilage from *S. capense* and the combination formulation have acid-neutralizing properties, since they showed an acid-neutralizing capacity above 3.5. Despite the presence of different bioactive, polysaccharides and proteins in the mucilage from *A. exuvialis*, this extract did not show acid-neutralizing properties, this could be due to the presence of an acid compound detected in this extract.

Table 5. The neutralizing effect of mucilage extracts on artificial gastric juice.

Samples	Initial pH of the gastric simulation	Final pH
Asparagus exuvialis	1.2	1.61 ± 0.01*
Sesamum capense	1.2	4.62 ± 0.01
Negative control (Water)	1.2	1.73 ± 0.22*
Positive control	1.2	5.7 ± 0.01
Asparagus exuvialis + *Sesamum capense*	1.2	4.64 ± 0.00

Values presented as mean ± SD of triplicate determinations; *p < 0.05 compared to Calcium carbonate (positive control).

Table 6. Acid neutralizing capacity (ANC) of different antacids.

Samples	Density	Volume of 1.0 N HCL (mL)	Volume of 0.5 N of NaOH (mL)	Total mgEq	ANC/gram of acid
Sesamum capense	0.72	30.0	48.5	5.75 ± 0.02	8.0.0 ± 0.01
Calcium carbonate	0.80	30.0	20.0	10 ± 0.01	12.5 ± 0.01
Asparagus exuvialis + *Sesamum capense*	0.74	30.0	48.5	5.6 ± 0.00	7.8 ± 0.00

Sample calculation using *S. capense* sample: Total mEq = (30 x N_{HCl}) − (V_{NaOH} x N_{NaOH}); N_{HCl} = 1.0 M N_{NaOH} = 0.5 M V_{NaOH} = 22.5 Ml; Total mEq = (30 × 1.0) − (48.5 × 0.5) = 5.75 mEq; Acid neutralizing capacity/gram of acid =Total mEq/Density of acid (5.57/0.72= 7.99)

Buffering capacity of different antacids

The buffering capacity represents the rate of pH change over a given time, as presented in Table 7. The initial pH for all samples ranged from 5.02 to 8.89. Calcium carbonate maintained its buffering capacity of 40 minutes. *S. capense* mucilage, on the other hand, maintained a buffering capacity of 30 minutes. However, the mixed formulation showed the lowest buffering capacity, 15 minutes. It can therefore be said that, according to the observations made on the *in vitro* antacid, acid neutralizing capacity, and buffering capacity, the addition of *A. exuvialis* to *S. capense* had reduced the neutralizing capacity of the combined formulation.

An imbalance between hydrochloric acid, pepsin gastrin, and gastric mucosal defenses can result in chronic recurrent gastric ulcers. Hydrochloric acid with a pH of 1.5 to 3.5 is a major component of gastric acid that is produced by parietal cells of the stomach. While acid production is important for chemical digestion, uncontrolled acid production can increase the acidity of the stomach and can result in peptic and duodenal ulceration (Dhawal & Barve, 2020). Gastric ulceration occurs in case of a rupture in the mucosa that allows the pepsin and hydrochloric acid to destroy the stomach wall. Most people in resource-poor settings with limited access to modern medicine use herbal medicine as an alternative treatment for many peptic ulcers and other gastrointestinal conditions (Pearson et al., 2018; Chaudhary et al., 2020). However, the lack of sufficient validation and guarantee for their rational uses limits the potential use of herbal medicine (Zhang, Onakpoya, Posadzki, & Eddouks, 2015).

In this study, although *A. exuvialis* mucilage did not show antacid activity, it showed higher foaming properties. Foaming is normally associated with higher presence of saponins. Foaming has the advantage of reducing the amount of acid that comes in contact with esophagus and this may help prevent gastroesophageal reflux (Antacids for Gastroesophageal Reflux Disease (GERD), 2014). Hence this property makes this mucilage a potential to be considered in relieving gastroesophageal reflux as a foaming agent. *A. exuvialis* also showed the presence of other health benefits given the bioactive compounds present in the mucilage. Other foaming agents, such as Gaviscon, also work by covering the stomach content with foam to prevent reflux (Gastroesophageal Reflux Disease, 2020).

The stability of *S. capense* mucilage as compared to calcium carbonate shows how stable and restrained this mucilage can be in an acidic environment. The physiochemical characteristics such as swelling index and low flow rate could have also contributed to the stability of *S. capense* mucilage (Deshmukh et al., 2013). Moreover, most physicochemical characteristics observed in these extracts offer added advantages to the

behavior of the mucilage in the gastrointestinal tract and should be further analyzed for the possibility of developing proton pump inhibitors, especially with antiulcer activities. The FDA-approved acid-neutralizing capacity of an antacid should be greater than or equal to 5 mEq per dose of the antacid (Ayensu et al., 2020). Therefore, based on this standard, *S. capense* mucilage and the combined formulation have potent acid-neutralizing capacity. The presence of bioactive compounds, proteins, and polysaccharides observed in *A. exuvialis* and *S. capense* could explain why their mucilage is popularly used as antacid decoctions in Namibia.

Table 7. Buffering capacity of different antacids.

Sample name	pH at different time points (Min)								
	0	5	10	15	20	25	30	35	40
Sesamum capense	6.95	4.71	3.91	2.94	2.81	2.74	2.2	ND	ND
Calcium carbonate	8.89	6.58	5.51	4.61	3.80	2.93	2.78	2.65	2.1
Asparagus exuvialis + *Sesamum capense*	5.02	3.56	2.76	2.41	ND	ND	ND	ND	ND

ND: Not Determined.

Cytotoxicity test

The cytotoxicity of the aqueous mucilage from *A. exuvialis* and *S. capense* was found to be dose-dependent. The aqueous mucilage of *S. capense* did not cause any significant cytotoxicity to 3T3 cell lines with an IC50 value of 91.5 ± 0.06 µg Ml^{-1}, confirming the safe nature of the mucilage (Figure1 A and C). *A. exuvialis*, however, showed moderate cytotoxicity to 3T3 cell lines with an IC50 value of 40.7 ± 0.04 µg mL^{-1} (Figures 1 B and C). These results are similar to those reported by Das and Devi (2015), who showed no cytotoxicity in *Terminalia belirica* ethanolic extracts. Moreover, *A. exuvialis* showed foaming properties This could be due to toxic compounds present in the mucilage. The results of the present study confirm that *S. capense* can be safely consumed however, *A. exuvialis* could be toxic, especially at higher concentrations.

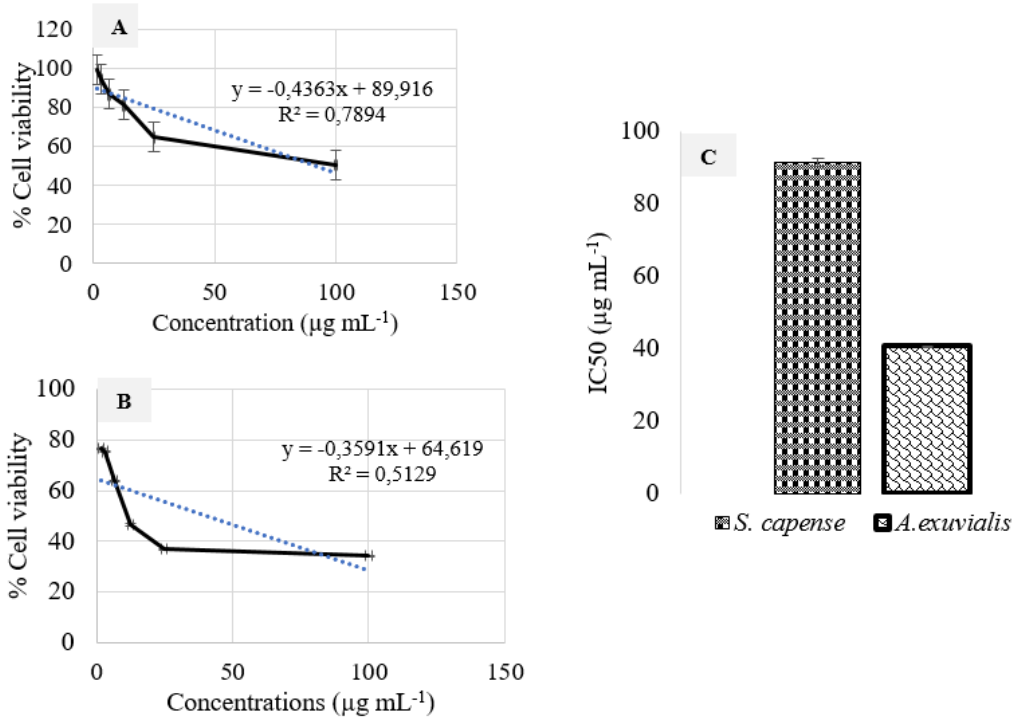

Figure 1. *In vitro* cytotoxic effect of A: *Sesamum capense*, B: *Asparagus exuvialis*, C: IC50 using MTT Assay, mean±SD, $p < 0.05$.

Conclusion

The mucilage extracted in this study has shown the presence of different therapeutic compounds reported in the literature to have gastroprotective properties. Moreover, *S. capense* showed potent acid-neutralizing and buffering capacities, which makes it a potential source for antacid mucilage compounds. Furthermore, the significant forming properties of *A. exuvialis* make it a potential source of mucilage that could relieve

gastroesophageal reflux. Hence, given the long history of ethnomedicinal uses of these plants, the mucilage should be further evaluated for toxicological profile and further developed into future alternative/complementary antacid.

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