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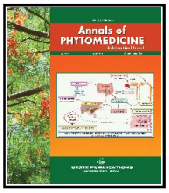

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Elucidation of nutritional, phytochemical and pharmacological activities of *Solanum mammosum* L.

Rina Ningthoujam*, Chandra Deo*, Siddhartha Singh, Arunkumar Phurailatpam***, B.N. Hazarika****, Nangsol Dolma Bhutia*****and Amit Kumar Singh****◆

* *Department of Vegetable Science, College of Horticulture and Forestry, Central Agricultural University, Pasighat-791102, Arunachal Pradesh, India*

** *Department of Basic Science and Humanities, College of Horticulture and Forestry, Central Agricultural University, Pasighat-791102, Arunachal Pradesh, India*

*** *Department of Medicinal and Aromatic Plants, College of Horticulture and Forestry, Central Agricultural University, Pasighat-791102, Arunachal Pradesh, India*

**** *Department of Fruit Science, College of Horticulture and Forestry, Central Agricultural University, Pasighat-791102, Arunachal Pradesh, India*

***** *Department of Vegetable Science, College of Horticulture, Central Agricultural University, Bermiok-737113, Sikkim, India*

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Abstract

Solanaceous crops are greatly diverse plant families, of which *Solanum* is the largest genera having 1,700 species. Among the *Solanum* species, *Solanum mammosum* L. stands out as a tropical and neglected fruit due to its elevated levels of steroidal alkaloids. However, due to the report on consumption at their vegetative stage in some Asian countries, the present investigation is done to monitor the amount of nutrients, phytochemicals, and pharmacological potential. The outcome of this study shows that the fruit of *S. mammosum* contains various nutrients such as free fatty acids (0.56 ± 0.008 mg/g), total amino acids (1.60 ± 0.031 mg/g), proline (173.16 ± 5.463 µmole/g), methionine (1.17 ± 0.004 mg/g), different biological pigments and several vitamins. It was also observed that the fruit has antioxidant, antifungal, and antidiabetic properties. It inhibits *Candida albicans* and *Cryptococcus neoformans* by showing the zone of inhibition at 4 ± 0.20 mm and 12 ± 0.62 mm, respectively. The inhibition of α-amylase and α-glucosidase shows that the fruit can reduce or control glucose levels in our body. Hence, this justifies that the plant has nutraceutical properties and further, it will support basic healthcare across the globe by exploiting it in drug development.

1. Introduction

Northeastern India is well known for the distinct culture and is regarded as the country's richest plant repository. It is made up of eight states; namely, Arunachal Pradesh, Sikkim, Tripura, Nagaland, Manipur, Assam, Meghalaya, and Mizoram. These states account for seven percent of India's total land area and constitute six agro-climatic zones (Koku *et al*., 2020). Due to the various agroclimatic conditions and topographical variances, this area is suitable for cultivating numerous plants or horticultural crops (vegetables, spices, fruits, flowers, medicinal plants, *etc.*). Many indigenous plants are eaten in addition to mainstream crops as a substitute or complement. They are rarely grown in fields; instead, they are collected or harvested from forests based on need. These wild edible plants are suppliers of carbohydrates, proteins, fats, vitamins, and mineral salts like zinc, iron, potassium, calcium, *etc*., just like other significant vegetable crops (Kanneboina *et al.,* 2023). In addition, they are used as food, drink, and medication. They are often used by conventional practitioners as natural healers.

Corresponding author: Dr. Amit Kumar Singh
Department of Basic Science and Humanities, College of Horticulture and Forestry, Central Agricultural University, Pasighat-791102, Arunachal Pradesh, India
E-mail: geneamit@gmail.com
Tel.: +91-9862567417

The Solanaceae family thrives very well in Northeast states and several wild species are grown in the region. Among the wild species, *S. mammosum* is one of the rare species which has less attention by the people and commonly known as nipple fruit, cow's udder, and apple of sodom because of their pyriform fruit shape. The plants were covered with hairy spiny thorns and bears inflorescence containing four to five flowers. The size of the fruits ranged from 4.5-5.5 cm which turns into yellowish orange when ripened. The ripened fruits contain a high concentration of steroidal alkaloids, which is undesirable for consumption. However, it is defined as a delectable indegineous vegetable with outstanding eating quality in a study presented by the Asian Vegetable Research and Development Center (AVRDC, 2002). In the Philippines, the fruits and leaves are consumed as vegetables after boiling. It is mainly planted as an ornamental plant for its unusual fruit rather than for human use. The fruit also contains solanine and solamargine, which, if consumed, may induce gastrointestinal distress, like many other plants in the Solanaceae family. The fruit's pulp is used medicinally to treat haemorrhoids, ulcers, and elephantiasis. Skin conditions can be treated by using the leaves. The seed oil is an anti-inflammatory and antiasthmatic treatment for constipation. Numerous pharmacological characteristics of the plant, such as antioxidant, anticancer, antimalarial, and molluscicidal effects, have been documented in other research (Cabanillas *et al*., 2021). It has also been used in the past to catch fish as a pesticide and as a rat poison (Levin*et al*., 2005).

Consequently, the current study aims to investigate the nutritional value, phytochemical composition, and significant pharmacological properties of *S. mammosum* fruit. The results of this study will aid in the assessment fordifferent constituents in the fruit and will also offer empirical proof of their importance. Thus, it is deemed worthwhile to do this study, and we firmly believe that it will pave the way for a wide rangein near future as nutraceutical species.

2. Materials and Methods

2.1 Plant materials

The ripe fruits of *S. mammosum* were collected from Medzhiphema, Nagaland and maintained at the experimental farm of Vegetable Science, College of Horticulture and Forestry, CAU, Pasighat, Arunachal Pradesh. The location of the experimental feildhas an altitude of 153 m above mean sea level with a latitude of 28°04′30′′N and longitude of 95°19′28′′E. The climatic condition of this area comes under subtropical humid which receives optimum rainfall throughout the year while maximum rainfall occurs in the month of May-August. The growing soil media is sandy loam with a pH of 6.5 and organic carbon of 2.1%.The taxonomical identification was done by Dr. Arunkumar Phurailatpam, Associate Professor, Department of Medicinal and Aromatic Plants, College of Horticulture and Forestry, CAU, Pasighat, Arunachal Pradesh under the Collection ID - CHF/CAU/Veg/Solanum-5.The studies were performed on the berries (fruit) of the plant at their vegetative stage. Nutrients and phytochemical analysis were carried out at Basic Science and Humanities Laboratory, College of Horticulture and Forestry, CAU, Pasighat, Arunachal Pradesh while antimicrobial and antidiabetic activities were screened at Institute of Bioresources and sustainable development, DBT, Takyelpat, Imphal, Manipur.

2.2 Nutritional profiling

2.2.1 Free fatty acid

The content of free fatty acid was analysed by following the steps of Hiremath *et al*. (2007). It was done by using 1% phenolphthalein as an indicator and titrated with 0.1 N potassium hydroxide. The titration was continued until the visualization of pink color which persisted for 10-15 sec.

2.2.2 Total free amino acid

The determination of total free amino acid in fruits of *S. mammosum* was performed by following the method described Azeez *et al.* (2020). It was done by using ninhydrin solution as a reagent and at 570 nm the reading of the sample was taken against the blank solution.

2.2.3 Proline

The amount of proline was also determined by referring the method given Kahlaoui *et al*.(2018). A sample amount of 0.5 g was homogenized with 10 ml of 3% aqueous sulphosalicyclic acid and collected an aliquot of 2 ml. Further, 2 ml of each glacial acetic acid and acid ninhydrin were added. The reaction was started by placing the test tube in boiling water bath for about 1 h. The reaction was terminated by keeping the tube in an ice bath andaddition of 4 ml toluene. The toluene layer was separated and formation of the red color was visualised. At the end, the color intensity was read in a UV visible spectrophoto meter at 520 nm against the blank solution.

2.2.4 Methionine

Quantification of methionine was performed by the procedure described Panwar *et al*. (2016). It was done by using 2 N HCl, 10 N NaOH, and orthophosphoric acid. Red color intensity was formed at the end of the reaction and the final reading was analyzed at 520 nm against the blank solution.

2.2.5 Total chlorophyll and total carotenoids

The amount of total chlorophyll as well as total carotenoids wascheckedby the procedure given by Sumanta *et al*. (2014). The pigment was extracted by using 80% acetone and quantification of total chlorophyll was done by using UV-VIS spectrophotometer at 663 nm and 645 nm. The content of total carotenoids was quantified at 470 nm.

2.2.6 Anthocyanin

Anthocyanin content was performed by following the methods of Oancea *et al*. (2012) with slight modifications. Extraction was done from the sample by using ethanolic HCl at a ratio of 85:15 and reading was taken at 535 nm in a UV spectrophotometer.

2.2.7 Vitamin A

It was estimatedby the colorimetric process as demonstratedby Kesuma *et al.* (2020) with slight modifications. Saponification of the sample was done by refluxing of the sample at 60°C for 20 min in 2 N KOH in 90% alcohol. Petroleum ether was used as solvent for the separation of vitamin A. From the extraction layer, amount of 5 ml was pipetted out for drying and then dissolved in 1 ml chloroform. At the end, trichloroacetic acid of 2 ml was added and absorbance was observed at 620 nm.

2.2.8 Vitamin B_1 (Thaimine)

The quantification of thiamine was performed by following the procedure (Sadasivam and Manickam, 2008). For extraction, sulphuric acid of 0.1 N was added to the sample and kept overnight. In a separating funnel, 10 ml extract, 3 ml of 15% NaOH, and ferricyanide (3-4 drops) were shaken gently, followed by adding 15 ml of isobutanol. After shaking, vigorously for 60 sec, the formation of the clear layer in the topmost was observed. It was collected and checked the absorbance at 366 nm.

2.2.9 Vitamin C

The determination of vitamin C or ascorbic acid was done using the processsuggested by Kumar *et al*. (2019). A sample amount of 0.5 g was crushedwith 5 ml of 3% metaphosphoric acid, and the volume was made up to 10 ml with again 3% metaphosphoric acid. The supernatant was collected after the content was centrifuged. As a final pocess, aliquot of 5 ml was measured and titration was done until the visualisation of light pink color.

2.2.10 Vitamin E

The content of vitamin E was determined by the procedure(Kumari and Achal, 2008) where 0.1 N sulphuric acids were used for extraction and 2, 2-dipyridyl was used as a reagent. In 0.5 g of sample 10 ml of 0.1 N sulphuric acids was added and kept overnight. In 1.5 ml of extract, 1.5 ml of ethanol and xylene was added. Then, 1 ml of 2, 2-dipyridyl reagent was added to 1 ml of xylene, and reading at 460 nm against the blank were recorded. Later, 0.33 ml of ferric chloride was added to the solution and a second reading was observed at 520 nm exactly after 15 min.

2.3 Determination of phytochemicals

2.3.1 Total phenols

The quantification of total phenol was checked by following the procedurerecommended by Siddiqui *et al*. (2017). Extraction of the crude was done by using 80% ethanol and folin-ciocalteau was used as the reagent and readings were taken at 650 nm.

2.3.2 Total flavonoids

The amount of total flavonoids was estimated by the methodology of Tambe and Bhambar, (2014). Quantificationwas performed by the aluminium chloride colorimetric assay. 1ml of extract, 4 ml of distilled water, and 0.3 ml of 10% aluminium chloridewere combined and allowed to stand for 5 min. Afterwards, 10 ml with distilled water were added to 2 ml of 1 M sodium hydroxide for treatment. By the help of UV spectrophotometer the absorbance for test and standard solutions was measured at 510 in relation to the reagent blank.

2.3.3 Tannins

Analysis of tannins was determined by following the procedure of the spectrophotometric methodology given by Nair *et al*. (2015). Folin-Denis was utilise as a reagent and reading was taken against blank at 700 nm.

2.3.4 Total alkaloids

Estimation of total alkaloids wasdoneusing the procedure of Tambe and Bhambar (2014). A sample amount of 0.5 g was macerated with 80% ethanol and the solvent was evaporated until it dries. Then, a volume of 1 ml and 0.1% of dimethyl sulphoxide were used to dissolve 5 mg of crude extract. The solution was transferred to the separating funnel and 1 ml of 2 N HCl was added. The solution was once more mixed with 5 ml of phosphate buffer and bromocresol green solution. The mixture was collected in a 10 ml volumetric flask and diluted to the volume with chloroform after being shaken with 4 ml of the solvent. At 470 nm, the spectrophotometric reading was measured in relation to the reagent blank.

2.3.5 Solasodine

The content of solasodine was done by following the methodology suggested by Kumar *et al*. (2017). It was started by refluxing the sample in 1 N HCl for 2 h in a water bath followed by adding 0.5 ml of 60% NaOH. Then, it was shaken continuously after adding 5 ml of chloroform. The chloroform layer was collected and the volume was made up to 5 ml with distilled water. Add 2.5 ml of bromothymol blue and shake for 10 sec. Straw colored from the lower layer was drawn out. To this, 1 ml of 0.2 M NaOH was added and visualised the formation of blue color and the reading was taken at 205 nm.

2.4 Pharmacological activities

2.4.1 Antioxidant properties

The activity of antioxidant was checked following the steps of Aoshima *et al.* (2004) with slight modification. Sample amount of 0.2 g was taken and ground in 5 ml of ethanol. The solution was centrifuged and pipetted out 0.5 ml of aliquot. 0.3 ml of DPPH reagent (0.5 mM in methanol) were added for further reaction and the solution was kept in the dark for 30 min at room temperature. The decoloration of DPPH was seen and quantified at 517 nm by the help of ultraviolet-visible (UV-Vis) spectrophotometer.

2.4.2 Antifungal activities

The agar well diffusion method was followed for antifungal activities by following the procedure of Gonelimali *et al*. (2018). It was checked from the crude extract of the fruit against the fungal pathogens *C. albicans* (ATCC 10231) and *C. neoformans* (ATCC 14116). The crude extract was prepared by macerating the sample in 70% methanol. Amount of 25 mg crude extract was dissolved in 1 ml of water and 100 µl of samples were poured in well. It was kept for 24 h to screen out their antimicrobial activity by observing the zone of inhibition.

2.4.3 Minimum inhibitory concentration (MIC)

It was also done by following the agar well diffusion method taking reference from Gonelimali *et al*. (2018). Crude extract of different concentrations ranging from 25, 20, 15, 10, and 5 mg/ml were used for MIC examination.

2.4.4 Antidiabetic properties

Their antidiabetic properties were checked by performing two enzyme assays, *i.e.*, the α-amylase inhibition assay and the β-glucosidase inhibition assay. α-amylase inhibition assay was determined by following the method described by Xiao *et al.* (2006) and α-glucosidase inhibition assay was determined by following the method described by Chanda *et al*. (2020).

2.5 Statistical analysis

The results of all the parameters were observed in triplicate numbers and the mean of three analyses were presented in Tables and Figures accompanied by the values of ± standard deviation (SD), following the procedure recommended by Gomez and Gomez (1984).

3. Results

3.1 Nutritional profiling

From the recorded results, it was revealed that the fruits of *S. mammosum* have various amounts of nutrient composition. It possesses a high content of total free amino acid (1.60 ± 0.031 mg/g) as depicted in Table 1. Other nutritional compositions such as free fatty acid (0.56 ± 0.008 mg/g), proline (173.16 ± 5.463 µmole/g), and methionine (1.17 ± 0.004 mg/g) were also obtained. Among the biological pigments, the highest amount of total chlorophyll (2.92 ± 0.005 mg/g) was recorded followed by anthocyanin (0.24 ± 0.011 mg/g) and total carotenoids (0.027 ± 0.002 mg/100 g). From the results obtained, it was also observed that the fruits have plentiful amount of vitamin C (0.09 ± 0.003 mg/g) than other vitamins such as vitamin A (3.30 ± 0.086 µg/g), vitamin B1 (0.10 ± 0.001 µg/100 g) and vitamin E (12.22 ± 0.143 µg/g).

3.2 Phytochemical contents

The quantification of phytochemical contents showed that a high amount of total alkaloids (14.02 ± 0.05 mg/g) was present as shown in Figure 1, followed by various compounds like total phenols (6.09 ± 0.137 mg/g), solasodine (3.05 ± 0.002 mg/g), tannins (2.95 ± 0.114 mg/g) and flavonoids (1.04 ± 0.046 mg/g).

Table 1: Nutritional composition of *S. mammosum* fruits with their SD (±) values of three measurements (n=3)

S.No.	Nutritional value of *S. mammosum* fruit	
1.	Free fatty acid	0.56 ± 0.008 mg/g
2.	Total free amino acid	1.60 ± 0.031mg/g
3.	Proline	173.16 ± 5.463 µmole/g
4.	Methionine	1.17 ± 0.004 mg/g
5.	Total chlorophyll	2.92 ± 0.005 mg/g
6.	Total carotenoids	0.027 ± 0.002 mg/100 g
7.	Anthocyanin	0.24 ± 0.011 mg/g
8.	Vitamin A	3.30 ± 0.086 µg/g
9.	Vitamin B_1(Thaimine)	0.100 ± 0.001µg/100 g
10.	Vitamin C	0.09 ± 0.003 mg/g
11.	Vitamin E	12.2 ± 0.143 µg/g

3.3 Pharmacological activities

As for the pharmacological properties, the obtained results are depicted in Table 2. It revealed that the fruit has less percentage of antioxidant properties (41.19 ± 0.631%). However, it has shown positive results on inhibiting fungal pathogens namely, *C. albicans* (ATCC 10231) and *C. neoformans* (ATCC 14116) as shown in Figure 2. However, better performance of inhibition was observed from *C. neoformans* (ATCC 14116) by forming an inhibition zone of 12 ± 0.62 mm with a MIC value of 10 ± 0.35 mg/ml. It has also shown the capability to depress *C. albicans* (ATCC 10231) by forming a zone size of 4 ± 0.20 mm with a MIC value of 15 ± 0.58 mg/ml. Further, it was also known from the observation that the fruit has antidiabetic properties as it has shown excellent results by inhibiting both α-amylase and α-glucosidase. Different concentrations of extract ranging from 0.1 to 1 mg/ml showed different inhibiting percentages which was depicted in Figures 3 and 4. However, more inhibition percentage of 70.84 ± 1.788 was achieved by inhibiting α-glucosidase at a concentration of 1 mg/ml. At the same concentration, 56.34 ± 1.808% was obtained by inhibiting α-amylase. The result of the acarbose drug also varied according to the methods employed for monitoring antidiabetic properties.

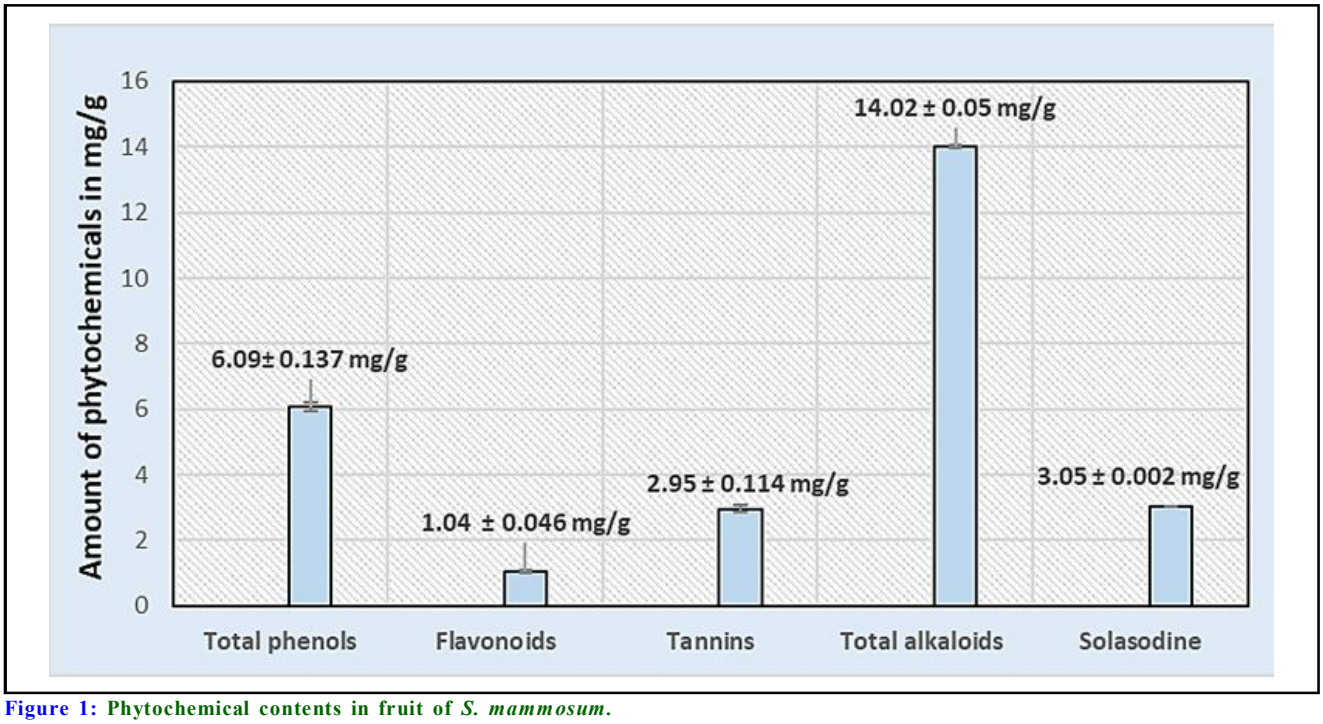

Figure 1: Phytochemical contents in fruit of *S. mammosum*.

An inhibition percentage of 96.01 ± 0.528 and 84.95 ± 1.378 was obtained on inhibiting α-glucosidase and α-amylase, respectively at a concentration of 1 mg/ml. From the results, it was also observed that the percentage of inhibition decreased with a decrease in concentration. Since the analysis was performed on different concentrations, the value of IC_{50} was also calculated and the results

pertained was represented in Table 2. $IC_{50}$of 0.01± 0.001 mg/ml and 0.02 ± 0.001 mg/ml were perceived from the positive control of α-glucosidase and α-amylase, respectively. As for the sample, $IC_{50}$of 0.34 ± 0.002 mg/ml and 0.79 ± 0.009 mg/ml were recorded in respective to α-glucosidase and α-amylase inhibition.

Table 2: Pharmacological properties of *S. mammosum* fruits with their SD (±) values of three measurements (n=3)

Pharmacological activities of *S. mammosum* fruit		
Inhibition% of antioxidant	41.19 ± 0.631%	
Antifungal properties	Zone of inhibition (mm)	MIC value (mg/ml)
C. albicans	4 ± 0.20	15 ± 0.58
C. neoformans	12 ± 0.62	10 ± 0.35
In vitro antidiabetic activities	IC_{50} of α-amylase	IC_{50} of acarbose on inhibiting α-amylase
	0.79 ± 0.009 mg/ml	0.02 ± 0.001 mg/ml
	IC_{50} of α-glucosidase	IC_{50} of acarbose on inhibiting α-glucosidase
	0.34 ± 0.002 mg/ml	0.01 ± 0.001 mg/ml

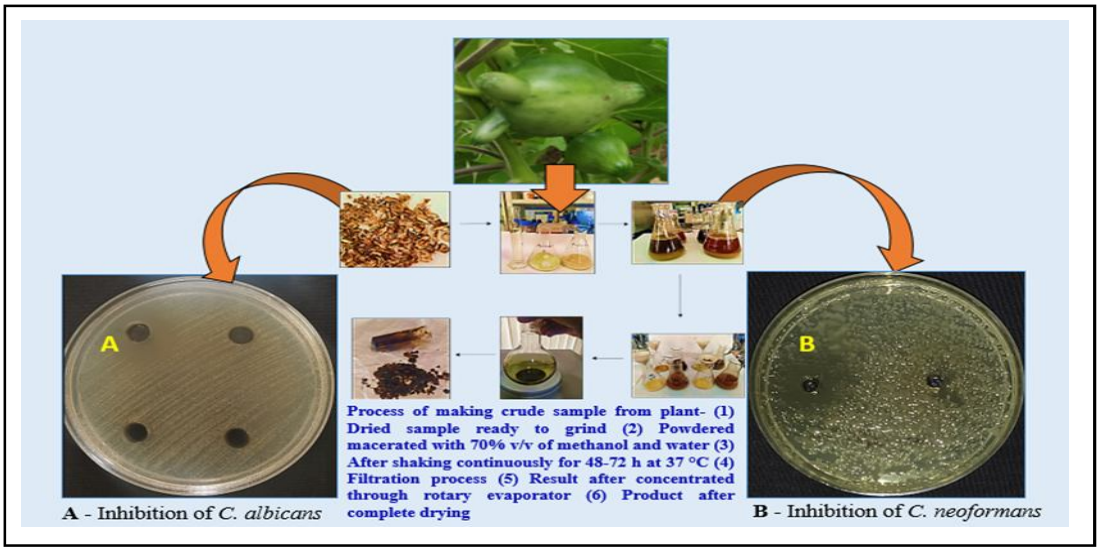

Figure 2: Brief description on the extraction process of crude and their inhibition upon *C. albicans* and *C. neoformans*.

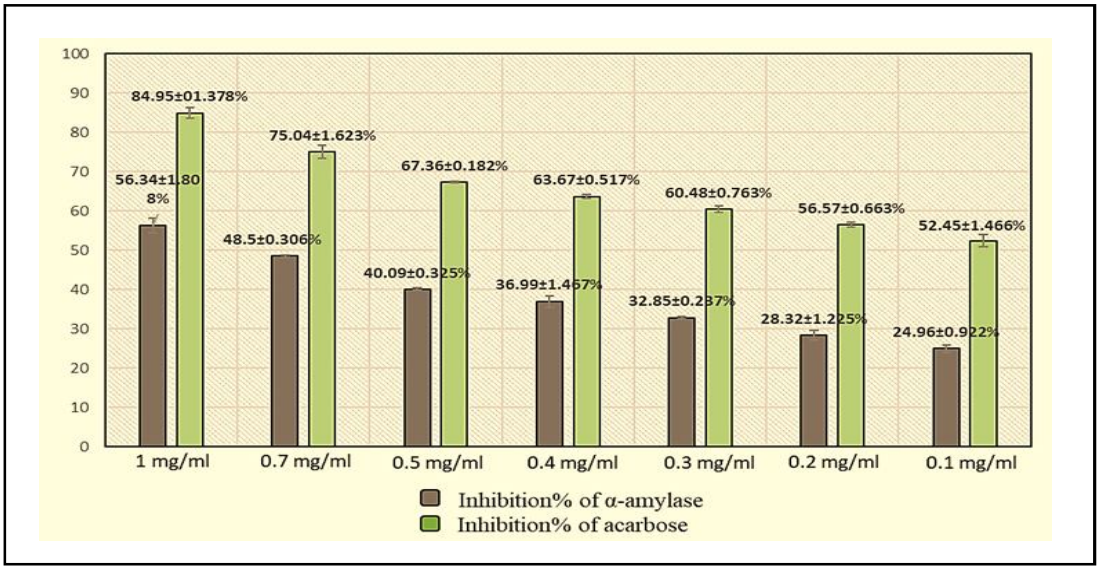

Figure 3: Inhibitory of α-amylaseby *S. mammosum* and acarbose (± control) at different concentrations.

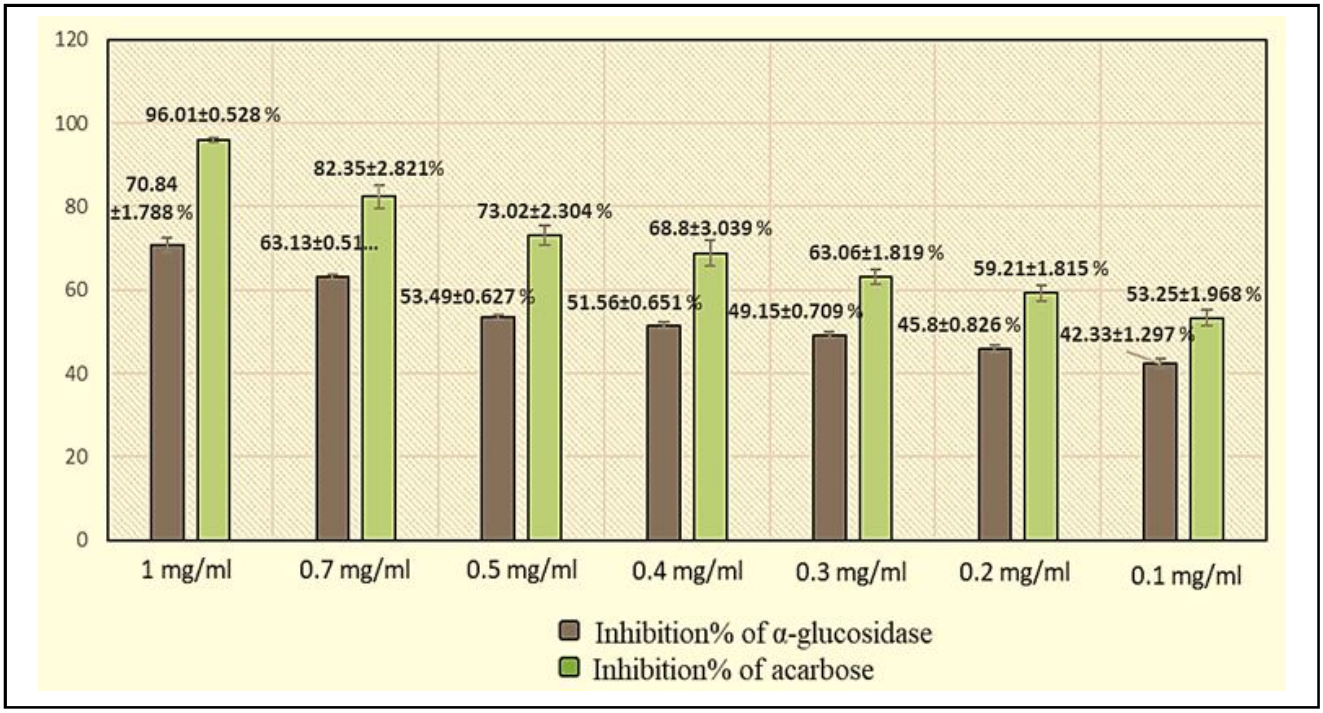

Figure 4: Inhibitory of α-glucosidase by *S. mammosum* and acarbose (± control) at different concentrations.

4. Discussion

The obtained results indicated that the fruits of *S. mammosum* contain several nutrients and phytochemicals, where they possess a high content of amino acids (1.60 ± 0.031 mg/g) and total alkaloids (14.02 ± 0.05 mg/g). This result is in conformity with the report submitted by Ravindran and Nandakumar (2015) who studied the distribution pattern of amino acid content in different tissues of *S. mammosum* fruits. They reported that the outer and inner pulp of fruit content was 2.70 ± 0.33 mg/g and 3.00 ± 0.34 mg/g of amino acids. Further, Aleman *et al*. (2023) also studied the chemical characterization of *S. mammosum* fruits and revealed its nutritional composition. They have mentioned that the fruits of *S. mammosum* contain total phenols (633.22 ± 10.67 µgGAE/ml) and total carotenoids (7.65 ± 0.32 mg/ml) where the amount differed with our study. This difference in results might be due to the selection of fruit for analysis at different stages and growing environmental conditions. As we know, growing plants in different soils and various geological horizons impact the numerous elements found in fruits. The secondary metabolites or phytochemicals in plants highly depend upon the surrounding factors and this is equivalent to the decision of Chinedu *et al*. (2011) and Sodipo *et al*. (2008). Additionally, the presence of phytochemicals also influence highly in the activities of pharmacological properties of a plant (Nidhya *et al.,* 2023). Similar comment has also been given by Srinivasan and Murali (2022).

Aleman *et al*. (2023) also reported the amount of antioxidants (41.56 ± 2.77%) in the fruit of *S. mammosum*, where the result is in aligned with our recorded inhibition percentage of antioxidants (41.19 ± 0.631%). The contain of low antioxidant might be due to the low amount of flavonoids (1.04 ± 0.046 mg/g) in the fruit. The compound in total flavonoids such as quercetin plays an important role in enhancing antioxidant (Shabnam *et al.,* 2022). From the results obtained, it is also visualized that the fruit has antifungal properties and this result is similar to the findings of Cabanillas *et al*. (2021), where they also reported the inhibition of *Trichophyton mentagrophytes* and *C. albicans*. The antifungal properties in fruit might be due presence of abundant plant by-products such as total phenols and total alkaloids. This is also in agreement with Gorlenko *et al*. (2020) who mentioned that the inhibition in the growth of microorganisms is due to the presence of plants' secondary metabolites. Furthermore, Cabanillas *et al*. (2021) disclosed that the presence of solamargine in *S. mammosum* acts as a main compound responsible for the inhibition of fungal pathogens. Interestingly, it was also found that fruits have good potential to reduce the sugar content in our body, and the control of sugar in the body by the use of phytomedicine is an alternative better way (Sanjeev and Divya, 2021). The inhibition of α-amylase and α-glucosidase proved that the fruit has antidiabetic properties and the main inducer of this property is the presence of solasodine in good amounts (3.05 ± 0.002 mg/g). This finding is similar to the report given by Lee *et al*. (2007) and Li *et al*. (2007), where they stated that glycoalkaloids such as solasodine and solamargine are responsible for the antidiabetic properties. Additionally, Jayant and Vijayakumar (2021) also reported that the content of several phytochemicals such as flavonoids, steroids, terpenoids, and phenols is responsible for antidiabetic activity in a plant. Therefore, the presence of phytochemicals in *S. mammosum* fruits may mimic the mechanism of insulin and function as a potent antihyperglycemic agent.

5. Conclusion

The findings indicated that *S. mammosum* contains a variety of nutrients and abundant phytochemical compounds, which are likely to contribute significantly to human metabolism. Moreover, the positive results regarding its pharmacological properties suggest its potential to enhance basic healthcare globally through drug development. However, further research is necessary to isolate the

specific compound responsible for the pharmacological properties. Importantly, highlighting the benefits of these plants opens up significant opportunities for Northeast farmers shortly. Simultaneously, it can be utilized in breeding programs and crop improvement due to its ability to thrive in adverse climatic conditions and disease tolerance. Additionally, it can employ as rootstock for biotic and abiotic susceptible *Solanum* plants after screening their compatiblility.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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