INVESTIGATION OF ANTIMICROBIAL ACTIVITY, ALPHA-AMYLASE INHIBITION ACTIVITY AND CYTOTOXIC ACTIVITY OF RHIZOME OF *BERGENIA CILIATA* (HAW) STERNB. (NATSEI GAMON)

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Abstract

This research aims to investigate some biological activities such as antimicrobial activity, α-amylase inhibition activity, and cytotoxic activity of the rhizome of Natsei gamon. The rhizome of Natsei gamon was collected from Lungpi Village, Falam Township, Chin State, Myanmar, in February 2019. In the present work, antimicrobial activity, α-amylase inhibition activity, and cytotoxicity have been determined. According to the results of antimicrobial activity, methanol and chloroform extracts of Natsei gamon did not show any antimicrobial activity against all of the microorganisms tested. But petroleum ether extract exhibited activity against six microorganisms, such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans*, and *Escherichia coli* with an inhibition zone diameter range of 13-15 mm. Ethyl acetate and ethanol extracts significantly inhibited only *Pseudomonas aeruginosa*, with an inhibition zone diameter of 20 mm. The α-amylase inhibition activity of ethanol and watery extracts of rhizome of Natsei gamon was determined by α-amylase inhibition assay or DNS reagent method. These two crude extracts were found to possess α-amylase inhibition activity. The ethanol extract (IC_{50} = 0.78 μg/mL) was found to have higher potency than that of watery extracts (IC_{50} = 0.85 μg/mL) in α- amylase inhibition activity. The cytotoxicity of watery and ethanol extracts of the rhizome of Natsei gamon is free from cytotoxic effect until a concentration of 1000 μg/mL.

Keywords: *Bergenia ciliata* (Haw.) Sternb., antimicrobial activity, α-amylase inhibition activity, cytotoxic activity

Introduction

The plants of the *Bergenia ciliata* (Haw.) Sternb. family belong to Saxifragaceae, a kind of perennial herb containing rich medicinal ingredients and having high application values. They are growing in India, Pakistan, China and Myanmar. It is a very important medicinal plant, widely applied in many fields (Guo *et al*., 2004). The plant of *Bergenia* has a height range of 10 to 80 cm, with short internodes. This is a kind of perennial herb, containing rich medicinal ingredients and having high application values. Its underground rhizomes grow creepingly, with radical branches. As a medicinal plant, *B. ciliata* (Haw.) Sternb. is being widely used for such things as cough, stopping bleeding, kidney stones, heart disease, pulmonary infection (especially asthma disease), menstrual disorder, stomach disorder, cold, and fever (Madiha *et al*., 2016).

People and other living organisms need certain nutrients to survive. Microorganisms are available naturally in the surrounding environment; they can easily reach food during harvesting, slaughtering, processing, and packaging (Hatab *et al*., 2016). Microbes are tiny living things that are found all around us and are too small to be seen by the naked eye. Microorganisms include bacteria, protozoa, algae, and fungi. In the present work, the antimicrobial activity of six crude extracts as petroleum ether, ethyl acetate, ethanol, methanol, chloroform, and water from the rhizome of Natsei gamon was determined against six strains of microorganisms such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans, and Escherichia coli* by employing the agar well diffusion method.

α-amylase (α-1,4 glucan-4-glucanohydrolase) is an endoglycosidase,which hydrolyses starch and related α-1,4-linked glycosyl polysaccharides. It is the major form of α-amylase found

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in human and other mammals (Garrison *et al*., 1986). The saliva in your mouth contains amylase, which is another starch digesting enzyme. Breaking down starch molecules into small glucose molecules, enables them to pass gut wall and into the bloodstream as an energy supply for the body's cells. The enzyme amylase is the biological catalyst for this reaction (Elsnoussi *et al.*, 2012). The inhibition of their activity in the digestive tract of humans is considered an effective tool to control diabetes (Hara and Honda, 1990).

Cytotoxicity studies are a useful initial step in determining the potential toxicity of a test substance, including plant extracts or biologically active compounds isolated from plants. Minimal to no toxicity is essential for the successful development of a pharmaceutical or cosmetic preparation, and in this regard, cellular toxicity studies play a crucial role (Tulay, 2018). In this study, the cytotoxicity of water and ethanol extracts of the rhizome of Natsei gamon was evaluated by a brine shrimp cytotoxicity bioassay.

Materials and Methods

Collection and Preparation of the sample

The rhizomes of Natsei gamon were collected from Lungpi Village, Falam Township, Chin State in February 2019. Then, the sample was identified at the Department of Botany, University of Yangon.

The fresh rhizomes were cleaned by washing with water and air-drying at room temperature for 2 weeks. The dried rhizomes were cut into small pieces and were ground into powder by using a grinding machine. The dried powdered samples were stored in an airtight container to prevent moisture changes and other contaminations. The dried powdered samples were used to investigate for their chemical and biological activities.

Preliminary Phytochemical Investigation of Rhizome of Natsei Gamon

Phytochemical investigation of rhizome of Natsei gamon was carried out according to the reported methods. In the present work, the plant sample tested alkaloids, α-amino acids, carbohydrates, flavonoids, cyanogenic glycosides, glycosides, phenolic compounds, organic acids, reducing sugars, saponins, starch, steroids, tannins and terpenoids (M-Tin Wa, 1972). The presence of these phytochemicals makes the plant useful for treating different ailments and have a potential of providing useful drugs of human use.

Determination of Nutritional Values

Nutritional values such as moisture content, ash content, fat content, fibre content, protein content, and energy value of the selected sample were determined by AOAC method (AOAC, 2000). Total carbohydrate content was determined by phenol-sulphuric acid method (Neeru *et al.*, 2015). The results were expressed as milligrammes of glucose, and then the percentage carbohydrate content was also calculated from the above results.

$$\text{percent of total carbohydrate} = \frac{\text{mg of glucose}}{\text{volume of test sample}} \times 100$$

Determination of Antimicrobial Activity of Crude Extracts of Rhizome of Natsei Gamon by Agar Well Diffusion Method

The powder of Natsei gamon rhizome was mixed with each of PE, EtOAc, EtOH, $CHCl_3$, MeOH, and H_2O to prepare six crude extracts for about 5 h, and this solution was evaporated on a water bath. The antimicrobial activity of these six crude extracts was screened by the agar-well diffusion method.

Crude extract (0.5 g), peptone (0.5 g), and sodium chloride (0.25 g) were mixed with distilled water and made up to 100 mL with distilled water. The pH of this solution was adjusted to 7.2 with a 0.1 M sodium hydroxide solution, and 1.5 g of agar was added.

Nutrient agar was prepared according to the method described by Mar Mar Nyein *et al.*, (1991). Briefly, nutrient agar was boiled, and 20-25 mL of the medium was poured into a test tube and plugged with cotton wool, and autoclaved at 121 ºC for 15 min. Then the suspension was also added to the dishes. After allowing the agar to set for 30 min, a 10 mm agar plate well was made with using a sterile cork border. Following that, 0.1 mL of sample was added to the agar-well and incubated at 37 ºC for 24 h. The zone of inhibition diameter was used to determine the extent of antimicrobial activity. The results are described in Figures 2 and 3, and Table 3.

Determination of α-Amylase Inhibition Activity of Crude Extracts of Rhizome of Natsei gamon by DNS Reagent Method

The α-amylase inhibition activity of ethanol and watery extracts of rhizomes of Natsei gamon was determined by using a UV-visible spectrophotometer. In a test tube, 500 µL of extract solution was added and followed by 500 µL of 0.02 M sodium phosphate buffer (pH 6.9) containing α-amylase solution (2 mg/mL). The contents of test tubes were pre-incubated at 25℃ for 15 min, after which 500 µL of a 1% starch solution with buffer (pH 6.9) was added at timed intervals. The reaction mixture was incubated at 50℃ for 20 min. The reaction was terminated by adding 1000 µL of dinitrosalicylic acid (DNS) reagent and further incubating in boiling water for 5 min, then cooling to room temperature. The contents of each test tubes were diluted with 5 mL distilled water, and the absorbance was measured at 540 nm in a spectrophotometer. A control was prepared using the same procedure but without extract. The α-amylase inhibitory activity was calculated by the following equation.

$$\% \text{ inhibition} = [\, A_{control} - (A_{sample} - A_{blank}) / A_{control} \times 100]$$

Where, % Inhibition = % α-amylase inhibition

A_{sample} = Absorbance of the sample +enzyme solution+ DNSA solution

B_{blank} = Absorbance of blank (sample + DNSA solution)

$A_{control}$ = Absorbance of control (without sample)

The α-amylase power (IC_{50}) is expressed as the test substance's concentration (µg/ mL) that results in a 50 % reduction of the initial absorbance of DNS solution and that allows to determine the concentration. IC_{50} (50 % inhibition concentration) values were calculated using the linear regression Excel programme. IC_{50} values of crude extracts of the rhizome of Natsei gamon are shown in Figure 4 and Tables 4 and 5.

Determination of Cytotoxicity by Brine Shrimp Lethality Bioassay of Rhizome of Natsei Gamon

The brine shrimp (*Artemia salina*) was used in this study for cytotoxicity bioassays. Brine shrimp eggs were purchased from a pet shop in Baho Road, Hlaing Township, Yangon Division. Brine shrimp eggs (ca. 0.25 g) were added to the beaker, along with 500 mL of sea water. The beaker was placed near a lamp. Light is essential to hatching. Brine shrimp eggs also required a constant supply of oxygen and a 24-h incubation period at room temperature (Ahmed *et al*., 2010).

Each chamber of the ice tray was filled with artificial sea water (9 mL), different concentrations of samples, and standard solutions. Alive brine shrimp (10 nauplii) were taken with a Pasteur pipette and placed into each chamber. They were incubated at room temperature for about 24 h. The number of dead or alive brine shrimp was counted after 24 h, and 50% of the lethal dose (LD_{50}) was calculated (Sahagal *et al*., 2010). The results are shown in Table 6.

Results and Discussion

Preliminary Phytochemical Investigation of Rhizome of Natsei Gamon

The phytochemical constituents of Natsei gamon rhizomes were investigated by the test tube method.

The phytochemical tests revealed that the sample contained alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, organic acids, reducing sugars, saponins, starch, steroids, tannins and terpenoids. However, cyanogenic glycosides were not detected in the rhizome of Natsei gamon. The results are summarized in Table 1.

Table 1. Phytochemical Results of Rhizome of Natsei Gamon

No	Test	Extract	Test Reagent	Observation	Results
1	Alkaloids	1 % HCl	Dragendroff's reagent Mayer's reagent Wagner's reagent Sodium picrate solution	Orange ppt White ppt Reddish brown ppt Yellow ppt	+ + + +
2	α- amino acids	H_2O	Ninhydrin reagent	Purple spot	+
3	Carbohydrates	H_2O	10 % α- naphthol, conc:H_2SO_4	Pink ring	+
4	Cyanogenic glycosides	H_2O	Sodium picrate solution	No brick red colour	-
5	Flavonoids	EtOH	Mg turning, conc: HCl	Orange colour	+
6	Glycosides	H_2O	10 % Lead acetate solution	White ppt	+
7	Organic acids	EtOH	Bromocresol green indicator	Blue colour	+
8	Phenolic compounds	EtOH	1 % $FeCl_3$ solution	Dark blue colour	+
9	Reducing sugars	H_2O	Benedict's solution	Green colour	+
10	Saponins	H_2O	Distilled water	Frothing	+
11	Starch	H_2O	1 % Iodine solution	Brown colour	+
12	Steroids	$CHCl_3$	Acetic anhydride, conc: H_2SO_4	Green colour	+
13	Tannins	H_2O	1 % Gelatin	White ppt colour	+
14	Terpenoids	$CHCl_3$	Acetic anhydride, conc: H_2SO_4	Pink colour	+

(+) = presence, (-) = absence, (ppt) = precipitate

Nutritional Values of Rhizome of Natsei gamon

The rhizome of Natsei gamon contains moisture (1.39 %), ash (5.56 %), fat (1 %), fibre (8.01 %), protein (3.66 %), carbohydrate (46.06 %), and energy values of 207.88 kilocalories in 100 g of sample.

The relatively high moisture content of the plant indicates a possible reduction in plant shelf life. The higher the ash content, the better the quality. Low-fat foods are known to reduce chlorosterol. Higher-fibre foods cause intestinal irritation and lower bioavailability. The protein content of these rhizomes, even though it appears to be low, has many medicinal properties.

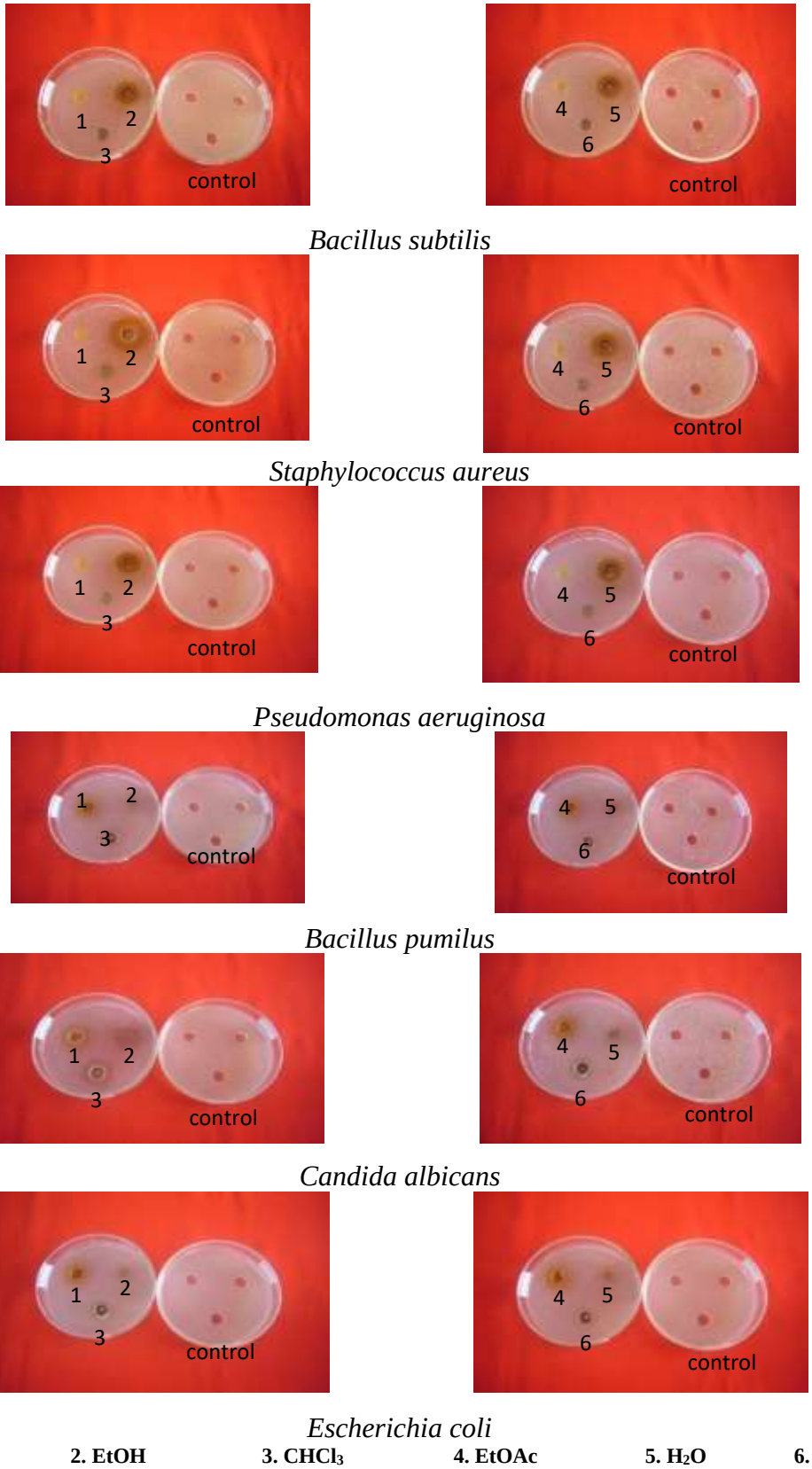
The presence of important nutrients like fats, fibre, proteins, and carbohydrates, and the physical properties such as moisture and ash indicate that the selected rhizome sample could be used as a nutritionally valuable and healthy ingredient to improve traditional medicinal formulations and to treat many diseases, according to the findings. The results are shown in Table 2.

Table 2. Nutritional Values of Rhizome of Natsei Gamon

No	Nutrients	Contents %
1	Moisture	1.39
2	Ash	5.56
3	Fat	1.00
4	Fibre	8.01
5	Protein	3.66
6	Total Carbohydrate	46.06
7	Energy Value (kcal/100 g)	207.88

Antimicrobial Activity of Crude Extracts of Natsei Gamon against Six Microorganisms

Investigation of the antimicrobial activity of six crude extracts, such as PE, EtOAc, EtOH, $CHCl_3$, MeOH, and H_2O, from the rhizome of the Natsei gamon sample was done by the agar well discussion method according to the procedure presented. In this investigation, these extracts were tested on six harmful microorganisms, including *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans,* and *Escherichia coli.* The diameter of the agar well was 10 mm. When comparing different antimicrobial agents to known concentrations, the inhibitory zone diameter is taken as a measure of antimicrobial activity (Figure 1). The larger the diameter, the higher the antimicrobial activity of the test agents.



1. PE **2. EtOH** **3. $CHCl_3$** **4. EtOAc** **5. H_2O** **6. MeOH**

Figure 1. Effect of different crude extracts from rhizome of Natsei gamon on six microorganisms

According to the results of antimicrobial activity, petroleum ether extract showed all strains of microorganisms with the inhibition zone diameter range of 13-15 mm. Watery, ethyl acetate, and ethanol extracts of the sample inhibited only one microorganism *Pseudomonas*

aeruginosa, with an inhibition zone diameter of 15-20 mm. Ethyl acetate and ethanol extracts significantly inhibited only *Pseudomonas aeruginosa*, with an inhibition zone diameter of 20 mm. The results are shown in Figure 2 and Table 3.

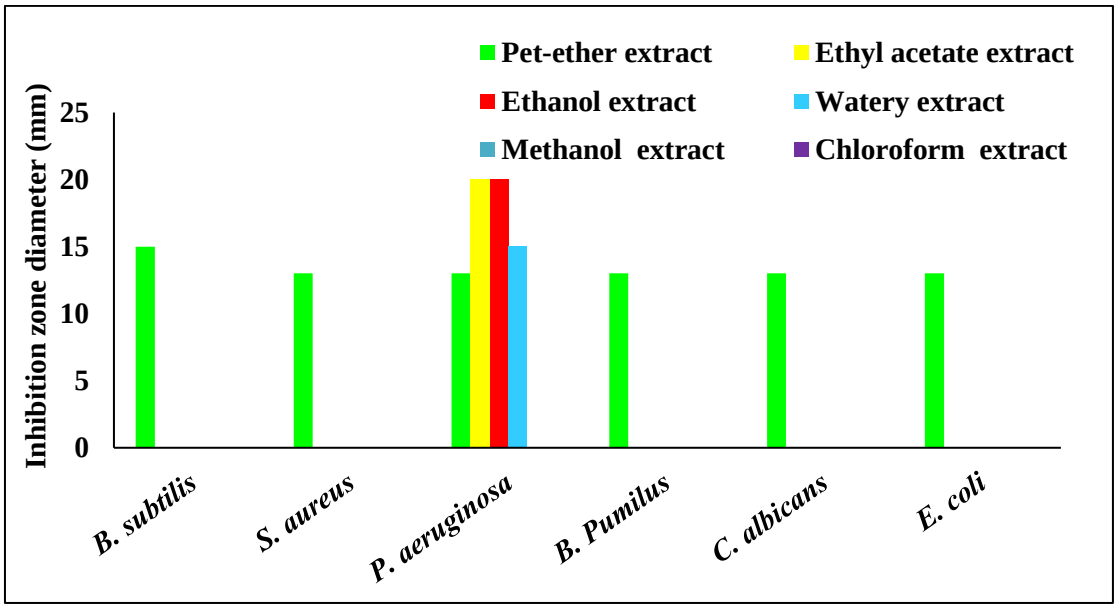


Figure 2. Inhibition zone diameters of crude extracts of rhizome of Natsei gamon against six microorganisms

Table 3. Antimicrobial Activity of Crude Extracts of Rhizome of Natsei Gamon Against Six Microorganisms

Microorganism	Inhibition zone diameter of extracts(mm)					
	PE	EtOAc	**EtOH**	**MeOH**	**$CHCl_3$**	**H_2O**
B.subtilis	15	-	-	-	-	-
S.aureus	13	-	-	-	-	-
P.aeruginosa	13	20	20	-	-	15
B. Pumilus	13	-	-	-	-	-
C. albicans	13	-	-	-	-	-
E.coli	13	-	-	-	-	-

Diameter of agar well = 10mm
10mm ~ 14 mm = (+)
15mm ~ 19mm = (++)
20 mm above = (+++)
No activity = (-)
Susceptible ≥ 21 = (+++)
Intermediate 17.20 = (+++)
Resistant ≤ 16 = (+)

α-Amylase Inhibition Activity of Crude Extracts of Natsei Gamon

The α-amylase inhibition activity of a crude extracts of the rhizome of Natsei gamon was measured by using DNS reagent method. In this study, *in vitro* α-amylase inhibitory activities of ethanol and water extracts from rhizomes of Natsei gamon were investigated. The percent inhibition of the α-amylase by ethanol and water extracts was studied in concentrations of (10, 5,

2.5, 1.25, 0.625 and 0.312 µg/mL) respectively. The percent inhibition of the sample on α-amylase enzyme activity increased with the increasing concentration.

From the percent inhibition, the respective IC_{50} values for the water and ethanol extracts were calculated. The ethanol and water extracts of the rhizome of Natsei gamon were investigated for *in vitro* α-amylase inhibition, and their activity was compared with that of the standard anti-diabetic drug, acarbose. The IC_{50} values of water and ethanol extracts of Natsei gamon were observed to be 0.85 µg/mL and 0.78 µg/mL respectively. These two extracts posses α-amylase inhibition activity. But these two extracts showed lower potent of α-amylase inhibition activity than the standard drug acarbose (IC_{50}= 0.59 µg/mL). These observations are illustrated with a bar graph diagram in Figure 3 and Tables 4 and 5.

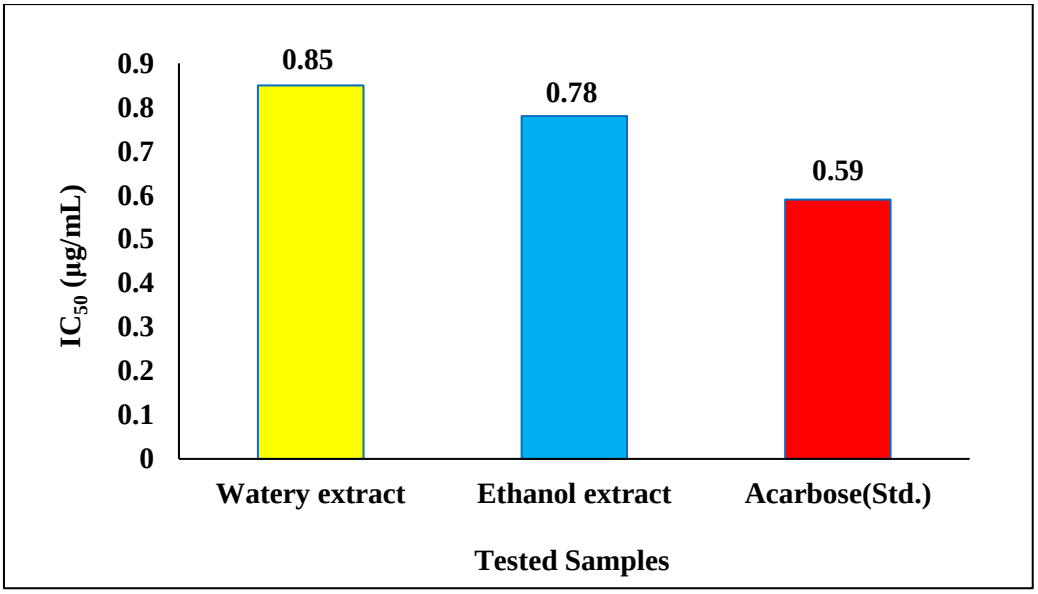


Figure 3. Comparison of IC_{50} values of watery and ethanol extracts from rhizome of Natsei gamon with standard acarbose

Table 4. α-Amylase Enzyme Inhibitions Activity of Ethanol and Watery Extracts of Rhizome of Natsei Gamon and Standard Acarbose

Sample	% Inhibition (Mean ± SD) In Different Concentration (µg/mL)					
	0.312	0.625	1.25	2.5	5	10
Water (extract)	48.83 ± 0.000	49.08 ± 0.001	51.56 ± 0.001	53.33 ± 0.001	56.61 ± 0.005	57.13 ± 0.002
Ethanol (extract)	40.19 ± 0.006	48.83 ± 0.000	53.52 ± 0.004	54.15 ± 0.000	56.67 ± 0.002	58.19 ± 0.002
Acarbose (Std.)	49.54 ± 0.000	50.04 ± 0.002	51.96 ± 0.003	52.27 ± 0.001	54.75 ± 0.001	58.15 ± 0.000

Table 5. IC_{50} Values of Crude Extracts from Rhizome of Natsei gamon and Standard Acarbose

Sample	IC_{50} (µg / mL)
Watery Extract	0.85
Ethanol Extract	0.78
Acarbose (Std.)	0.59

Cytotoxicity of Crude Extracts of Natsei Gamon by Brine Shrimp Assay

The cytotoxicity of watery and ethanol extracts of the rhizome of Natsei gamon was evaluated by a brine shrimp cytotoxicity bioassay. Ten brine shrimp (*Artemia salina*) are used for each chamber. The cytotoxicity effect was expressed as LD_{50} values (50 % lethal doses). In this bioassay, potassium dichromate and caffeine were used as cytotoxic standards.

According to these results, the LD_{50} values of water and ethanol extracts of the rhizome of Natsei gamon and standard caffeine did not show any cytotoxicity until 1000 µg/mL concentration, whereas the LD_{50} values of standard potassium dichromate were found to have a cytotoxic effect at less than 1 µg/mL of concentration. These results are shown in Table 6.

Table 6. Cytotoxicity Effect of Ethanol and Watery Crude Extracts of Rhizome of Natsei Gamon with Standard Caffeine and Potassium Dichromate

Sample	% of dead brine shrimp (Mean±SD) in various concentration (µg/mL)				LD_{50} (µg/mL)
	1	10	100	1000	
Ethanol Extract	6.66 ± 0.577	26.66 ± 0.577	36.66 ± 0.577	46.66 ± 0.577	>1000
Watery Extract	10.00 ± 1	20.00 ± 1	40.00 ± 1	46.66 ± 0.577	>1000
*Caffeine	0 ± 0	0 ± 0	26.66 ± 0.577	36.66 ± 0.577	>1000
*$K_2Cr_2O_7$	63.33 ± 0.577	66.66 ± 0.577	76.66 ± 1.154	100 ± 0	<1

* Used as cytotoxic standard

Conclusion

From the overall assessments of a chemical and biological investigation of the rhizome of Natsei gamon, the following inferences could be deduced.

In the preliminary photochemical investigation, it was found that alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, organic acids, steroids, phenolic compounds, reducing sugars, saponins, starch, tannins and terpenoids were present in the sample. The plant is reported to contain numerous phytochemical medicinal properties.

The nutritional values are 1.39% of moisture, 5.56% of ash, 3.66% of proteins, 1% of fats, 8.01% of fibre, 46.06 % of carbohydrates, and 207.88 (kcal/100g) of energy value were determined in the rhizome of Natsei gamon. The plants are rich sources of fibre and carbohydrates.

According to the results of antimicrobial activity, petroleum ether extract showed the all strains of microorganisms with the inhibition zone diameters range of 13-15 mm. Ethyl acetate, and ethanol extracts of the sample significantly inhibited only *Pseudomonas aeruginosa* with zone diameter of 20 mm. Watery extract inhibited only *Pseudomonas aeruginosa* with–an inhibition zone diameter of 15 mm.

The α-amylase inhibition activity of ethanol and water extracts of the rhizome of Natsei gamon was evaluated by the DNS reagent method. The α-amylase activity of ethanol extracts (IC_{50} = 0.78 μg/mL) is higher than watery extracts (IC_{50} = 0.85 μg/mL). α-amylase inhibition activity of both extracts was found to be lower potency than that of standard acarbose (drug) (IC_{50} = 0.59 μg/mL). From this observation, ethanol extract showed higher potency of antioxidant and α-amylase inhibition activities than watery extract of the rhizome of Natsei gamon.

From this research, watery and ethanol extracts are free from cytotoxic effects until 1000 μg/mL concentration. Therefore, these crude extracts of rhizome of Natsei gamon were evaluated for their non-cytotoxic effect.

Therefore, this research programme may contribute to the scientific development of Myanmar traditional medicine, especially in the areas concerned with α-amylase inhibition properties (antidiabetic disease), oxidative stress, non-toxics and the bacterial infections.

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