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Phytochemical Screening, *In Vitro* Antidiabetic and Antioxidant Activity of *Rabdosia rugosa* (Wall. ex Benth.) H. Hara Extract from Kinnaur District, Himachal Pradesh

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ABSTRACT

Rabdosia rugosa (Wall. ex Benth.) H. Hara. (Syn. *Plectranthusrugosus* Wall.) belongs to the Lamiaceae family and is used by local communities of the Kinnaur district to cure diabetes. The selection of the plant material is based upon data that aerial parts of the plant (leaves along with flowers) are used in folklore to manage the postprandial hike in blood sugar levels. Therefore, in the present investigation, antiradical and antidiabetic activities of the ethanolic extract of the plant were checked. Preliminary tests for phytochemicals, total phenols, and flavenoids were also determined. Our results manifested that *R. rugosa* (*Rabdosia rugosa*) extract exhibited superior antioxidant activity ($p<0.05$) by DPPH and H_2O_2 assays with IC_{50} = 9.62 ± 2.70 µg/ml and IC_{50} = 28.22 ± 2.94 µg/ml. This plant also exhibited preeminent repression action against the enzyme, *i.e.,* alpha-amylase and alpha-glucosidase ($p<0.05$) with IC_{50} = 10.49 ± 6.17 µg/ml (Porcine α – amylase), IC_{50} = 1.87 ± 1.79 µg/ml (Yeast α - glucosidase), (IC_{50} = 16.89 ± 0. 06 µg/ml(Intestinal rat α - glucosidase) Hence, we concluded that present plant possessed excellent antiradical and antidiabetic activity. The antidiabetic activity was estimated higher than the standard Acarbose. As per our information, this may be the first time to report on the extract of *R. rugosa* for its antioxidant and antidiabetic capacity. The current findings divulge that this plant can further be studied to verify its therapeutic activities.

Keywords: Antioxidant; Alpha-amylase; Alpha-glucosidase; Kinnaur; *Rabdosia rugosa*; Phytochemicals; Antidiabetic.

1. INTRODUCTION

The human body's metabolism of fats, carbohydrates, and proteins is mainly affected by chronic diabetes mellitus. The annihilation of pancreatic beta cells accelerates abnormal rises in blood sugar levels that occur due to insulin resistance, or insulin deficiency is considered the leading cause of this syndrome. The inflation of glucose molecules in the blood disrupts metabolic processes inside the human body.[1]

The major limitation of DM is associated with an increase in long-term micro vascular and macro vascular complications. If not treated properly at the right time, it will result in irreversible damage to the heart, peripheral arterial system, eyes, nerves, and kidney failure.[2] Moreover, people with uncontrolled hyperglycemia are more susceptible to developing infectious diseases (bacterial, viral). In addition, people with diabetes are more vulnerable to COVID - 19 severity than non-diabetic patients caused of a novel corona virus that has disrupted the global health and economic structure in recent times.[3]

Several synthetic antidiabetic medications are available such as sulfonylureas, biguanides, α–glucosidase inhibitors, etc.[4] However, these drugs have severe complications with undesirable side effects. The frequently detected symptoms are hypoglycemia, weight gain, and gastrointestinal disturbance (bloating, flatulence, abdominal discomfort, diarrhea).[5] The global demand for herbal medicines has recently increased due to growing interest in the therapeutic effect of natural antioxidants. So, plant-based drugs have been considered to effectively treat DM as they are less toxic compared to synthetic drugs.[5-6] The existence of immense bioactive compounds in plants leads to the antioxidant property that plays an extensive role in suppressing diseases induced by free radicals. Phenolic compounds, alkaloids, terpenoids, saponins, tannins, sterols, and quinines are among these compounds.[7] An adequate amount of antioxidants provides a shield from hazardous free radicals.

The presence of phytochemicals proclaims various pharmaceutical activities, such as antimicrobial, anti-inflammatory, antihyper glycemic and anticancer, which is why they form an essential part of the formulation of pharmaceutical products and can also be used as raw materials for the research and development of new drugs.[8]

R. rugosa is an aromatic plant that belongs to the Lamiaceae family. It is widely spread around the northern temperate Himalayas up to 1500-3000 m. However, its population is scanty in higher-altitude regions. The researchers

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Figure 1. Image of R.rugosa.

have focused most of the study on investigating its essential oil. The essential oil of the plant has shown significant antioxidant activity.Several supporting pharmacological activities, *viz.,* anti-inflammatory, analgesic, antipyretic, myorelaxant, antibacterial, and antifungal, have been reported on its essential oil.[9-10]

Previous studies reported that the existence of monoterpenoids and triterpenoids, beta-sitosterol, ursolicacid, oleanolic acid, botulin, and hexacosanol had been investigated in the leaf extracts.[11] The leaves of *R.rugosa* were traditionally practiced against diabetes by inhabitants of the Kiwai region of Pakistan.[12]

Apart from the literature studies relevant to our work, an extensive survey was conducted in different parts of the Kinnaur district to gather valuable information. *R.rugosa* is locally known as Chichiri in the Kinnaur dialect. After interacting with local inhabitants, especially with old age people and shepherds, valuable information was gathered. It was revealed that to cure diabetes, they took a half cup of plant extract twice a day, which was prepared by boiling plant parts (leaves and inflorescence) for 5-10 min. As per our knowledge, no prior reports on this plant's antidiabetic and antioxidant activities are available. Thereafter, the current study was examined to find out the antidiabetic efficacy of this plant through *invitro* study. Therefore, our investigation's main objective was to test plant extract's antioxidant and antidiabetic potential. The total phenols, flavonoid content, and preliminary phytochemical analysis were also carried out.

2. METHODOLOGY

2.1 Method of Extraction

R. rugosa was picked up from the Kinnaur district of Himachal Pradesh during its blossoming season in September 2019. Plant tentatively identified with the help of keys and description given in various floras and monographs. Authentication of plant material by comparing with the identified specimens lying in the Herbarium of Panjab University Chandigarh (PAN) and deposited (accession number 22397). The collected plants were properly washed to remove all impurities with normal water. The plant's aerial parts (leaves and flowers) used in the present study were dried in shadow at ambient temperature (27 °C) for two weeks. A grinder was used to generate fine powder from plant material. In 50 ml of 90 % ethanol, 20 g of powdered material was extracted from the shaker for 22-24 h. The obtained material was filtered through filter paper (What man no. 1). The filtrate was retained in a partially covered petri dish until obtained the concentrated extract (was 3-6 days). The extracted material should be adequately covered to avoid contamination and conserved in a freezer at 4 °C to further use.

2.2 Chemicals

2, 2-diphenyl-1-picrylhydrazy, Porcine alpha-amylase; alpha-glucosidase (Saccharomyces cerevisiae); p-nitrophenol- α-D-glucopyranosideof Sigma Aldrich. Dimethyl sulphonate, sodium chloride, starch, Sodium potassium tartrate, Dinitrosalicylic acid, Sodium carbonate, H_2O_2, Ascorbic acid, Folin–Ciocalteu (F–C) reagent, Gallic acid, Aluminum chloride, Quercetin, HCl, mercuric chloride, H_2SO_4, Chloroform, ferric chloride, NaOH, ethanol, methanol, glacial acetic acid purchased from GK enterprises, Chandigarh.

2.3 Analysis of Phytoconstituents

Screening of phytoconstituents of plant samples in ethanolic, methanolic, and aqueous solvents to check the existence of different phytochemicals, i.e., terpenoids, phenolic compounds, flavonoids, tannins, saponins, alkaloids, and glycosides by following the standard methods.[13]

2.4 TPC (Total Phenolics Content)

TPC was investigated using Folin - Ciocalteau's reagent using the standard technique with little modification.[14] Approximately 2.5 ml of F-C reagent mixed in 0.5 ml of extract of diverse concentrations (50-250 µg/ml), incubated for 5 min. After that, 2.5 ml Na_2CO_3 solution (7.5 %) was poured and then reincubated at 45 °C for an hour.The absorbance of the samples was read against blank at 765 nm.TPC was articulated as the extract's equivalent of gallic acid (mg/g) by applying a calibration curve.

2.5 TFC (Total Flavonoid Content)

The TFC of *R.rugosa* was ascertained by succeeding the standard protocol using aluminium chloride colourimetric assay.[15] Firstly, different plant extracts (50-250 µg/ml) were prepared in triplicates, making them a final volume of 0.5 ml by adding deionised water. 0.1ml $AlCl_3$ solution was poured into all test tubes except the blank. Subsequently, 0.1ml potassium acetate was mixed in each test tube except the blank. 3 ml of total volume was made by pouring 2.3 ml of 80 % ethanol. After vigorously blending, the mixture is incubated for 30 min. The samples reading were determined against

blank using a spectrophotometer at 420 nm. Using a calibration curve, the TFC was explicated as equivalent to the extract's quercetin acid (mg/g).

2.6 Antioxidant Activity

2.6.1 By DPPH Method

The anti-DPPH action of *R.rugosa* was confirmed by following the standard methodwith some alteration.[16] Different concentrations of extracts (50-250 µg/ml in triplicates) were dissolved in DMSO. Subsequently, 0.1 mM DPPH solution of volume 2.5ml was mixed with varying concentrations of extracts containing glass tubes. After 40 min of incubation at 28°C, the optical density of the samples was read against a blank. Ascorbic acid is referred to as standard. Hindering of free radicals in per cent was measured as appended below:

$$\text{Antiradical activity (\%)} = [(A\text{b}\hat{} - A\text{b}\hat{}\hat{}) / A\text{b}\hat{} \times 100]$$

where A$\hat{}$and A$\hat{}\hat{}$ are the optical densityof the control and the test sample.

A linear regression model was implied to calculate the IC_{50} value, which was used and subsequently obtained by applying the fitted line, i.e., Y = a**X* + b *IC*50 = (0.5 - b) / a

2.6.2 Hydrogen Peroxide Assay

The H_2O_2 radical scavenging capacity of *R.rugosa*extract was directed by following the standard method.[17] The different extract volumes were prepared in triplicate form (50-250 µg/ml). Induction of 0.6 ml of H_2O_2 solution (40 mM Phosphate buffer; pH 7.4) in all test tubes except a blank. Subsequently, after ten min of incubation, the final reading of samples was recorded at 230 nm against the blank. Ascorbic acid is referred to as standard. The scavenging of H_2O_2 per cent was measured as follows:

$$\text{Antiradical activity (\%)} = [(A\text{b}\hat{} - A\text{b}\hat{}\hat{}) / A\text{b}\hat{} \times 100]$$

where, Ab$\hat{}$and Ab$\hat{}\hat{}$ are the optical density of the control and the test.

2.7 *In-virto* Antidiabetic Study

2.7.1 The α-amylase Inhibition Assay

Alpha-amylase suppression capacity of *R.rugosa* extracts is accomplished by following the previously described method with little modification.[18] The extract of different concentrations (50 – 250 µg/ml in triplicates) was dissolved in DMSO. In each test sample, 250 µl alpha-amylase (0.001 g in 20 mM phosphate buffer with 6.7 mM NaCl, pH 6.9) was mixed. The preincubation of the commixture was carried out at 37 °C for half an hour. Induction of 500 µl volume of starch solution (1%) to the different aliquots (50 – 250 µl)and reincubated for 12 min at 37 °C. Reaction ceased by induction of 1 ml of DNSA solution (Prepared with 1g of 3,5-dinitro salicylic acid, 30g of sodium potassium tartrate, and 20 ml of 0.5 M NaOH), followed by a hot water bath for 5 min. Afterwards, all the samples were placed in a beaker containing ice-cold water. 5ml of double purified water was poured into all test tubes, and absorbance was read at 540 nm against the blank. Blank was formulated by adding 250 µl of buffer (no enzyme added). Control which exhibited 100 % enzymatic activity, 250 µl of DMSO was added instead of extract. Acarbose is referred to as standard. Percentage inhibition capacity was determined by applying the formula:

$$\text{\% inhibition} = [(A\text{b}\hat{} - A\text{b}\hat{}\hat{}) / A\text{b}\hat{} \times 100]$$

where, Ab$\hat{}$and Ab$\hat{}\hat{}$ are the optical density of control and the test.

2.7.2 The α-glucosidase Inhibition Assay (Saccharomyces Cerevisiae)

The α-glucosidase repression capacity of plant material was investigated by following the standard protocol with some alteration.[19] 50 µl (1 mg/ml) of the sample was assembled at various concentrations (10 µl – 50 µl), and each sample was taken in a triplicate form. In each glass tube added 100 µl of α-amylase solution (0.1 U/ml in 0.1M Phosphate buffer; pH 6.9). Preincubated for 10 min at 37 °C, followed by mixing 50 µl of p -NPG (5 mM p-nitrophenyl-α-D-glucopyranoside in 0.1M phosphate buffer; pH 6.9) in all test tubes. To halt the reaction, 2 ml of Na_2CO_3 (0.1M) was mixed with the mixture at 37 °C for half an hour. Subsequently, at 405 nm, absorbance was read against a blank, and values were compared with a control containing DMSO as a substitute for plant extract. A carbose is referred to as standard. The calculation of percentage inhibition activity by following the expression given below:

$$\text{\% inhibition} = [(A\text{b}\hat{} - A\text{b}\hat{}\hat{}) / A\text{b}\hat{} \times 100]$$

where, Ab$\hat{}$and Ab$\hat{}\hat{}$ are the optical density of the control and the test.

2.7.3 Suppression of Rat Intestinal α–glucosidase

Small intestines were collected from male Wistar rats after sacrificing the animal under anaesthesia (with the approval of the ethical committee Central Animal House Panjab University Chandigarh (45/99/CPCSEA). Extracted part of the intestine was thoroughly washed with saline (0.9%) while all the protocols proceeded at the temperature of 4 °C. The part of the intestine was homogenized in 5 mM of phosphate buffer with pH 7 for 5 min. The centrifugation of the homogenate mixture was carried out at 4000 rpm for 10 min. The resultant supernatant was utilised as a source of α-glucosidase in our *in vitro* analysis. The procedure for inhibition activity was the same as that of yeast alpha-glucosidase.In most previous studies, Yeast alpha-amylase has been used. However, alpha-glucosidase extracted from rat intestinal closely imitates mammalian

enzymes.[20] It could be a superior model to develop antidiabetic agents specifically for regulating postprandial hyperglycemia. Therefore, in the present study, we have tested both enzymes to compare if there is any difference in their enzymatic activity.

2.7.4 Statistical Study

The data demonstrated as a mean ± SEM of a clone of three independent values. Calculation of the IC_{50} value was done by using a non-linear regression curve in MS Excel. Mean differences were estimated by adopting a one-way analysis of variance.To observe the significant comparability among the mean Tukey post hoc multiple comparison test was applied. The Pearson Correlation Coefficient test was implied to investigate any correlation between TPC and TFC with the antioxidant property. The value was expected to be significant if $p \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1 Qualitative Investigation of Phytochemicals

Qualitative analysis of plant samples in powdered form was done in methanolic, ethanolic, and aqueous solvents for various phytochemical constituents such as flavonoids,phenols, terpenoids, saponins, tannins, and glycosides was done (Table 1). We observed the best results in the ethanolic solvent. Therefore, further extraction was done in the ethanolic solvent. The per cent yield (% w/w) of ethanolic extract was 15 %. Therefore, further *in vitro* was conducted in ethanolic extract only. Where

(-) confirms the absence of phytochemicals.

(+) confirms the presence of phytochemicals (+++) confirms the strongest indication of phytochemicals

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(+++) confirms the strongest indication of phytochemicals

Medicinal plants are rich in different phytoconstituents, making them highly recommended to treat chronic diseases, i.e., diabetes mellitus, anticancer, anti-ageing, cardiovascular, obesity, and neurodegenerative diseases.[21]

Table 1. Results of phytoconstituents analysis of *R.rugosa* extract

Test	Ethanolic	Methanolic	Aqueous
Alkaloids	-	-	-
Terpenoids	+	+	+
Phenols	+++	+	+
Flavanoids	+	+	+
Tannins	+	+	+
Saponins	++	+	+++
Glycosides	+	+	+

Table 2. Shows results of TPC and TFC

Concentration (µg /ml)	TPC (Gallic acid (mg/g) of the extract)	TFC (Quercetin (mg/g) of the extract)
50	62.03 ± 0.66	49.48 ± 0.21
100	72.18 ± 0.57	58.05 ± 0.16
150	88.88 ± 0. 67	64.08 ± 0. 26
200	103.58 ± 0. 66	73.75 ± 0.13
250	150 ±7.56	87.14 ± 0.42

3.2 Assessment of TPC and TFC

The TPC and TFC in the ethanolic extract were measured by adopting a linear regression equation of total phenols ($y=0.009x-0.446$; $R^2=0.986$) were expressed as GAE/g froma calibration curve of standard gallic acid, and total flavonoids ($y=0.011x-0.480$; $R^2 = 0.991$) indicated in terms of QE/g as demonstrated in Table 2. Secondary metabolites such as phenolics and flavonoids are potent sources of antioxidants; phenols act as metal chelators fought against free radicals due to their antioxidant properties.[22] Flavonoids are considered the most promising polyphenolic compounds among secondary metabolites.[23] The ethanolic extract contains an ample amount of phenols and flavonoid content of 150 ±7.56 mg GAE/g of extract and 87.14 ± 0.42 mg QE/g of extract, indicating its health-promoting activity.

DPPH free radical scavenging activity

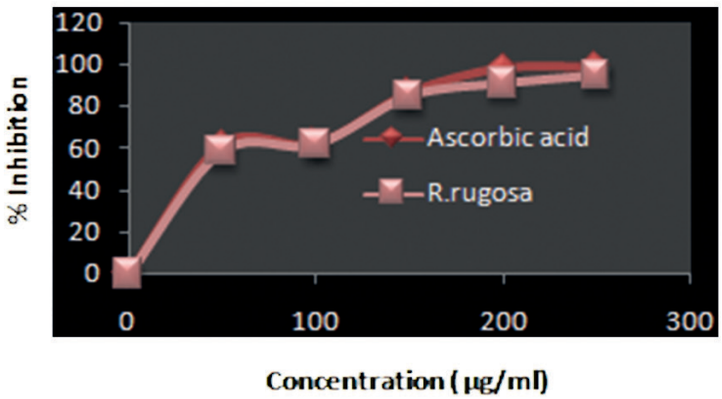


Figure 2. DPPH suppressing activity of *R. rugosa* extract and ascorbic acid.

Table 3. Results of % inhibition of DPPH by ethanolic extracts of *R.rugosa* and standard Acarbose. (n=3)

Concentration (µg/ml)	% inhibition by *R. rugosa*	IC_{50} (µg/ml) of (*R.rugosa*)	% Inhibition by Standard (Ascorbic acid)	IC_{50}(µg/ml) of Ascorbic acid
50	58.87 ± 0.50[a]		61.88 ± 0. 13[a]	
100	62.06 ± 1.39[a]		62.27 ± 0.14[b]	
150	85. 12 ± 0.10[b]		86.47 ± 0. 12[c]	
200	91.19 ± 1.23[c]	9.62 ± 2.70	98.22 ± 0. 24[d]	6.05 ± 0. 61
250	94.97 ± 0.12[c]		98.58 ± 0.04[d]	

Different letters above the results in each column (a-d) indicated significant (p≤0.05), a value shown as Mean ± SEM of triplicates.

3.3 DPPH Free Radical Scavenging Assay

Previously many scientific investigations have exhibited that free radicals could lead to the instigation of various diseases. Antioxidants can eliminate unstable free radicals by putting down the oxidation process. Figure 1. Shows the scavenging activity of *R.rugosa* ethanolic extract and standard ascorbic acid. Percentage inhibition activity was highest for both the extract and ascorbic acid, representing 94.97 ± 0.12 % at 250 µg/ml (concentration). At the same concentration, 98.58 ± 0.04 % was estimated for Ascorbic acid. The antioxidants had a noticeable impact on DPPH primarily due to their hydrogen-donating ability. In this method, deep violet colour DPPH is reduced to a light yellow product, *i.e.,* diphenyl picrylhydrazine, which is now free from free radicals after reacting with plant extracts. The scavenging activity is mainly due to its potential to neutralize the deleterious role of free radicals.[24] IC 50 value of plant extracts was 9.62 ± 2.70 µg/ml and for ascorbic acid was 6.05 ± 0.61 µg/ml, as shown in Table 3. Our findings indicate that plant extracts and standards exhibited more than 90 % antiradical activity. Polyphenolic compounds can hunt harmful free radicals through their hydrogen-donating activity.[25]

3.4 H_2O_2 Scavenging Assay

The H_2O_2 hindrance effect of the ethanolic extract of *R.rugosa* was experimented with at a different dose (50-250 µg/ml), as shown in Figure 2. *R.rugosa* ethanolic extract possessed higher H_2O_2 scavenging activity, with IC_{50} = 96.85 ± 4.88 µg/ml) comparable to the standard (ascorbic acid) with IC_{50} value= 94.77 ± 10.5 µg/ml. Though, the hindering effect of the extract was recorded as lower than the ascorbic acid. However, our sample displayed more than 90 % inhibition which is very close to the positive control, i.e., Ascorbic acid. "The H_2O_2 is not pernicious; sometimes, it may become toxic to the cell by giving it a hydroxyl radical. Introduction of H_2O_2 in a cell accelerates the metal ion-dependent OH radical-mediated oxidative destruction of DNA".[26] Therefore, it is foremost to remove the superoxide anion that could lead to the generation of various kinds of secondary complications. The results clearly show the H_2O_2 scavenging potential of *R. rugosa* extract containing a high amount of antioxidant compounds.

H_2O_2 scavenging activity

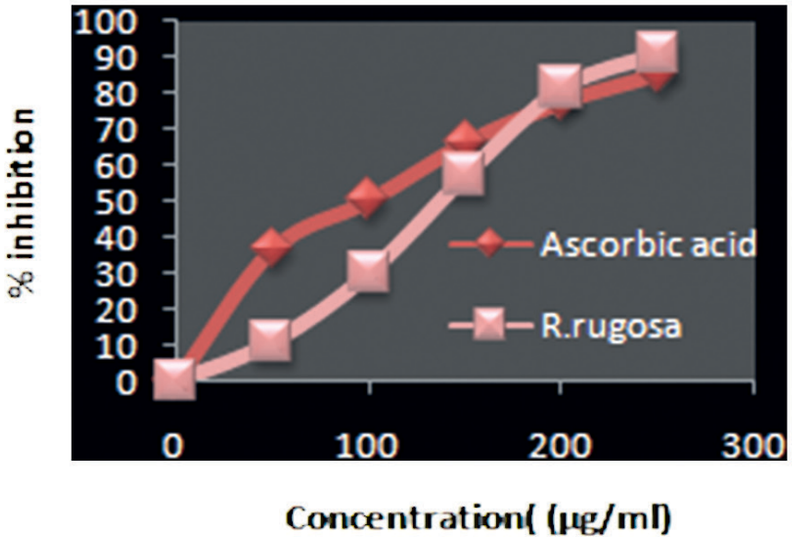


Figure 3. H_2O_2 scavenging activity of *R.rugosa* and standard Acarbose.

3.5 Correlations

R.rugosa extract showed a statistically significant correlation between antioxidant activity by H_2O_2 assay with TPC ($p<0.05$), $R^2 = 0.822$. Its activity is highly correlated with TFC ($p<0.01$), $R^2 = 0.927$. It showed no significant correlation between DPPH and TPC, $R^2 = 0.73$. However, it significantly correlated with TFC ($p\leq0.05$), $R^2=0.826$.

3.6 Suppression of Alpha-amylase Activity

The potential hindering response of *R.rugosa* against the alpha-amylase enzyme is presented in Figure 3. The ethanolic extract exhibited concentration-dependent inhibition of α–amylase performance. Our outcome confirmed that the inhibition potential of *R.rugosa* is around 46 folds higher with an IC_{50} value of 2.65 ± 2.54 in comparison to the standard Acarbose, i.e., positive control with an IC_{50} value of 122.34 ± 4.28. Several drugs that lower postprandial excursion of blood sugar levels by declining the process of starch digestion, e.g., α-amylase inhibitors, have been found beneficial for treating hyperglycemia.[27] So inhibiting the activities of enzymes is a promising approach for managing high

Table 4. Results of % inhibition of H_2O_2 by ethanolic extracts of *R.rugosa* and standard (Ascorbic acid)

Concentration (µg/ml)	% H_2O_2scavenging activity of *R.rugosa*	IC_{50}(µg/ml) value of (*R.rugosa*)	% H_2O_2 scavenging activity of Ascorbic acid	IC_{50} (µg/ml) of standard (Ascorbic acid)
50	10.96 ± 1.67[a]		36.72 ± 4.76[a]	
100	30.06± 1.00[b]		50.33 ± 2.34[ab]	
150	57.18 ± 0.70[c]		66.39 ± 3.69[bc]	
200	81.29 ± 0.58[d]	96.85 ± 4.88	77.78 ± 2.01[cd]	94.77 ± 10.55
250	90.84 ± 0.17[e]		85.53 ± 4.10[d]	

Different letters above the results in each column (a-e) indicate the significant changes at $p<0.05$; the value is shown as the Mean ± SEM of triplicates.

Table 5. Results of % inhibition of alpha-amylase by ethanolic extract of *R.rugosa* and standard drug Acarbose

Concentration (µg/ml)	α – amylase inhibition by *R.rugosa*	IC_{50}(µg/ml) of (*R. rugosa*)	α – amylase inhibition by Acarbose	IC_{50} (µg/ml) of (Acarbose)
50	50.92 ± 1.87[a]		45.14 ± 0.14[a]	
100	70.87 ± 0.40[b]		47.23 ± 0.33[a, b]	
150	83.78 ± 0.21[c]		49.10 ± 0.61[b]	
200	87.11± 0. 41[c,d]	10.49±6.17	53.97 ± 1.78[c]	122.34± 4.28
250	90. 78 ± 0.46[d]		71.02 ± 0.86[d]	

Table 6. Results of % inhibition of yeast and intestinal rat alpha-glucosidase by ethanolic extracts of *R.rugosa* and Standard drug Acarbose

Concentration (µg/ml)	Inhibition of Yeast alpha glucosidase by *R. rugosa*	IC_{50} (µg/ml) (*R.rugosa*)	Inhibition by Acarbose	IC_{50} (µg/ml) (Acarbose)	% inhibition of Intestinal rat alpha glucosidase by *R.rugosa*	IC_{50} (µg/ml) (*R.rugosa*)	Inhibition by Acarbose	IC_{50} (µg/ml) (Acarbose)
10	61.30 ± 1.26[a]		31.87 ± 1.37[a]		30.96 ± 0.21[a]		42.39 ± 1.53[a]	
20	69.16 ±1.83[b]		58.33 ± 2. 88[b]		58.08 ± 0.14[b]		50.60 ± 2.26[a,b]	
30	70.01±1.50[b]		68.54 ± 2.29[b,c]		81.20 ± 0.21[c]		60.41 ± 4.01[c]	
40	95.32 ± 1.10[c]	1.87 ± 1.7	73.04 ± 1.68[c, d]	9.02±1.111	88.84 ± 0.46[d]	16. 89 ± 0.06	84.18 ± 1.13[d]	17.74 ± 1.86
50	98. 08± 0. 29[c]		83.54 ± 2.93[d]		94.56 ± 0. 28[e]		85.78 ± 1.08[d]	

Different letters above the results in each column (a-e) denote the significant changes ($p<0.05$), IC_{50} value calculated as mean ± SEM of triplicates.

blood sugar levels. The human pancreatic α-amylase in the small intestine is closely associated with an increase in postprandial blood sugar levels, which have been reported in previous studies.[28] *R.rugosa* could be a lead extract for the regimen of diabetes. The plant extract exhibited a lower IC_{50} value than Acarbose, i.e., 50 % inhibitory activity at a minimum extract concentration. The ethanolic extract of *R.rugosa* extract possessed significant porcinepancreatic amylase-inhibiting activity.

Different letters above the results in each column (a-d) indicate the significant changes at $p<0.05$ of Mean ± SEM triplicates.

3.7 Suppression of Alpha-glucosidase Activity

The alpha-glucosidase is found in the jejunum on the brush borders of the small intestine. Its functional role catalyses the disaccharides into simple sugar molecules, the final step in digestion or intestinal absorption. Hyperglycemia could be controlled by hindering the activity of α – glucosidase.[29] The obstructive activity of the extract against the enzyme was evaluated by calculating the IC_{50} value of theextract, which showed the most potent inhibition as compared to Acarbose, *i.e*., positive controlfor both enzymes (alpha-glucosidase and rat intestinal alpha-glucosidase), as shown in Table 5. Both plant extracts and a positive control showed dose-dependent percentage inhibition, as delineated in Figure 4. By delaying the consumption of dietary starch in the small intestine, hyperglycemia can be managed, a practical approach for the formulation of antidiabetic drugs.[30] The selected plant extract showed hindering

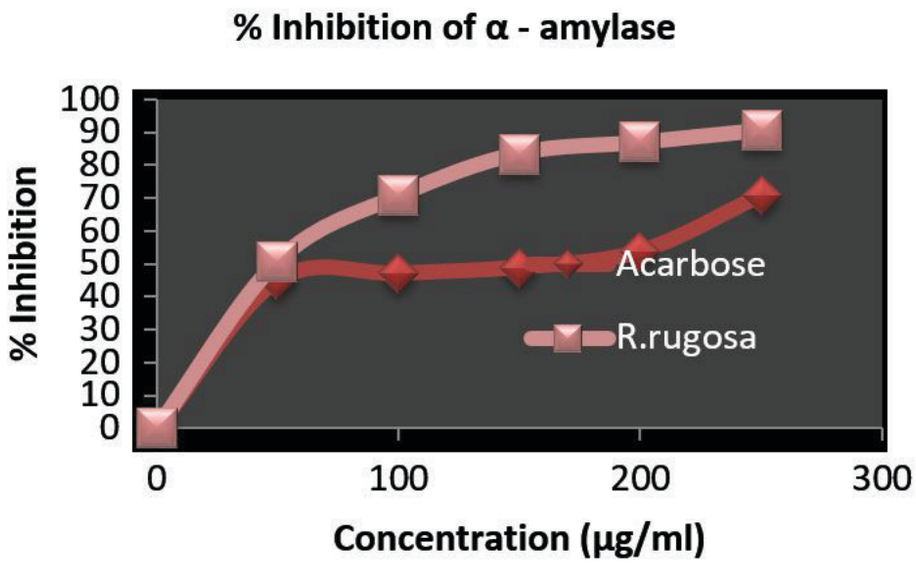
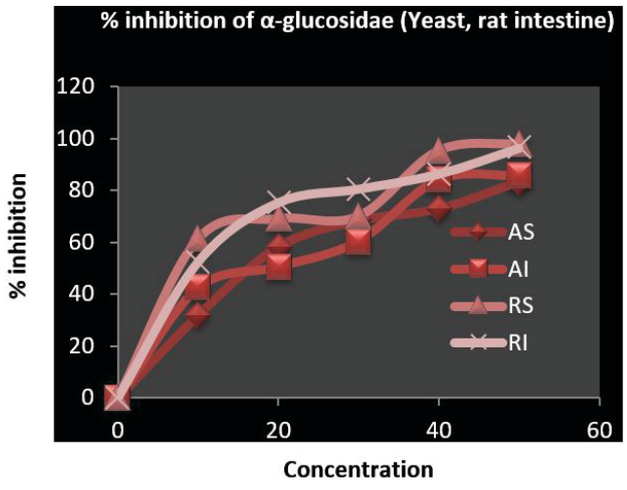


Figure 4. Shows % inhibition of alpha-amylase by extracts of *R.rugosa* and Standard Acarbose.



AS:- % inhibition of α – glucosidase (*Saccharomyces cerevisiae*) by standard (Acarbose).

AI:- % Inhibition α – glucosidase (rat intestinal) by standard (Acarbose).

RI:- % Inhibition of α – glucosidase (rat intestinal) by ethanolic extracts of *R. rugosa.*

RS:- % inhibition of α – glucosidase (*Saccharomyces cerevisiae*) by ethanolic extracts of *R. rugosa.*

Figure 5. Result of % inhibition of α-glucosidase and rat intestine α-glucosidase of *R. rugosa* and Acarbose

activity against the enzymes, i.e., alpha-glucosidase (yeast) and rat intestinal alpha-glucosidase.

4. CONCLUSION

The knowledge of the potential inhi-bition of carbohydrate metabolic enzymes is significant for managing postprandial spikes in blood sugar levels. The best-approaching action mechanism proclaimed by this plant is the suppression of alpha-amylase and alpha-glucosidase to regulate the abnormal rise of blood glucose levels. It also showed great activity against DPPH and H_2O_2 free radicals. Our Research is a first-hand delineation of the antioxidant and antidiabetic activities of *R.rugosa*. Moreover, the presence of various phytochemicals with a good amount of phenolics and flavonoid content indicates that this plant has antioxidant properties. To justify the findings in the present study, we have also performed *in-vivo* study, which we will discuss in the continuation of this article. With this, we have concluded that the present study could be beneficial in future Research on the application of traditional medicinal plants in the formulation of pharmaceutical drugs for the long-term management of hyperglycemia.

REFERENCES

1. Lacroix, I. M. & Li-Chan, E.C. Overview of food products and dietary constituents with antidiabetic properties and their putative mechanisms of action: A natural approach to complement pharmacotherapy in the management of diabetes. *Mol. Nutr. Food Res.*, 2014, **58**(1), 61-78. doi: 10.1002/mnfr.201300223.
2. Fowler, M. J. Microvascular and macrovascular complications of diabetes. *Clin. Diabetes.*, 2008, **26**(2), 77-82. doi: 10.2337/diaclin.26.2.77.
3. Erener, S. Diabetes, infection risk and COVID -19. *Mol. Metab.,* 2020, **39**, 101044. doi: 10.1016/j.molmet.2020.101044.
4. DiStefano, J.K. & Watanabe, R.M. Pharmacogenetics of anti-diabetes drugs. *Pharm.*, 2010, **3**(8), 2610-2646. doi: 10.3390/ph3082610.
5. Cheng, A.Y. & Fantus, I.G. Oral antihyperglycemic therapy for type 2 diabetes mellitus. *Can. Med. Assoc. J.*, 2005, **172(**2), 213-226. doi: 10.1503/cmaj.1031414.
6. Grover, J.K.; Yadav, S. & Vats, V. Medicinal plants of India with antidiabetic potential. *J. Ethnopharmacol.*, 2002, **81(**1), 81-100. doi: 10.1016/S0378-8741(02)00059-4.
7. Durazzo, A.; Lucarini, M.; Novellino, E.; Souto, E.B.; Daliu, P. & Santini, A. Abelmoschus esculentus (L.): Bioactive components' beneficial properties-Focused on the antidiabetic role-For sustainable health applications. *Mol.*, 2018, **24**(1), 38. doi: 10.3390/molecules24010038.
8. Zhang, Y.J.; Gan, R.Y.; Li, S.; Zhou, Y.; Li, A.N.; Xu, D.P. & Li, H.B. Antioxidant phytochemicals for the prevention and treatment of chronic diseases. *Mol.*, 2015, **20**(12), 21138-21156. doi: 10.3390/molecules201219753.
9. Misra, P.S.; Misra, G.; Nigam, S.K. & Mitra, C.R. Constituents of Plectranthusrugosus. *Lloydia*,1971, 265-266.
10. Singh, P.; Kumar, R.; Pant, A.; Kumar, M.; Isidorov, V. & Szczepaniak, L. Chemical composition, anti-inflammatory, analgesic, antipyretic, myorelaxant, antibacterial, and antifungal activity of Rabdosia rugosa wall. (Syn. Plectranthus rugosus Wall). *J. Med. Herbs Ethnomed.,* 2019, **5**, 8-15. doi:10.25081/jmhe.2019.v5.383.
11. Razdan, T.K., Kachroo, V., Harkar, S., Koul, G.L. & Dhart, K.L. (1982). Plectranthoic acid, acetylplectranthoic acid and plectranthadiol, are three triterpenoids from Plectranthusrugosus. *Phytochem.*, **21**(2), 409-412. doi: 10.1016/S0031-9422(00)95277-4.
12. Jabeen, T.; Akhtar, A.; Shah, A.H.; Khan, K.R. & Farooq, M. Qualitative and quantitative ethnobotanical evaluation of plant resources of Kiwai, Kaghan valley, district Mansehra, Pakistan. *Indian J. Trad. Knowl.*, 2021, **20(**1), 141-153. doi: 10.56042/ijtk.v20i1.29424.
13. Krishnaiah, D.; Devi, T.; Bono, A. & Sarbatly, R. Studies on phytochemical constituents of six Malaysian medicinal plants. *J. Med. Plants Res.*, 2009, **3**(2), 067-072. doi: 10.5897/JMPR.9001153.
14. Saeed, N.; Khan, M.R. & Shabbir, M. Antioxidant activity, total phenolic and total flavonoid contents of the whole plant extracts *Torilisleptophylla* L. *BMC Complement. alternative med.*, 2012, **12(**1), 1-12.
15. Chang, C.C.; Yang, M.H.; Wen, H.M. & Chern, J.C. Estimation of total flavonoid content in propolis by two complementary colourimetric methods. *J. Food Drug Anal.*, 2002, **10**(3).
16. Brand-Williams, W.; Cuvelier, M.E. & Berset, C.L.W.T. Use of a free radical method to evaluate antioxidant activity. *LWT-Food Sci. Technol.*, 1995, **28**(1), 25-30. doi: 10.1016/S0023-6438(95)80008-5.
17. Bozin, B.; Mimica-Dukic, N.; Samojlik, I.; Goran, A. & Igic, R. Phenolics as antioxidants in garlic (Allium sativum L., Alliaceae). *Food Chem.*, 2008, **111**(4), 925-929. doi: 10.1016/j.foodchem.2008.04.071.
18. Ali, H.; Houghton, P. J. & Soumyanath, A. α-Amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to Phyllanthus amarus. *J. Ethnopharmacol.*, 2006, **107**(3), 449-455. doi: 10.1016/j.jep.2006.04.004.
19. Shanghai, S.; Thiantongin, P.; Sontimuang, C.; Ovatlarnporn, C. & Puttarak, P. α-Glucosidase and α-amylase inhibitory activities of medicinal plants in Thai antidiabetic recipes and bioactive compounds from Vitex glabrata R. Br. stem bark. *J. Herb. Med.*, 2020, **19**, 100302. doi: 10.1016/j.hermed.2019.100302.
20. Ohta, T.; Sasaki, S.; Oohori, T.; Yoshikawa, S. & Kurihara, H. α-Glucosidase inhibitory activity of a 70% methanol extract from Ezoishige (Pelvetia babingtonii de Toni) and its effect on the elevation of blood glucose level in rats. *Biosci. Biotechnol. Biochem.*, 2002, **66**(7), 1552-1554.

doi: 10.1271/bbb.66.1552.
21. Zhang, Y.J.; Gan, R.Y.; Li, S.; Zhou, Y.; Li, A.N.; Xu, D.P. & Li, H.B. Antioxidant phytochemicals for the prevention and treatment of chronic diseases. *Mol.*, 2015, **20**(12), 21138-21156. doi: 10.3390/molecules201219753.
22. Al-Owaisi, M.; Al-Hadiwi, N. & Khan, S.A. GC-MS analysis, determination of total phenolics, flavonoid content and free radical scavenging activities of various crude extracts of Moringa peregrina (Forssk.) Fiori leaves. *Asian Pac. J. Trop. Biomed.*, 2014, **4**(12), 964-970. doi: 10.12980/APJTB.4.201414B295.
23. Tomczyk, M.; Pleszczyńska, M. & Wiater, A. Variation in total polyphenolics contents of aerial parts of Potentilla species and their anti-cariogenic activity. *Mol.*, 2010, **15**(7), 4639-4651. doi: 10.3390/molecules15074639.
24. Rahman, M.M.; Islam, M.B.; Biswas, M. & Alam, A.K. *In vitro* antioxidant and free radical scavenging activity of different parts of Tabebuia pallida growing in Bangladesh. *BMC Res. Notes*, 2015, **8**(1), 1-9. doi: 10.1186/s13104-015-1618-6.
25. Huang, D.; Ou, B. & Prior, R.L. The chemistry behind antioxidant capacity assays. *J. Agric. food chem.*, 2005, **53**(6), 1841-1856. doi: 10.1021/jf030723c.
26. Suhartono, E.; Viani, E.; Rahmadhan, M.A.; Gultom, I.S.; Rakhman, M.F. & Indrawardhana, D. Total flavonoid and antioxidant activity of some selected medicinal plants in South Kalimantan of Indonesian. *APCBEE Procedia*, 2012, **4,** 235-239. doi: 10.1016/j.apcbee.2012.11.039.
27. Tundis, R.; Loizzo, M.R. & Menichini, F. Natural products as α-amylase and α-glucosidase inhibitors and their hypoglycaemic potential in the treatment of diabetes: An update. *Mini Rev. Med. Chem.*, 2010, **10**(4), 315-331. doi: 10.2174/138955710791331007.
28. Eichler, H.G.; Korn, A.; Gasic, S.; Pirson, W. & Businger, J. The effect of a new specific α-amylase inhibitor on postprandial glucose and insulin excursions in normal subjects and type 2 (non-insulin-dependent) diabetic patients. *Diabetol.,*1984, **26**(4), 278-281.
29. Mechchate, H.; Es-Safi, I.; Louba, A.; Alqahtani, A.S.; Nasr, F.A.; Noman, O.M. & Bousta, D. *In vitro* alpha-amylase and alpha-glucosidase inhibitory activity and in vivo antidiabetic activity of Withania frutescens L. Foliar extract. *Mol.*, 2021, **26(**2), 293. doi: 10.3390/molecules2602029.
30. Toeller, M. α-Glucosidase inhibitors in diabetes: efficacy in NIDDM subjects. Eur. *J. Clin. Investig*., 1994, **24(**S3), 31-35. doi: 10.1111/j.1365-2362.1994.tb02253.

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