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Antihyperglycemic and antioxidant activities of *Rhododendron schlippenbachii* Maxim. bark and its various fractions

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In this research, we evaluated the antidiabetic and antioxidant potential of 80% aqueous methanolic extract of the bark of *Rhododendron schlippenbachii* Maxim. along with its various fractions (*n*-hexane, dichloromethane, ethyl ethanoate, *n*-butyl alcohol and distilled water). The antihyperglycemic activity of all the fractions was investigated *in vitro* by α -glucosidase inhibition (using two types of enzyme sources) and *in vivo* in normal rats by evaluating the blood glucose lowering effect after maltose administration (1 g/kg bw). In addition, all these fractions were also evaluated by five diverse *in vitro* antioxidant assay systems, which include, ferrous ion chelating (FIC), 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferric-reducing antioxidant power (FRAP), 2,20-azinobis(3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) and lipid peroxidation inhibition (using rat liver) assays. We also examined the total phenolic and flavonoid contents for all of these fractions. In all, ethyl ethanoate and dichloromethane fractions exhibited potent antihyperglycemic and antioxidant effects *in vitro* and *in vivo*.

Key words: *Rhododendron schlippenbachii*, α -glucosidase, oral glucose tolerance test (OGTT), rats, antioxidant.

INTRODUCTION

Diabetes mellitus is a metabolic complication. It is mainly described by hyperglycemia and alterations in carbohydrate, fat and protein metabolism. In the current era, the prevalence of type 2 diabetes has increased due to endorsement of high-grade meals, swiftly altering lifestyles, and hereditary factors (Itoh et al., 2009). The major symptom of this type 2 diabetes is postprandial hyperglycemia, which is illustrated as a rapid increase in the blood glucose levels after the meals. These postprandial "hyperglycemic spikes" might prove responsible for the patho-physiological conditions of late diabetes, including various micro- and macro-vascular diseases, cardiovascular diseases (Bonora and Muggeo, 2001;

Ceriello, 2005). Therefore, management of this critical parameter might help in treating diabetes and preventing cardiovascular complications.

One of the medications available for the treatment of postprandial hyperglycemia are α -glucosidase inhibitors, which have been proved to be the efficient glucose-lowering agents by slowing down the uptake of dietary carbohydrates in diabetic patients. The α -glucosidase enzyme, situated in the brush border of the small intestine, is essential for the breakdown of disaccharides and polysaccharides into absorbable monosaccharides (Stuart et al., 2004). Also, free radicals have been associated in the development of diabetes and its complications (including retinopathy and atherosclerotic vascular disease) and the agents possessing potent free radical scavenging ability could prove beneficial in improving this disease progression (Sabu and Kuttan, 2002). Thus, antioxidants have been found to play an important role in the treatment

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of diabetes by protecting the human body against damage by these reactive oxygen species (Sabu and Kuttan, 2002; McCune and Johns, 2002).

For many years, plants are the most common form of medicine. In addition, the World Health Organization has also suggested the assessment of the efficacy of plants for the diseases/disorders, in which safe modern drugs are lacking.

A great attempt has been made to find effectual and nontoxic α -glucosidase inhibitors having potent antioxidant effects from natural source, mainly plants, in order to develop physiological functional compound(s) to employ as antidiabetic agents. While evaluating active α -glucosidase inhibitors from natural medicines in Korea, we identified that the 80% aqueous methanolic extract of the bark of *Rhododendron schlippenbachii* Maxim. (RSME) exhibited the most potent α -glucosidase (baker's yeast) inhibitory activity *in vitro*.

Rhododendron species are widespread garden plants with lustrous, evergreen leaves and large, flashy flower presentations (Carballeira et al., 2008).

In The Black Sea Region, toxic honey from many of these species is used as an alternative medicine for treating various ailments (Silici et al., 2010). *R. schlippenbachii* Maxim. (*R. schlippenbachii*) family Ericaceae, is evergreen in mild winters, but deciduous in cold winter climates. Its leaves are used to reduce high blood pressure (Kim, 2008) and the whole plant was evaluated as a strong cholinesterase inhibitor (Sancheti et al., 2010). But, no previous research on the antioxidant and antidiabetic capability of this plant was reported.

Taking this into consideration, the purpose of the current investigation was to assess the antioxidant and antidiabetic potential of RSME and its fractions using several *in vitro* assay techniques, together with 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferrous ion chelating (FIC), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), nitric oxide scavenging (NOS), ferric reducing antioxidant power (FRAP), lipid peroxidation (on rat liver), α -glucosidase (using baker's yeast and rat intestinal enzyme sources) assays. Following these analyses, *in vivo* evaluations of RSME and its fractions were carried out in normal rats after maltose administration to scrutinize postprandial anti-hyperglycemic effect.

MATERIALS AND METHODS

Plant material

The bark of *R. schlippenbachii* (2 kg, dry weight) was received from "Korean Collection of Herbal Extracts" a Biotech company in Korea. A set of voucher specimens is available in the company (Korea Collection of Herbal Extracts, 2000).

Extraction

The bark (2 kg, dry weight) was sliced into small parts and immersed

immersed in 80% aqueous *n*-methyl alcohol for about 72 h at 20 \pm 2°C. The obtained extract was filtered and concentrated with the help of rota-evaporator \leq 40°C to get the dried crude bark extract (RSME) (46.52 g), which was added in distilled water (approximately 1 L) and partitioned with *n*-hexane, dichloromethane, ethyl ethanoate and *n*-butyl alcohol to yield *n*-hexane (RSME-H), dichloromethane (RSME-C), ethyl ethanoate (RSME-E), *n*-butyl alcohol (RSME-B) and aqueous (RSME-W) fractions, respectively.

Reagents

All the essential chemicals and enzymes used were procured from Sigma-Aldrich (St. Louis, MO, USA). Other commercially available reagents and solvents were utilized as obtained.

Determination of total phenolic content (TPC)

TPC of RSME and its fractions was determined using Folin-Ciocalteu assay as illustrated by (Zhang et al., 2006). In this assay, 10 μ l extract and 100 μ l Folin-Ciocalteu reagent were mixed together and the reaction mix was allowed to stand for 5 min followed by the addition of 80 μ l of 7.5% sodium carbonate solution and mixed well. This reaction mixture was kept in the dark at room temperature for 30 min, and the absorbance was then measured at 750 nm. TPC was expressed as gallic acid equivalent (GAE) in mg/g dry extract.

Determination of total flavonoid content (TFC)

TFC of RSME and its fractions was determined using aluminium chloride colorimetric assay as described by Chang et al. (2002) with minor modifications. In this, 10 μ l of extract, 60 μ l of methanol, 10 μ l aluminium chloride (10% w/v), 10 μ l of potassium acetate (1 M) and 120 μ l of distilled water were mixed and incubated at room temperature for 30 min. The absorbance was measured at 415 nm. TFC was expressed as quercetin equivalent (QE) in mg/g dry extract.

DPPH free radical scavenging assay

The free radical scavenging activity of RSME and its fractions at different concentrations was measured by the previously described method of Blois, 1958. In this assay, 50 μ l of 0.5 mM DPPH in methanol, 10 μ l of test sample at different concentrations and 50 μ l of 0.1 M tris HCl buffer (pH 7.0) were added in the 96-well microplate and the change in absorbance was measured at 517 nm 30 min later. The positive control contained 10 μ l of methanol instead of test sample. L-ascorbic acid was used as a reference standard. All experiments were carried out in triplicates. The % scavenging activity was calculated using the following formula:

$$\% \text{ scavenging activity} = [1 - (\text{sample absorbance}/\text{control absorbance})] \times 100$$

Nitric oxide scavenging assay

The nitric oxide (NO) scavenging activity of RSME and its fractions was measured according to the method described by Bafna et al. (2010). This method consisted addition of 50 μ l of sample solution with 50 μ l of 10 mM sodium nitroprusside solution into a 96-well flat-

bottomed plate and the plate was incubated under light at room temperature for 90 min. Finally, an equal volume of Griess reagent (1% of sulphanilamide and 0.1% of naphthylethylenediamine in 2.5% HPO₃) was added to each well to measure the nitrite content immediately at 546 nm. L-Ascorbic acid was evaluated as a reference standard.

ABTS radical cation decolorization assay

ABTS radical cation decolorization assay was carried out using the method reported by Bafna et al. (2010). ABTS^{•+} was generated by oxidation of ABTS with potassium persulfate. The ABTS stock solution was prepared by adding 0.0768 g of ABTS salt and 0.0132 g of potassium persulfate in 20 ml of distilled water. Stock solution was kept in dark for 12 to 16 h (overnight) at room temperature prior to use. The ABTS^{•+} solution was diluted with methanol to an absorbance of 0.700 ± 0.020 at 734 nm. After addition of 200 µl of diluted ABTS solution (A_{734 nm} = 0.700 ± 0.020) to 10 µl of RSME and its fractions at various concentrations, the absorbance was read at 734 nm at 30°C using microplate reader exactly after 6 min after initial mixing. The positive control contained 10 µl of methanol instead of test sample. L-Ascorbic acid served as a reference standard.

Ferric (Fe³⁺) reducing antioxidant power (FRAP) assay

The reducing power of RSME and its fractions was determined according to the method of Bafna et al. (2010). Briefly, 10 µl of sample was mixed with 15 µl of 0.1 M sodium phosphate buffer (pH 6.6) and 15 µl of potassium ferricyanide (1% w/v). This reaction mixture was incubated at 50°C for 20 min. After 20 min incubation, the reaction mixture was acidified with 15 µl of trichloroacetic acid (10%) and mixed well. To this, 55 µl of distilled water and 110 µl of ferric chloride (0.1%w/v) were added and the absorbance was measured at 700 nm in a spectrophotometer. Increased absorbance of the reaction mixture indicated increased reducing capability. L-ascorbic acid was taken as a reference standard. All experiments were carried out in triplicates.

Ferrous ion-chelating assay

The ferrous ion chelating potential of RSME and its fractions at different concentrations was investigated according to the method of Bafna et al. (2010). In this, 2 mM ferrous sulfate (FeSO₄) solution and 5 mM ferrozine solution were prepared and diluted 20 times at the time of the experiment. 50 µl of diluted FeSO₄ and 50 µl of the extract were mixed in a microplate and the reaction was initiated by the addition of 50 µl of diluted ferrozine. The solutions were well mixed and allowed to stand at 25°C for 10 min. After incubation, the absorbance was measured at 562 nm. Methanol was used as positive control instead of sample. Distilled water was used as blank instead of ferrozine, which was used for error correction. Citric acid was used as reference standard.

Determination of lipid peroxidation

The assay was determined according to the method of Bafna et al. (2010) with slight modifications. Briefly, the reaction mixture was composed of 0.5 ml of each liver homogenate, 0.9 ml phosphate buffer (50 mM, pH 7.4), 0.25 ml FeSO₄ (0.01 mM), 0.20 ml ascorbic acid (0.1 mM) and 0.1 ml of different concentrations of RSME and its fractions. The reaction mixture was incubated for 30 min at 37°C.

The extent of lipid peroxidation of the rat liver homogenate in the presence and absence of RSME and its fractions was evaluated by measuring the product of thiobarbituric acid reactive substances (TBARS) using the Cayman's TBARS assay kit (Seoul, Korea). The MDA (malondialdehyde)-TBA adduct formed by the reaction of MDA and TBA under high temperature and acidic conditions was measured colorimetrically at 532 nm. The amount of TBARS formed was calculated using the MDA standard curve. Quercetin was used as a reference standard.

Baker's yeast α -glucosidase inhibition assay

The α -glucosidase inhibition activity for RSME and its fractions was estimated according to the previously described method of Shibano et al. (1997), with little variations. In this, 50 µl phosphate buffer (0.1 M, pH 7.0), 25 µl PNPG (0.5 mM, in 0.1 M phosphate buffer, pH 7.0), 10 µl test sample/standard (acarbose) (0.02 to 3 mg/ml) and 25 µl α -glucosidase solution were added together. This reaction blend was kept for 30 min at 37 °C. This reaction was stopped by adding 100 µl 0.2 M sodium carbonate solution. The enzymatic hydrolysis of the substrate was examined on the basis of the quantity of *p*-nitrophenol liberated in the reaction mix by observation at 410 nm. All tests were performed in triplicate and the results are expressed as the mean ± S.D. of three findings.

Rat intestinal α -glucosidase inhibition assay

Rat intestinal α -glucosidase assay was performed according to the method of Kim et al., (2009) with little amendments. In this, the enzyme source was prepared as follows: 0.5 g of rat-intestinal acetone powder was suspended in 10 ml of 0.9% saline. This suspension was sonicated at 4°C for 12 times, 30 s each time. This step was followed by centrifugation at 10000 g for 30 min at 4°C. The resultant supernatant was utilized as an enzyme source for the assay system. The reaction blend possessed 50 µl phosphate buffer (0.1 M, pH 7.0), 30 µl PNPG (0.5 mM, in 0.1 M phosphate buffer, pH 7.0), 10 µl of test sample/standard (acarbose) (0.1 to 0.7 mg/ml) and 15 µl enzyme solution. This mixture was kept for 30 min at 37°C. The reaction was stopped by adding 100 µl sodium carbonate solution (0.2 M). The enzymatic hydrolysis of the substrate was examined on the basis of the amount of *p*-nitrophenol liberated in the reaction mix at 410 nm. All experiments were executed in a set of three tests at the same time and the outcomes are expressed as the mean ± S.D. for three determinations.

In vivo scrutinizing in normal rats after maltose loading for blood glucose level

Male Sprague Dawley rats (180 to 200 g each) were procured from Daehan Biolink Co., Chungcheongbuk-Do, Korea. The animals were housed in polycarbonate cages under 12/12 h light/dark cycles at 20 ± 2°C. Free access to a laboratory chow diet and water was provided to all animals. The animal usage and study design protocol used in this study was granted by the University's Animal Ethics Committee.

Maltose, acarbose and RSME and its fractions were dissolved in (RSME-C)); Group 5 (1 g/kg body wt. of maltose and 300 mg/kg body wt. of RSME ethyl ethanoate fraction (RSME-E)); Group 6 (1 g/kg body wt. of maltose and 300 mg/kg body wt. of RSME *n*-butyl alcohol fraction (RSME-B)), and Group 7 (1 g/kg body wt. of maltose and 300 mg/kg body wt. of RSME distilled water fraction (RSME-W)).

Statistical analyses

Every assay was carried out for not less than three times with triplicate samples. All results are expressed as mean \pm S. D. In all of the assay systems, the inhibition rates were determined as a percentage of control (buffer possessing *n*-methanol) without the addition of inhibitor.

RESULTS AND DISCUSSION

Total phenolic (TPC) and flavonoid (TFC) content

To estimate the quantity of total phenolic compounds present in the various fractions of RSME, the Folin–Ciocalteu phenol reagent was utilized and the total flavonoid content was determined using aluminium chloride colorimetric assay. TPC and TFC of RSME and its fractions ranged from 22.75 to 339.47 mg of gallic acid equivalent/g of extract and 1.07 to 8.95 mg of quercetin equivalent/g of extract, respectively (Table 1). In all of the fractions, ethyl ethanoate fraction (RSME-E) was found to contain the highest TPC and TFC values; this might be due to the fractionation of RSME with non polar to increasing polarity solvents (*n*-hexane, dichloromethane, ethyl ethanoate, *n*-butyl alcohol and water), which might have helped in purification and concentration of phenol in ethyl ethanoate fraction. On the other hand, water fraction of RSME (RSME-W) exhibited the lowest amounts of total phenolics and flavonoids.

Antioxidant and free radical scavenging assays

Various methods have been developed to determine antioxidant potential of the extracts. The chemical intricacy of extracts, which are generally a mixture of numerous compounds possessing various functional groups, different polarities and varied chemical behavior, could give rise to scattered results, depending upon the assay methods employed. Therefore, for evaluating the antioxidant potential of extracts, an approach of using multiple assay systems might prove more informative and even necessary (Öztürk et al., 2007; Muraina et al., 2009). Taking this fact into consideration, in this experiment, we performed six various free radical scavenging and antioxidant assays.

DPPH, a stable radical, is used extensively for the purpose of evaluation of primary antioxidant activity, that is, the free radical scavenging activity of pure antioxidant compounds, plant extracts and food materials (Wong et al., 2006). The basis of this method is to reduce alcoholic DPPH solution in the attendance of a hydrogen-donating antioxidant owing to the formation of the non-radical DPPH–H, which results an absorbance drop at 517 nm (Öztürk et al., 2007). In DPPH assay system, the strongest activity was shown by RSME-E, which was in concurrence with the total phenolic content present in this

fraction (Sahreem et al., 2010). The overall ranking for free radical scavenging ability of all the fractions including crude extract is: Ascorbic acid < RSME < RSME-E < RSME-C < RSME-B < RSME-W < RSME-H (Figure 1).

Ferric reduction is frequently used as a marker of electron-donating activity and considered as a significant mechanism of phenolic antioxidant action (Nabavi et al., 2008). In the ferric reducing antioxidant power (FRAP) assay, the antioxidants in the extracts, if any, would result in reducing Fe^{3+} to Fe^{2+} by donating an electron. Amount of this Fe^{2+} complex can be determined at 700 nm by evaluating the formation of Perle's Prussian blue. The reductive ability is directly proportional with the absorbance of the extract at 700 nm (Ebrahimzadeh et al., 2010). The ferric reducing power of RSME extract and its fractions is given in Figure 2. The dose-response graph exhibited that the reducing powers of all of the test samples were enhanced in a concentration dependent manner. The Fe^{3+} reducing capacity for the standard RSME extract and its fractions in descending order is: Ascorbic acid < RSME-E < RSME-B < RSME-C < RSME-H < RSME-W. The RSME-E fraction exhibited nearly equivalent reduction pattern as that of ascorbic acid, whereas all the remaining samples showed considerably lower activities.

Chelating agents are valuable as secondary antioxidants as they reduce the redox potential, thus stabilizing the metal ion's oxidized form (Peksel et al., 2010). In ferrous ion chelating assay, a violet complex is formed due to reaction of ferrozine with Fe^{2+} ion. This complex formation is disrupted in the presence of a chelating agent, thus resulting in reduced violet colored complex formation. Based on this principle, in the present study, RSME-C was found to possess the most potent ferrous ion chelating activity, whereas RSME-E exhibited the lowest activity (Table 2). Overall, the results expressed that RSME fractions have considerable iron binding ability. This intimates that antioxidant activity might relate to their iron binding capacity.

The IC_{50} values of RSME and its fractions for nitric oxide scavenging activity are given in Table 2. Sodium nitroprusside after decomposition produces NO. Under aerobic conditions, nitric oxide reacts with oxygen to form nitrate and nitrite, and these can be determined using Griess reagent (Peksel et al., 2010). A dose-dependent inhibition of nitrite formation was observed for RSME-H, RSME-C and RSME-E, in which the highest inhibition was observed by RSME-H (higher than that of ascorbic acid). RSME, RSME-B and RSME-W showed very low NOS activity.

The ABTS derived radical cation is one of the most frequently employed organic radicals for the determination of antioxidant efficacy of various chemical compounds and their complex mixtures. The assessment criterion for the relative antioxidant capacities of these samples was based on the determination of quenching ability of ABTS

Table 1. Total phenolic and flavonoid contents of RSME and its fractions.

Fraction	Total phenolic content (mg gallic acid/g of extract)	Total flavonoid content (mg quercetin/g of extract)
RSME	76.39 ± 2.04	1.14 ± 0.23
RSME-H	48.84 ± 1.24	3.68 ± 0.18
RSME-C	67.65 ± 0.73	2.60 ± 0.33
RSME-E	339.47 ± 3.95	8.95 ± 0.21
RSME-B	135.77 ± 1.81	1.96 ± 0.34
RSME-W	22.75 ± 2.09	1.07 ± 0.06

Table 2. IC₅₀ values of RSME and its fractions for various antioxidant assays.

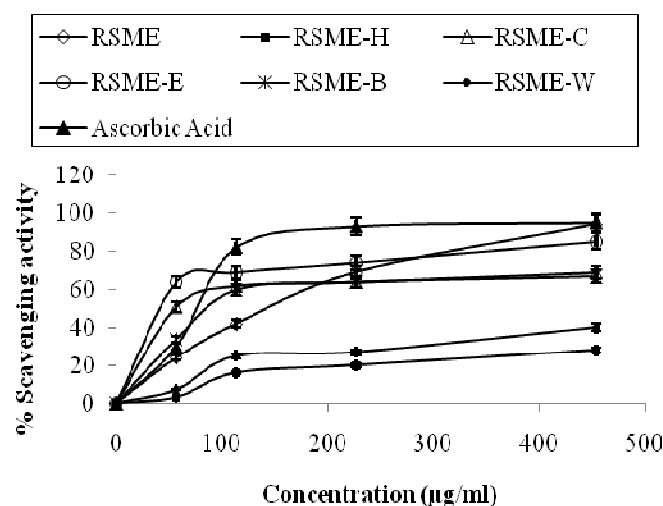
Antioxidant assay	IC ₅₀ value (µg/ml)							AA	Citric acid
	RSME	RSME-H	RSME-C	RSME-E	RSME-B	RSME-W			
ABTS	18.7 ± 0.2	37.2 ± 0.2	13.3 ± 0.1	8.5 ± 0.1	17.1 ± 0.3	139.4 ± 0.6	6.9 ± 0.1	-	
FIC	938.5 ± 7.8	480.3 ± 6.7	206.2 ± 8.4	2387.7 ± 5.9	1832.2 ± 6.3	550.9 ± 6.8	-	9764.8 ± 9.9	
NOS	>1250	191.5 ± 9.4	1129.6 ± 8.1	700.9 ± 5.9	>1250	>1250	220.0 ± 5.2	-	

Table 3. Lipid peroxidation assay of RSME and its fractions using rat liver homogenate.

Sample	Concentration (µg/ml)	MDA equivalents (µmole/liter)
Oxidative stress	-	24.21 ± 0.25
RSME	250	8.47 ± 0.13
RSME-H	250	6.01 ± 0.29
RSME-C	250	4.92 ± 0.08
RSME-E	250	9.21 ± 0.23
RSME-B	250	5.22 ± 0.41
RSME-W	250	8.60 ± 0.34
Quercetin	100	5.12 ± 0.12

al., 2010). Based on this, the results obtained are ranked here in descending order: Ascorbic acid < RSME-E < RSME-C < RSME-B < RSME < RSME-H < RSME-W (Table 2), which was nearly in agreement with those of the results obtained for total phenolic content.

When unsaturated lipids in liver tissue are exposed to reactive oxygen species (ROS), they are strongly susceptible to peroxidation (Rajeshwar et al., 2005). In this study, the liver tissue was incubated with ascorbate/FeSO₄ (ROS generating system), which produces malondialdehyde (MDA) in rat liver microsomes. This MDA forms a pink chromogen with thiobarbituric acid, which can be measured at 532 nm. Based on this assay principle, the data obtained revealed that, RSME-C demonstrated the highest lipid peroxidation inhibition and in all, the lowest inhibition was observed in RSME-E (Table 3).

**Figure 1.** DPPH free radical scavenging ability of RSME, its fractions and L-ascorbic acid (56.82 to 454.56 µg/ml).

α-Glucosidase inhibition assays using baker's yeast and rat intestinal enzyme source

The α-glucosidase inhibition assay of all the samples (at 240 µg/ml concentration) was performed by using two different enzyme sources. In both the assay systems, RSME-E exhibited the most potent activity. In addition, all other fractions also revealed very strong inhibitions for both the enzyme sources (Figure 3). Based on these results, postprandial blood glucose levels after maltose load in normal rats were determined at various time points.

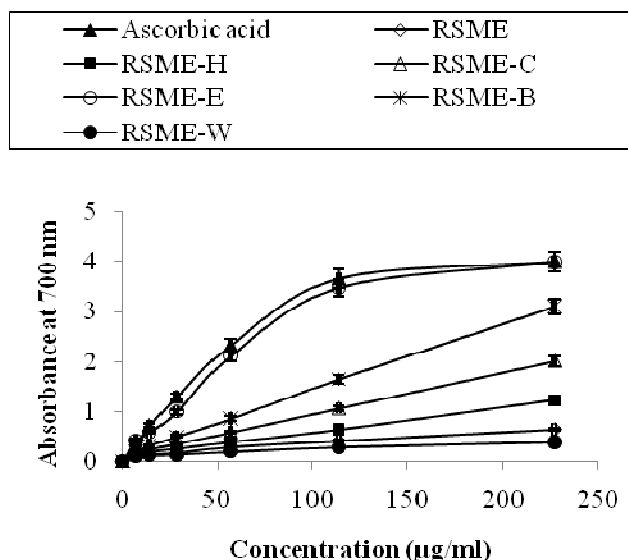


Figure 2. Reducing power of RSME, its fractions and L-ascorbic acid (7.1 to 227.27 µg/ml) by FRAP assay.

Postprandial blood glucose determination in normal rats after maltose loading

In Figure 4, different fractions of RSME at 300 mg/kg and acarbose at 50 mg/kg body weight are presented. Thirty minute after a maltose load (1 g/kg), the blood glucose levels in all groups increased rapidly and gradually decreased thereafter. Both fractions, RSME-C and RSME-E (dichloromethane and ethyl ethanoate) at 300 mg/kg demonstrated noteworthy diminution in the blood glucose levels, increase (123.34 ± 8.22 and 122 ± 7.65 mg/dl, respectively) after 30 min when compared to the other doses. In addition, crude extract of *R. schlippenbachii* (RSME) at a dose of 300 mg/kg exhibited effective blood glucose lowering effect (157.23 ± 8.43 mg/dl). On the other hand, RSME-H, RSME-B and RSME-W (that is, hexane, butanol and water layer) fractions did not show any significant blood glucose lowering effects (Figure 4). Interestingly, the effects of RSME-C and RSME-E at 300 mg/kg were nearly equivalent to that of standard drug (acarbose at 50 mg/kg, used as a reference).

Glucosidase inhibitors are considered as the drugs of choice for first-line treatment, as they have established their effectiveness in the reduction of postprandial hyperglycemic excursion in types I and II diabetes (Tiwari et al., 2008).

They are necessary for carbohydrate digestion and also play a vital role in the processing of glycoproteins and glycolipids. These inhibitors are as well implicated in different metabolic ailments and other diseases such as viral attachment (Gruters et al., 1987) and cancer formation (Dennis et al., 1987). Because of the aforementioned

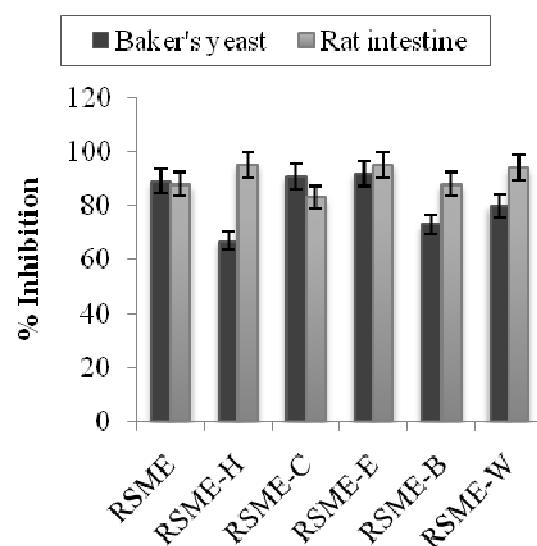


Figure 3. α -Glucosidase inhibitory effect of RSME and its fractions at 240 µg/ml. Each value is mean of three observations in triplicate. Acarbose was taken as reference standard, IC_{50} value = 2.5 µg/ml.

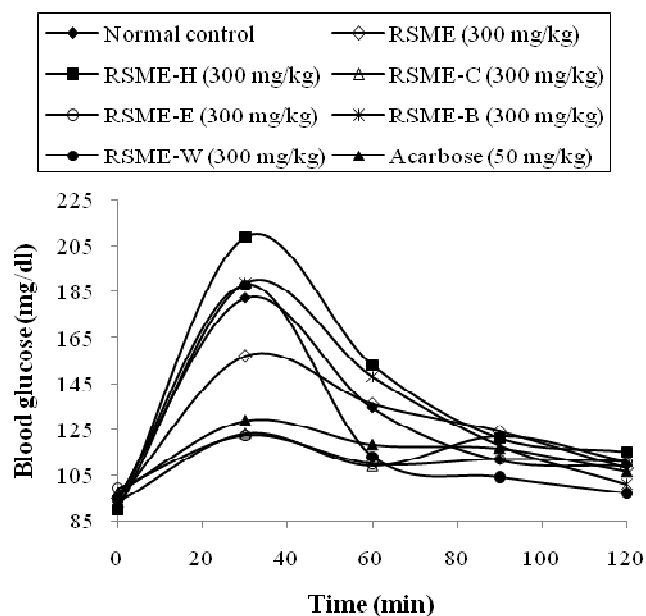


Figure 4. Effect of RSME and its fractions (300 mg/kg bw) on blood glucose levels in normal rats after maltose administration. Acarbose (50 mg/kg bw) was used as a reference standard. All the samples were administered orally immediately after maltose load (1 g/kg bw) to the respective groups of animals. Blood glucose levels were measured at different time points for 120 min (0, 30, 60, 90, 120 min). Data are expressed as mean \pm S. E. M.; n = 6 rats per group.

reasons, glycosidase inhibitors can be measured as important tools for understanding the mechanisms of action

action on glycosidases and are also forthcoming therapeutic agents for some degenerative diseases (Winchester and Fleet, 1992).

In the present study, RSME-C and RSME-E proved to be most potent in all fractions. Both fractions after maltose load effectively retarded the rise in blood glucose levels (30 min). The presence of high phenolic and flavonoid contents might be responsible for these strong antihyperglycemic effects (Table 1) in both fractions. Previously, some of the quercetin derivatives (quercetin 3-O- β -D-galactopyranoside and quercetin 3-O- α -L-arabofuranoside) and triterpenoids (Taraxerol) have been isolated from *R. schlippenbachii*. Quercetin and its derivatives along with 3 β Taraxerol are known to possess antidiabetic potential (Jo et al., 2009). In addition, potent antioxidant activity possessed by RSME and RSME-E might be helpful in diabetes treatment as previously considerable quantities of antioxidants (vitamin E, carotenoids, ascorbic acid, flavonoids and tannins) have been (McCune and Johns, 2002) found to be effective medicines associated with diabetes. However, further experiments are required to identify the active compounds from the bark of *R. schlippenbachii*.

In conclusion, the present study demonstrated the antihyperglycemic effect of RSME and its fractions (RSME-C and RSME-E), which could be associated with α -glucosidase inhibition and their ability to scavenge free radicals. Our research gives a preliminary understanding regarding the effects and mechanism of action of crude extract and its fractions as a suitable candidate for treating postprandial blood glucose rise. However, further in detailed long-term animal experiments along with toxicity studies are necessary to establish any therapeutic advantage.

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