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RESEARCH ARTICLE

Inhibitory effect of *Terminalia chebula* Retz. fruit extracts on digestive enzyme related to diabetes and oxidative stress

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Abstract

Terminalia chebula fruit extracts were prepared sequentially with hexane, ethyl acetate, methanol and methanol-water (70:30) and tested for their α -glucosidase inhibitory and antioxidant potential. The study resulted in the formulation of an extract with high α -glucosidase inhibitory potential (IC_{50} $0.19 \pm 0.03 \mu\text{g mL}^{-1}$) enriched with hydrolysable tannins. Also, each of the extract was chemically characterized by reversed-phase high-performance liquid chromatography on the basis of their marker compounds chebulagic acid, chebulinic acid and corilagin in order to give explanation to the significant activity shown by the extracts. The antioxidant potential of the highly active extract was evaluated in the cellular level also using superoxide dismutase, glutathione S-transferase and induced oxidative stress assays. The results indicated the possibility of using the extract as a nutraceutical health supplement in the management of type 2 diabetes.

Keywords: α -glucosidase inhibition, chebulinic acid, HepG2 cell line, HPLC, flow cytometry

Introduction

Diabetes mellitus is a major chronic disease caused by the improper balance of glucose homeostasis. An abnormal postprandial increase in blood glucose level has been linked to the onset of type 2 diabetes and associated with oxidative dysfunction and failure of various organs. Hydrolysis of dietary carbohydrate such as starch is a major source of glucose in blood. This hydrolysis is carried out by a group of enzymes that include pancreatic α -amylase and intestinal α -glucosidase. It is believed that inhibition of this enzyme activity should be an important strategy for management of type 2 diabetes, wherein α -glucosidase inhibitors could retard the rapid utilization of dietary carbohydrate and suppress postprandial hyperglycemia. Currently, therapeutic drugs, such as acarbose are shown to effectively reduce intestinal absorption of sugars in human¹. Oxidative stress due to hyperglycemia and hyperlipidemia also play an important role in complications and other adverse effect due to diabetes.

Herbal medicine is a major component in all indigenous people's traditional medicine and a common element in ayurvedic, homeopathic and naturopathic medicine. World health organization notes that out of 119 plant-derived pharmaceutical medicines, about 74% are used in modern medicine. Medicinal plants have the advantage of having little or no side effects. Long before, till the use of insulin became common, indigenous remedies were used for the treatment of diabetes mellitus that made scientist look towards hypoglycemic agents of plant origin.

Free radicals have been implicated in the causation of several diseases such as liver cirrhosis, atherosclerosis, cancer, diabetes and compounds that can scavenge free radicals have great potential in ameliorating these disease processes². Antioxidants thus play an important role to protect the human body against damage by reactive oxygen species (ROS)³. Increased oxidative stress has been postulated in the diabetic state. Oxygen free radical activity can initiate peroxidation of lipids,

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which in turn stimulates glycation of protein, inactivation of enzymes and alterations in the structure and function of collagen, basement and other membranes and plays a role in the long-term complications of diabetes⁴. Oxidative stress in diabetes coexists with a reduction in the antioxidant status⁵, which can increase the deleterious effects of free radicals. Supplementation with non-toxic antioxidants may have a chemoprotective role in the diabetes⁶.

Terminalia chebula belongs to the family *Combretaceae* and is found throughout India. Fruits of *T. chebula* contain 25–30% of hydrolysable tannins⁷. The chief constituents are chebulic acid, chebulagic acid, corilagin and gallic acid^{8,9}. *T. chebula* has been reported to exhibit a variety of biological activity, including anticancer¹⁰, antidiabetic¹¹, antibacterial¹², antifungal¹³, antiviral^{14,15}, antimutagenic¹⁶ activities, etc. *T. chebula* fruits are the part of well-known traditional formulation Triphala used in the treatment of many chronic diseases such as ageing, heart ailments and hepatic diseases^{11,17}. Most of the earlier studies regarding the biological activities of *T. chebula* fruits were carried out using crude extracts. In the present study, we have sequentially extracted the fruit powder with different solvents in the increasing order of polarity and studied how these extracts affected the α -glucosidase inhibitory and antioxidant activity.

Materials and methods

Plant material

T. chebula fruits were collected from forest near Vithura, Trivandrum, Kerala. The samples were identified by Dr. A.G. Pandurangan in tropical botanical garden and research institute (Palode, Trivandrum, Kerala, India) and a voucher specimen (No: 76487/AGP/09) was kept in herbarium for future reference. The samples were powdered to an average particle size of 0.5 mm and stored at 4°C in air tight containers.

Chemicals and reagents

The compounds 2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH) as free radical, α -glucosidase (from *Saccharomyces cerevisiae*), *p*-nitrophenyl α -D-glucopyranoside (PNPG), gallic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic Acid (Trolox), phenazine methosulphate (PMS), b-nicotinamide adenine dinucleotide (NADH), 20,70-dichlorodihydrofluoresceindiacetate (DCFH-DA), catechin hydrate, gallic acid were supplied from Sigma-Aldrich (St. Louis, MO, USA). Acarbose was obtained from SERVA Electrophoresis GmbH (Mannheim, Germany). Glutathione S-Transferase (GST) Assay Kit was procured from Cayman chemical company, Ann Arbor, USA. Folin-Ciocalteu reagent, potassium persulphate, sodium phosphate, and sodium carbonate, ethyl alcohol, hexane and chloroform were analytical grade and procured from

Merck (Darmstadt, Germany). Reference compounds chebulagic acid, chebulinic acid and corilagin were purchased from Natural Remedies (Bangalore, India). All other reagents and solvents used were of Analytical and AR grade.

Extraction of plant material

Powdered *T. chebula* fruits (500 g) were extracted successively with 1 Litre each of hexane, ethyl acetate, methanol and methanol-water (70:30) in the increasing order of polarity. The hexane (TC-1), ethyl acetate (TC-2) and methanol (TC-3) extracts were then filtered and concentrated under reduced pressure below 45°C. The methanol-water extract (TC-4) was lyophilised and kept at -20°C in sealed bottles till analysed.

Total phenolics assay

The total phenolic content of the extracts were determined by the Folin-Ciocalteu method with gallic acid as standard¹⁸. Stock solution (1 mg mL⁻¹) was diluted with methanol to have sample solutions of varying dilutions (2–10 μ g mL⁻¹). Sample solutions (0.5 mL) were mixed with 0.5 mL of Folin-Ciocalteu reagent and 1 mL of sodium carbonate (20% w/v), adjusting with water so as to have a total volume of 5 mL, the contents were mixed and allowed to stand for 30 min. Absorption at 760 nm was measured in a Shimadzu 1601 UV-VIS Spectrophotometer. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per gram of sample.

α -Glucosidase inhibition assay

The enzyme reaction¹⁹ was performed using PNPG as a substrate in phosphate salt buffer. The α -glucosidase inhibiting effect of the extractant was assayed according to the following procedure. Different volume of the extractant (0.1 mg mL⁻¹) was added into the phosphate salt buffer (pH 6.8), after adding 20 μ L α -glucosidase (1 U mL⁻¹), the mixture was incubated at 37°C for 5 min. Then 200 μ L PNPG was added as a substrate into the buffer, and after 20-min reaction, 200 μ L sodium carbonate was added to terminate the reaction. Enzymatic activity was quantized by measuring the *p*-nitrophenol released from PNPG at 405 nm wavelength using multiplate reader (Synergy 4, BioTek Instruments Inc., Vermont, USA). Acarbose was taken as reference compound.

Evaluation of antioxidant activity

DPPH radical scavenging activity

DPPH is a free radical compound and has been widely used to test the free radical scavenging ability of samples. The hydrogen atom or electron donation ability of the extracts was measured from the bleaching of purple coloured methanol solution of 2,2-diphenyl-1-picrylhydrazyl²⁰. Methanolic stock solutions (5.0 mg mL⁻¹) of extracts were diluted to final concentrations ranging from 2 to 10 μ g mL⁻¹. Various concentrations (100 μ L) of the extracts were mixed with 2.9 mL of a 0.3 mM DPPH

solution and allowed to react at room temperature in dark. After 30 min, the absorbance values were measured at 517 nm. Gallic acid was used as positive control and the percentage radical scavenging activity is calculated using the following equation.

$$\%RSA = \frac{A_0 - A_1}{A_0} \times 100$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

Experiments were carried out in triplicate.

ABTS radical cation decolourisation assay

An improved ABTS decolorization assay²¹ in which ABTS^{•+} were generated by the oxidation of ABTS with potassium persulphate. The ABTS^{•+} solution was diluted to an absorbance of 0.7 ± 0.05 at 734 nm (Shimadzu UV-VIS Spectrophotometer, Model 2100). Absorbance was measured 7 min after the initial mixing of different concentrations of the methanolic bark extracts with 1 mL of ABTS^{•+} solution. Trolox was used as a reference standard.

HPLC estimation of major phenolics

Stock solutions (5 mg mL^{-1}) of TC-2, TC-3, and TC-4 were prepared and the solutions were filtered through a $0.45\text{-}\mu\text{m}$ filter. The stock solutions were then diluted to a concentration of $1 \mu\text{g } \mu\text{L}^{-1}$ and $20 \mu\text{L}$ of the solutions were injected into the high-performance liquid chromatography (HPLC) column. Reference compounds chebulagic acid, chebulinic acid, corilagin and gallic acid in methanol were used to calibrate standard curves and retention times.

Cell culture and treatment

HepG2 cells (hepatocellular carcinoma cell line) obtained from the National Centre for Cell Science (Pune, India) were maintained in culture in 75 cm^2 polystyrene flasks (Falcon) with DMEM containing 10% fetal bovine serum and $100 \mu\text{g mL}^{-1}$ penicillin-streptomycin at 37°C in a humidified atmosphere of 95% air and 5% CO_2 . Cultures (2.5×10^6 cells per well) were grown for 3 days in complete DMEM until they reached the post-confluence stage. Cells were incubated with different concentrations of TC-2 extract (10, 50, 100 and $200 \mu\text{g mL}^{-1}$) medium for 24 h.

Superoxide radical scavenging and SOD activity

Superoxide radicals are generated non-enzymatically in PMS-NADH systems by the oxidation of NADH and assayed by the reduction of NBT²². The superoxide radicals were generated in 1 mL of Tris-HCl buffer (16 mM, pH 8.0) containing NBT ($50 \mu\text{M}$) and NADH ($78 \mu\text{M}$), PMS ($10 \mu\text{M}$) was added to the mixture and incubated at 25°C for 5 min, and the absorbance was measured at 560 nm against blank samples.

To test whether TC-2 extract can alter the activity of intracellular superoxide dismutases (SODs), SOD activity

in the cell lysates was measured using the nitroblue tetrazolium assay (NBT assay, which measures the conversion of NBT to blue formazan²³). Superoxide radicals were generated intracellularly by phorbol myristate acetate (PMA). The formazan formed after incubation of the cells with 0.5 mg mL^{-1} NBT was solubilized by adding 300 μL of KOH/dimethylsulphoxide (DMSO) per well (mix 1 part of 2 M KOH with 1.167 parts of DMSO just before use) and absorbance was measured at 620 nm using a microplate reader (Synergy 4, BioTek Instruments Inc., Vermont, USA).

Assay of glutathione S-transferase activity

GST activity of the samples was measured using the Cayman Chemical GST Assay Kit (Cayman chemical company, Ann Arbor, USA). Total GST activity (cytosolic and microsomal) was measured by the conjugation of 1-chloro-2,4-dinitrobenzene with reduced glutathione. The conjugation is accompanied by an increase in absorbance at 340 nm. The rate of increase is directly proportional to the GST activity in the sample²⁴.

Evaluation of induced oxidative stress inhibition

Cell culture and treatment

C2C12 cells purchased from the National Centre for Cell Science (Pune, India) were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 100 U mL^{-1} penicillin and 100 mg mL^{-1} streptomycin. Cultures were maintained at 37°C in a 5% carbon dioxide incubator. When the cells were about to cover 80% of the flask area, they were detached and seeded on 24-well plates. After attaining, 70–80% confluency, the cells were rinsed twice with phosphate-buffered saline (PBS) and changed with medium containing extracts at different concentrations. After 24 h incubation, the cells were washed twice with PBS and $50 \text{ mM H}_2\text{O}_2$ was maintained in individual wells for 1 h at 37°C . These cells were detached by trypsin to assay by flow cytometry.

Evaluation of oxidative stress inhibition

Cytoprotective effect against the oxidative stress-induced by H_2O_2 was measured by determining the intracellular content of ROS. Intracellular ROS levels were measured employing DCFH-DA²⁵. Non-fluorescent DCFH-DA, after cellular incorporation, is hydrolysed by cellular esterase to DCFH, which in the presence of strong oxidants such as H_2O_2 and others, is oxidised to fluorescent 2',7'-dichlorofluorescein which were analysed with Fluorescence-activated cell sorting (FACS) Aria II (BD Bioscience, San Jose, CA, USA). C2C12 cells pre-treated with TC-2 extract were incubated with DCFH-DA at 37°C for 20 min and then analysed by the FACS Aria II.

Statistical analysis

The results were analysed using Microsoft excel 2007 and Origin Pro 8 softwares. All the data are expressed

as mean \pm SD ($n=3$). Student's *t*-test was used to compare means, and values were considered significant at $p<0.05$.

Results and discussion

Total phenolic content of *Terminalia chebula* extracts

The phenolic content was found to be maximum ($718 \pm 24.5 \text{ mg g}^{-1}$ GAE) in TC-2 followed by TC-3 ($623.8 \pm 12 \text{ mg g}^{-1}$ GAE) (Table 1). But the hexane extract TC-1 was found to be devoid of phenolic compounds. Therefore, it was exempted from further studies.

Inhibitory effect on α -glucosidase

The hydrolysable tannins rich extracts of *T. chebula* were tested for their ability to inhibit α -glucosidase.

It was found that all the three extracts showed strong inhibition against α -glucosidase. A low IC_{50} translates to a stronger α -glucosidase inhibition. The IC_{50} values of different extracts were shown in Table 2. α -Glucosidase inhibitory potential of extracts varies as TC-2>TC-3>TC-4 (Figure 1). As compared to the standard acarbose ($\text{IC}_{50} 38.5 \pm 3.2 \text{ } \mu\text{g mL}^{-1}$), all the extracts of *T. chebula* showed significantly higher inhibition against α -glucosidase. However, among the extracts, TC-2 showed highest inhibitory potential against α -glucosidase ($\text{IC}_{50} 0.19 \pm 0.03 \text{ } \mu\text{g mL}^{-1}$) followed by TC-3 with an IC_{50} value of $0.28 \pm 0.02 \text{ } \mu\text{g mL}^{-1}$. This may be attributed to the high content of chebulagic and chebulinic acids. HPLC analysis showed that the TC-2 extract contained the highest quantity of chebulagic ($242.8 \pm 6.03 \text{ mg g}^{-1}$) and chebulinic acid

Table 1. TPC content and HPLC analysis of individual phenolic compounds in ethyl acetate, methanol and 70% methanol-water extracts of *T. chebula*.

Extract	TPC (mg g^{-1} GAE)	Chebulagic acid (mg g^{-1})	Chebulinic acid (mg g^{-1})	Corilagin (mg g^{-1})	Gallic acid (mg g^{-1})
TC-2	718.8 ± 25	242.8 ± 6.03	358.6 ± 8.6	24.8 ± 0.58	36.8 ± 1.2
TC-3	623.8 ± 14	159.0 ± 5.8	233.9 ± 3.4	26.2 ± 0.62	5.5 ± 0.14
TC-4	460.8 ± 8	127.2 ± 7.3	99.4 ± 0.98	49.6 ± 2.3	7.7 ± 0.88

GAE-gallic acid equivalent.

Table 2. α -glucosidase inhibitory effect and antioxidant activity of different extracts of *T. chebula* on *in vitro* models, as expressed ($\mu\text{g mL}^{-1}$) by inhibitory concentration (IC_{50}). Each value represents the mean \pm SD of three replicates.

Assay	$\text{IC}_{50} (\mu\text{g mL}^{-1})$		
	TC-2	TC-3	TC-4
α -Glucosidase inhibition	0.19 ± 0.03	0.28 ± 0.02	1.81 ± 0.10
DPPH	2.01 ± 0.08	3.91 ± 0.05	3.99 ± 0.10
ABTS	2.52 ± 0.09	4.65 ± 0.08	4.52 ± 0.10
Superoxide scavenging	7.03 ± 0.33	12.51 ± 1.2	8.25 ± 0.65

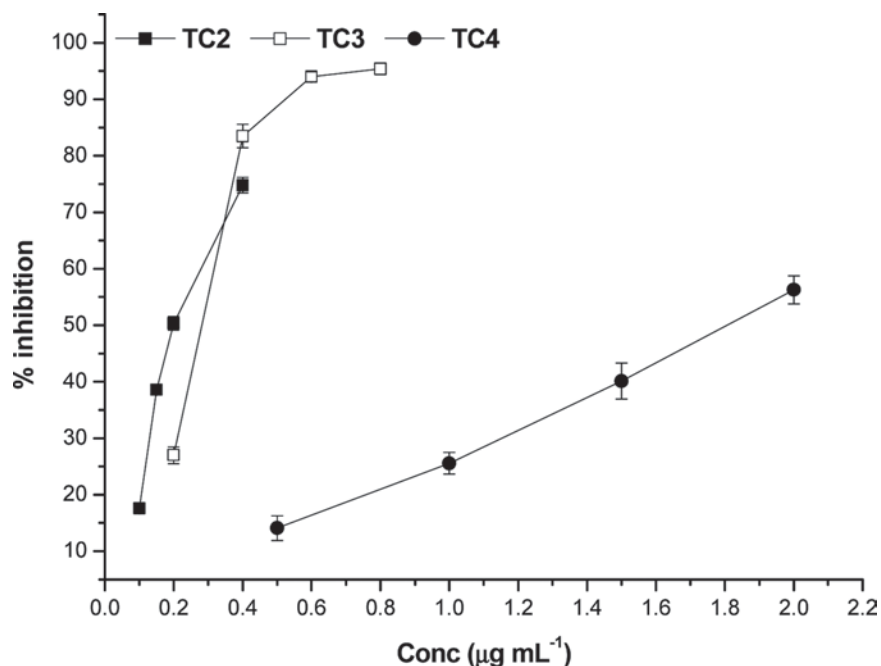


Figure 1. Dose-response curve for the inhibitory effect of *T. chebula* extracts on the activity of α -glucosidase.

($358.6 \pm 8.6 \text{ mg g}^{-1}$) followed by the TC-3 extract (chebulagic acid – $159.0 \pm 5.8 \text{ mg g}^{-1}$ and chebulinic acid – $233.9 \pm 7.3 \text{ mg g}^{-1}$). The α -glucosidase inhibitory potential of hydrolysable tannins is directly proportional to the number of galloyl and chebuloyl groups²⁶. Chebulinic acid contains three galloyl and one chebuloyl groups whereas chebulagic acid contains a galloyl, a chebuloyl and a hexahydroxydiphenoyl (HHDP) group. The HHDP unit have only a weak influence on the α -glucosidase inhibition in the case of monomeric hydrolysable tannins²⁷. Therefore, it can be inferred that as the amount of chebulagic and chebulinic acid increases the α -glucosidase inhibitory potential also increases. This is clearly evident from the IC_{50} values obtained for different extracts (Table 2). A recent study on α -glucosidase inhibitory potential of *Terminalia* species²⁸ showed that *T. chebula* ethanol extract has an IC_{50} value of $6.32 \pm 0.40 \text{ } \mu\text{g mL}^{-1}$. Also, the α -glucosidase inhibitory power of chebulagic acid, one of the active principles in *T. chebula*, was studied by Gao et al²⁹ and the IC_{50} value is given as $47.7 \text{ } \mu\text{g mL}^{-1}$.

Normally, the inhibition of α -glucosidase enzyme reduces starch digestion and absorption, consequently lowering a postprandial hyperglycaemic response. This hypoglycemic effect is an established and effective target for type 2 diabetes prevention and treatment³⁰. All the extracts studied here showed high level of α -glucosidase inhibition in comparison with the synthetic inhibitor, acarbose. But, these synthetic inhibitors have some side effects also. Therefore, the possibility of preventing the onset of diabetes using dietary supplements and herbal medicines has attracted increasing attention. Thus, making these extracts as suitable

candidates as nutraceutical supplements for diabetes prevention.

DPPH radical scavenging activity

T. chebula extracts were subjected to the DPPH assay along with reference standards gallic acid and BHT. The DPPH scavenging activity with concentration is depicted in Figure 2. The decrease in absorbance of the DPPH radical at 517 nm caused by antioxidant was due to the scavenging of the radical by hydrogen donation. A lower value of inhibitory concentration indicates a higher antioxidant activity. The samples showed high scavenging activity against DPPH. The lowest IC_{50} was obtained in the case of TC-2 ($2.01 \pm 0.08 \text{ } \mu\text{g mL}^{-1}$) and it may be attributed to its high phenolic content ($718 \pm 24.5 \text{ mg g}^{-1} \text{ GAE}$). The reference compounds gallic acid and BHT has IC_{50} values 1.4 and $7 \text{ } \mu\text{g mL}^{-1}$, respectively. Linear correlation was found between the total phenolic content and DPPH IC_{50} of *T. chebula* extracts. IC_{50} values of extracts are given in Table 2.

ABTS radical cation decolourization assay

This method is an excellent tool for determining the antioxidant activity of hydrogen donating antioxidants and of chain breaking antioxidants. The *T. chebula* extracts showed good scavenging activity against $\text{ABTS}^{\bullet+}$. The IC_{50} values of the extracts were in the concentration range $2\text{--}5 \text{ } \mu\text{g mL}^{-1}$ (Table 2). The values were compared with that of the reference compounds trolox (an ideal ABTS scavenger) ($\text{IC}_{50} = 2.2 \text{ } \mu\text{g mL}^{-1}$) and ascorbic acid ($\text{IC}_{50} = 2.78 \text{ } \mu\text{g mL}^{-1}$) and found to statistically significant ($p \leq 0.05$) (Figure 3). The higher activity of extracts against $\text{ABTS}^{\bullet+}$ can be due to the presence rich tannin content which is evident from the HPLC analysis of the extracts.

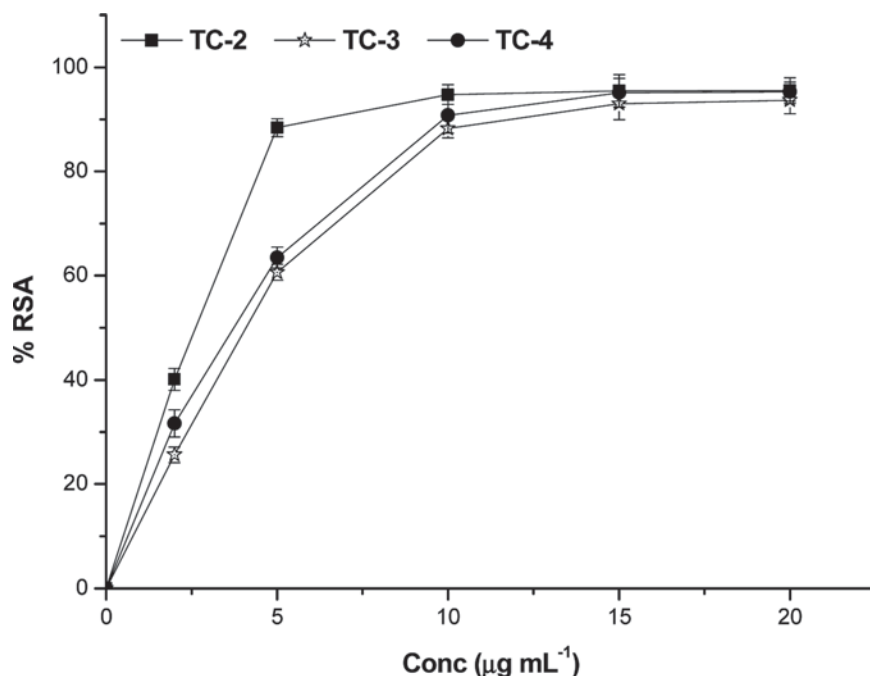


Figure 2. DPPH radical scavenging activity of *T. chebula* extracts. The values were expressed as \pm SD of triplicate measurements.

Superoxide radical anion scavenging assay

All the extracts showed scavenging activity in a dose-dependent manner. In the PMS/NADH-NBT system, superoxide anions derived from dissolved oxygen by PMS/NADH coupling reaction reduce NBT. The decrease of absorbance at 560 nm with antioxidants thus indicates the consumption of superoxide anions in the reaction mixture. The extracts exhibited superoxide scavenging activity (Figure 4) at all the concentrations (10–50

$\mu\text{g mL}^{-1}$). The superoxide scavenging capacities of the extracts follow the order TC-2>TC-4>TC-3>catechin. Catechin, the natural superoxide scavenger has an IC_{50} value of $14.6 \pm 0.08 \mu\text{g mL}^{-1}$.

HPLC analysis

The effect of *T. chebula* extracts on α -glucosidase inhibition and *in vitro* antioxidant chemical assays were very impressive. Therefore, HPLC analysis was carried out

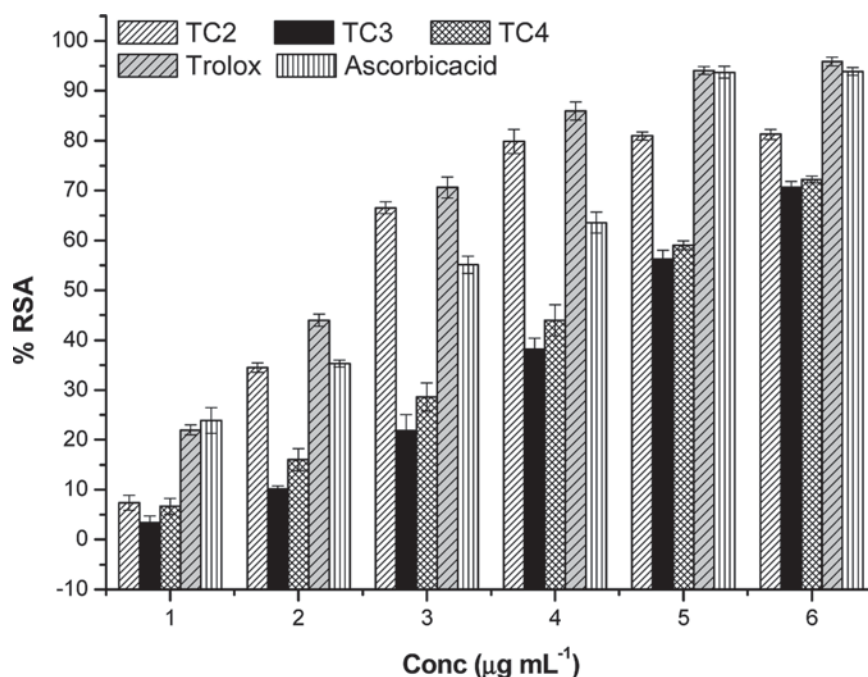


Figure 3. ABTS radical cation decolourization assay of *T. chebula* extracts along with the reference compounds trolox and ascorbic acid. All values were represented as \pm SD of three parallel measurements ($p \leq 0.05$).

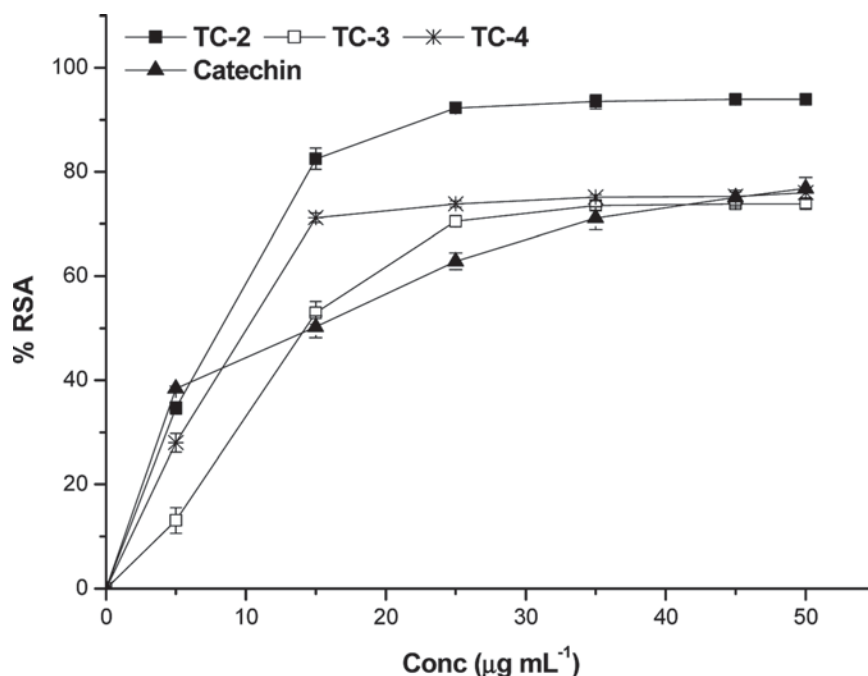


Figure 4. Superoxide radical scavenging activity of extracts and reference compound catechin.

in order to characterize these extracts in terms of their chemical composition. Four compounds namely chebulagic acid, chebulinic acid, corilagin and gallic acid were identified and quantified by the HPLC analysis. Both chebulagic and chebulinic acid were present in considerable amounts in all the three extracts. The results are tabulated in Table 1. The TC-2 extract showed the highest amount of these two compounds.

Since the TC-2 extract showed strongest activity in all the *in vitro* chemical antioxidant assays, it was subjected to antioxidant activity studies using cell lines.

Superoxide dismutase assay

The effect of TC-2 extract on the SOD activity was measured in terms of the intracellular ROS levels. The ROS or superoxide anion levels in the normal cells were taken as zero. When these cells are treated with PMA (control cells), the ROS levels were increased by $+24 \pm 1.4\%$. The variation of ROS levels in the extract pre-treated cells were shown in the Figure 5. Concentrated extract ($200 \mu\text{L}$) reduced the superoxide levels up to $-19.5 \pm 0.3\%$.

Glutathione S-transferase assay

The phase II metabolizing enzyme GST plays an important role in the conjugative detoxification of electrophiles as well as being an essential component of cellular antioxidant defence mechanism. The effect of *T. chebula* extract on the GST modulation was presented in Figure 6. The normal untreated cells has shown GST activity of $7.38 \text{ nmol/mL/min/mL}$, whereas in the H_2O_2 -treated control cells has reduced GST activity of $1.8 \text{ nmol/mL/min/mL}$ the extract pre-treated cells has shown very significant increase in the GST activity. Extracts at different concentration ($10, 50, 100$ and $200 \mu\text{g mL}^{-1}$) showed a dose-dependent increase in the GST activity of 3.2 times higher activity than the control cells ($5.8 \text{ nmol/mL/min/mL}$).

Oxidative stress-induced ROS production

Oxidative stress-induced ROS production detected with DCFH-DA was significantly decreased by TC-2 extract in a dose-dependent manner. Based on this particular experiment, the TC-2 extract in $10\text{--}100 \mu\text{g mL}^{-1}$ concentration range reduced oxidative stress in C2C12 cells in a dose-dependent manner (Figure 7). The generation of ROS is increased in both types of diabetes and the onset of diabetes is closely associated with oxidative stress. Abnormally high levels of ROS and simultaneous decline of antioxidant defense systems can lead to the damage of cellular organelles and enzymes, increased lipid peroxidation and development of complications of diabetes mellitus. In the present experiment, no evidence of any increase or decrease in 2,7-dichlorofluorescein fluorescence was observed in cells incubated with extracts alone.

In diabetic condition due to hyperglycemia, there is excess generation of ROS. Overproduction and insufficient removal of ROS result in vascular dysfunction, damage

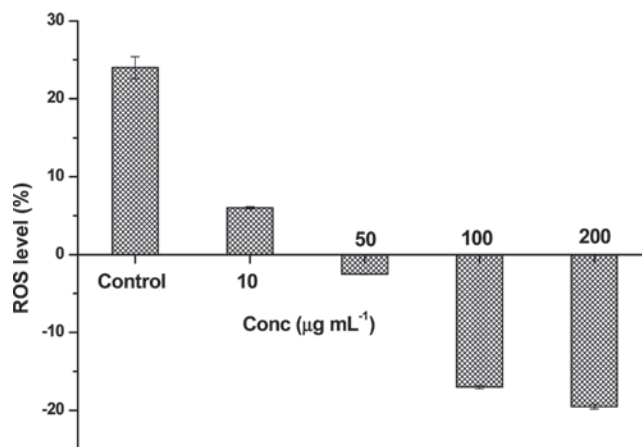


Figure 5. Effect of TC-2 extract treatment on the intracellular ROS levels in HepG2 cells. Cells were treated with indicated concentrations of the extract for 2 h. Data were expressed as mean \pm SD of triplicate experiments.

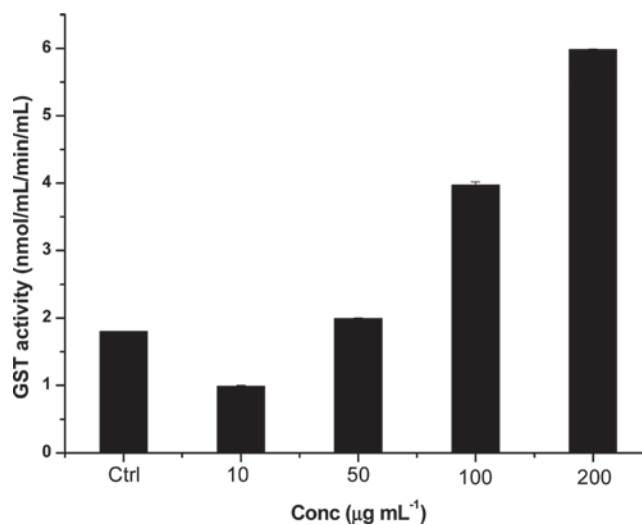


Figure 6. Level of total glutathione S-transferase (GST) after treatment of HepG2 cells with TC-2 extract for 24 h. The results are expressed relative to the control and represent mean values \pm SD of GST measurements ($n=3$).

to cellular proteins, membrane lipids and nucleic acids. The present study revealed that the *T. chebula* extract (TC-2) has a very high *in vitro* radical scavenging activity and which further confirmed by the cell line studies. This indicates that the extracts effectively reduces the ROS levels and thus helps in the management of diabetes.

Conclusion

The ethyl acetate (TC-2), methanol (TC-3) and methanol-water (70:30) (TC-4) extracts of *T. chebula* showed inhibitory potential against the key enzyme (α -glucosidase) linked to type 2 diabetes and also strong antioxidant activity. Out of the three extracts, the TC-2 fraction showed very low IC_{50} value for α -glucosidase inhibition. This may be attributed to its high hydrolysable tannin content comprising mainly chebulagic and chebulinic acids.

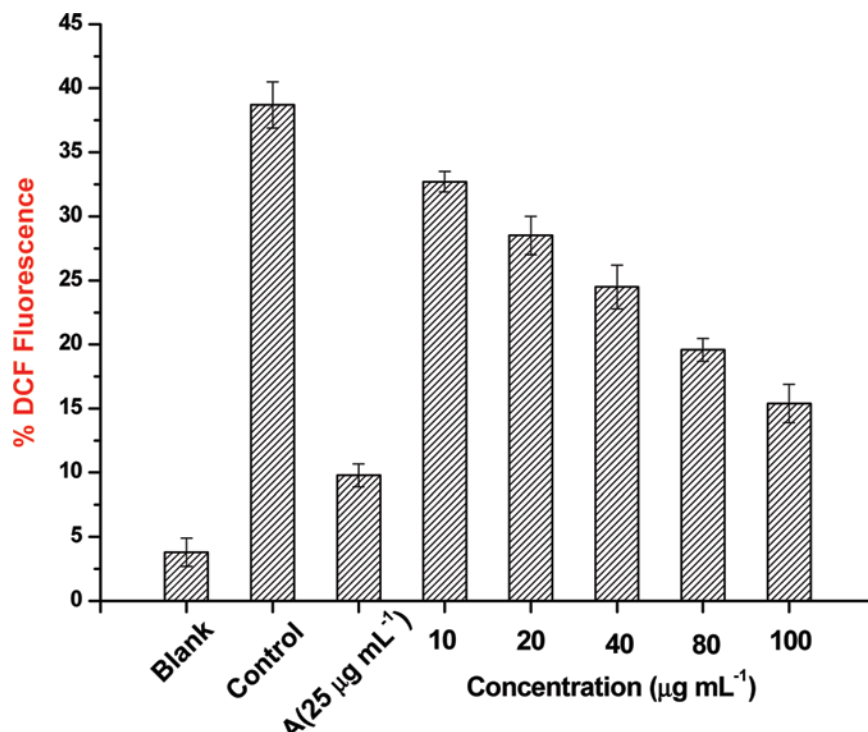


Figure 7. Evaluation of oxidative stress in C2C12 cell lines by flow cytometry. Blank – cells without any treatment; control – cells treated with hydrogen peroxide; A – cells treated with hydrogen peroxide and ascorbic acid (25 µg mL⁻¹); others are cells treated with hydrogen peroxide and different concentrations (10, 20, 40, 80 and 100 µg mL⁻¹) of TC-2 extract. Values are represented as mean value ± SD ($n=3$).

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Declaration of interest

The authors report no declarations of interest.

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