



## Antidiabetic Activity of Phenolic Compounds from *Desmodium triflorum* Leaves in Streptozotocin-induced Diabetic Rats

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### ABSTRACT

The present study was to evaluate the effect of active compounds from *Desmodium triflorum* (DT) leaves on serum glucose in normal and diabetic rats. Diabetes was induced by Streptozotocin (STZ) and High Fat Diet (HFD) in wistar rats. An isolated fraction of ethanolic extracts of *Desmodium triflorum* (ETDT) was administered orally at a dose of 100 mg/kg, p.o. Metformin was used as standard anti diabetic drug (50 mg/kg, p.o). An isolated fractions showing for higher anti diabetic activity was subjected to column chromatography that led to isolation of a pure compound, which was given trivial name DT-1. The interesting results of our preliminary studies with the ETDT have motivated to isolate anti-diabetic active compounds from the leaves of DT for the management of hypoglycemic activities. Collected fractions were subjected to anti-diabetic activity in STZ and HFD induced wistar rats. The particular fraction from ETDT showed strong anti-diabetic activity on a par with the standard drug Metformin. To ensure the compounds responsible for anti-diabetic activities associated with Fraction respectively. In addition a column chromatographic analysis was carried out with Fraction using various solvent systems and isolated compound named as DT-1 from the column which was amorphous powders with decomposition point. DT-1 is phenolic compound nature confirmed by spectral analysis. Reduction in the FBG by DT-1 indicates that DT-1 has anti diabetic efficacy and provides a scientific rationale for the use as an anti diabetic agent.

**Keywords:** Blood glucose, *Desmodium triflorum*, Phenolic compound, Spectrum analysis.

### INTRODUCTION

The chemistry of natural products is an emerging area in drug development activity. The secondary metabolite derived from plant and animal sources are proved to be an effective therapeutic agent in various diseases.<sup>1</sup> Naturally the secondary metabolites of the plant provide defence mechanisms against predators, pathogens, and for self protection against herbivore and microbes.<sup>2</sup> The scientists are exploiting the natural products of the plant and they are focusing their attention to isolate the secondary metabolites of the plant and animals for treating various ailments.<sup>3</sup> The important plant secondary metabolites are namely alkaloids, glycosides, tannins, lignins, flavanoids, terpenes, volatile oils, fixed oils, steroids so on.<sup>4</sup> The chemistry of natural products helps the scientists to find out the structure of the secondary metabolites by using various separation techniques such as Column chromatography, thin layer chromatography (TLC) and sophisticated analytical techniques such as UV, IR, NMR and Mass spectroscopy. Among these some of the successful drugs are isolated from the natural sources such as antibiotic "penicillin" from *Penicillium notatum*, antimalarial agent "quinine" from *Cinchona succirubra*, narcotic analgesic aspirin precursor "salicin" from white willow bark *Salix alba*, cardiac glycoside "digoxin" from *digitalis purpurea* and so on.<sup>5</sup> More recently, anticancer agents "vincristine and vinblastine" are isolated from the *Catharanthus roseus*; and these agents are successfully prescribed by the physicians for the treatment of cancer.<sup>6</sup>

The interesting results of our preliminary studies with the ethanolic extracts of *Desmodium triflorum* (ETDT) have motivated to isolate anti-diabetic active compounds from the leaves of DT for the management hypoglycaemic and hypolipidemic activities. *Desmodium triflorum*, a medicinal plant from the Fabaceae family and also known as san-dam-jin-cao, is commonly used by traditional Indian medicine clinicians in India for the treatment of dysmenorrhoea, muscle spasm, cough, pain and poisoning. The Chinese also called this plant "wings of fly" because of the shape and arrangement of the leaves. This research focuses on evaluating the antidiabetic activity of the ethanolic extract of the *Desmodium triflorum* to verify the presence of the phenolic compound (antidiabetic molecule) by a method using IR, NMR and Liquid chromatography coupled with mass spectrometry. The purpose of this paper is to confirm the antidiabetic activity of *Desmodium triflorum* leaves.

### MATERIALS AND METHODS

#### Preparation of different plant extracts

*Desmodium triflorum* leaves were collected from the forest of Kalakatu, Tirunelveli District, India. Taxonomic identification was made from botanical survey of medicinal plants, Siddha Unit, Government of India, Palayamkottai authenticated by Chelladurai Botanist. A voucher specimen No (CCRAS-1154/2017). Fresh plant leaves were shade dried at room temperature, ground into fine powder and stored in airtight containers. Then extracted (amount 500 g) with solvents of increasing



polarity such as petroleum ether, ethyl acetate, and ethanol, for 72 hours with each solvent, by continuous hot extraction using the soxhlet apparatus at a temperature of 60°C. The extracts were concentrated under reduced pressure using a rotary evaporator to constant weight. The extracts were collected and preserved in a desiccator until used for further studies.

### Study design

In order to carry out column chromatography, a solvent system was established by developing TLC technique. The silica gel (100-200 mesh size) slurry was made with the solvent system established earlier. The slurry was poured time to time into the column very carefully and the silica gel was allowed to settle down to form a uniform packing. Then the stop-cock of the column was opened and the excess of solvent over the column head was allowed to run. The dry crude ethanol extract (10 g) was mixed with small amount of silica gel in a mortar to get a free flowing powder. The powdered sample was then applied carefully on the top of the prepared column and successfully eluted with solvent/solvent system using various solvent systems such as petroleum ether, petroleum ether: chloroform, chloroform: ethyl acetate, ethyl acetate, ethyl acetate: methanol and methanol alone to separate the eluate. The eluate with same  $R_f$  value are pooled together and evaporated to dryness. When the mixture of solvent system used, the ratio of mixtures are prepared as 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80 and 10:90. Elutes were collected in a number of conical flasks marked from fractions 1-100. Elutes were spotted successfully on TLC plate and the flasks having similar spots were combined together.

### Animals

Male Wistar rats each weighing 180–220 g was obtained from Aditya Bangalore Institute of Pharmacy Education and Research, Bangalore, India. The guidelines of the Committee for the Purpose of control and Supervision of Experiments on Animals (CPCSEA) of the Government of India were followed, and prior permission was granted from the Institutional Animal Ethics Committee No.115/1611/CPCSEA. Rodent laboratory chow and water were accessed *ad libitum*, and rats were maintained on a 12 h light/dark cycle in a temperature regulated room (20–25 °C) during the experimental procedures.<sup>7</sup>

### Effect of ETDT on fasting blood glucose in STZ induced diabetic rats

Various isolated fractions of ETDT (100 mg/kg) were evaluated for their anti-diabetic effect in fed with high energy diet of 20% sucrose and 10% lard.<sup>8</sup> The STZ was freshly dissolved in citrate buffer (0.01 mol/L, pH 4.5) and kept on ice prior to use. One week later STZ inductions of diabetes in wistar rats, the fasting blood glucose levels were measured.<sup>9</sup> The hyperglycemic rats (blood glucose >240 mg/dl) were divided into 10 groups (each with 3 rats). Distilled water, metformin and various isolated fractions of ETDT (100 mg/kg) daily administered orally to

normal control, diabetic control and the treatment groups respectively for 3 weeks.

### Purification of Isolated Fraction

Nearly 1 gm of the fraction is weighed and mixed with silica gel and poured into the column. The column is eluted with different solvents by polarity basis. Aliphatic fractions are separated by the solvent petroleum ether. In this solvent system, some distinct bands are formed. That fraction is collected and evaporated to dryness. The aromatic fractions are separated by the n-hexane: ethyl acetate mixture in the ratio of 1:1. The polar fraction present in the extract is eluted by using chloroform. The second polar fraction of the extract is eluted by using methanol. The column fraction which is collected and evaporated to dryness is used for further studies.

### Analysis of fraction

The fraction was characterized by spectroscopy techniques like Perkin-Elmer Vector 22 model FT-IR Spectrophotometer (Nujol), <sup>1</sup>H NMR spectra were recorded in a BRUKER DPX-200 MHz using TMS as internal standard and Mass spectrometer spectra was recorded in SHIMADZU QP 50000 and was given a trivial name DT - 1.

### Statistical analysis

Data expressed as  $\bar{x} \pm SEM$ . Statistical analysis was performed by one-way analysis of variance (ANOVA). The least significant difference test was used for mean comparisons and  $P < 0.05$  was considered to be statistically significant.

## RESULTS

### Column Chromatography Study with ETDT

The column chromatography study was carried out with ETDT to separate the eluates namely F1 – 40 using petroleum ether as a solvent system. F 41 – 75 are the eluates isolated using petroleum ether: chloroform, F 76 – 105 are the eluates isolated using chloroform, F 106 – 140 are the eluates isolated using chloroform: ethyl acetate, F 141 – 160 are the eluates isolated using ethyl acetate, F 161 – 183 are the eluates isolated using ethyl acetate: methanol and finally methanol alone is used the eluate F 184 – 200. The volume of the each eluate is 50 ml. The eluates with same  $R_f$  value were pooled together and evaporated to dryness. The pooled fraction of ETDV such as F 1 – 40, F 41 – 75, F 76 – 105, F 106 – 140, F 141 – 160, F 161 – 183 and F 184 – 200 are named as A, B, C, D, E, F and G respectively. The pooled eluates of A, B, C, D, E, F, G were tested in fasting blood glucose level in STZ induced diabetic rats.

### Effects of 3-week administration of various isolated fractions ETDT (100 mg/kg), and Metformin (50 mg/kg) on FBG in STZ induced diabetic Rats

From the study it was observed that the fraction “F” showed significant ( $P < 0.05$ ) decrease in blood glucose but the other fractions did not show significant effect of



blood glucose when compared with normal control. The results of the effect of various isolated fractions of ETDT (100 mg/kg) on the blood glucose level in STZ induced diabetic rats are shown in Table-1.

**Table 1:** Effect of Various Isolated Fractions of ETDT (100 mg/kg) on the Blood Glucose Level in STZ Induced Diabetic Rats

Treatment	Fasting blood glucose			
	0 day	7 <sup>th</sup> day	14 <sup>th</sup> day	21 <sup>st</sup> day
Normal control	78.4 ± 3.7	77.9 ± 4.2	78.5 ± 2.7	76.6 ± 3.6
Diabetic control	67.3 ± 5.8	261.8 ± 5.3	259.3 ± 4.8	251.4 ± 2.8
Fraction – A	68.9 ± 3.4	263.7 ± 4.7	257.4 ± 2.3	228.8 ± 1.6
Fraction – B	73.6 ± 4.1	259.5 ± 3.9	251.3 ± 3.4	217.4 ± 3.5
Fraction – C	79.8 ± 2.5	265.4 ± 2.8	249.5 ± 2.3	198.8 ± 1.6
Fraction – D	69.3 ± 1.6	245.3 ± 1.5	218.4 ± 2.6	181.1 ± 5.3
Fraction – E	67.4 ± 1.9	255.4 ± 4.9	221.5 ± 2.5	167.4 ± 1.2
Fraction – F	77.5 ± 0.9	248.3 ± 1.1	150.4 ± 0.8	86.1 ± 2.6*
Fraction – G	76.4 ± 7.6	254.4 ± 1.5	185.3 ± 4.6	149.3 ± 5.2
Metformin	80.3 ± 3.4	251.4 ± 5.5	142.3 ± 3.1	88.4 ± 1.1*

n=3. \*  $P < 0.05$  vs control group

#### Purification of pooled column fraction of ETDT by column chromatography

From the results of anti-diabetic effect, the fraction F from ETDT showed promising results. Hence this fraction was subjected to further purify using column chromatography and followed by TLC. The natures of fractions obtained are listed in Table-2.

**Table 2:** The Column Chromatographic Fractions of F from ETDT and their TLC Analysis

S. No	Solvent system used in column elution	Eluates	Volume of pooled eluate (ml)	Solvent system used for TLC	Nature of compound
1.	Ethyl acetate: Hexane(25: 75)	F 1 – F5	300	Methanol: Ethyl acetate (20: 20)	
2.	Ethyl acetate: Hexane(50: 50)	F6 –F10	300	Methanol: Ethyl acetate (20: 20)	
3.	Ethyl acetate: Hexane(75: 25)	F11-F 19	300	Methanol: Ethyl acetate (20: 20)	
4.	Ethyl acetate alone (100)	F20-F25	300	Methanol: Ethyl acetate (20: 20)	
5.	Methanol: Ethyl acetate (5: 95)	F26-F 31	300	Methanol: Ethyl acetate (20: 20)	
6.	Methanol: Ethyl acetate (10: 90)	F32-F 41	300	Methanol: Ethyl acetate (20: 20)	
7.	Methanol: Ethyl acetate (20: 80)	F42-F 45	300	Methanol: Ethyl acetate (20: 20)	
8.	Methanol: Ethyl acetate (50: 50)	F46-F 47	300	Methanol: Ethyl acetate (20: 20)	Amorphous powder with decomposition point *
9.	<b>Methanol alone (100)</b>	<b>F48-F 50</b>	<b>100</b>	Methanol: Ethyl acetate (20: 20)	

\* is the compound from the fractions of (F 46 - 47) named as DT -1.

#### Characterization of compounds using various analytical techniques

##### IR studies with DT – 1

The IR spectra exhibit characteristic absorption bands at 3332  $\text{cm}^{-1}$  for a –OH stretching, Characteristic absorption

bands at 1622  $\text{cm}^{-1}$  which show that the compound have C=O group. The IR spectra exhibits characteristics absorption bands at 1648  $\text{cm}^{-1}$  for a C=C group. The spectrum is presented in Figure-1.



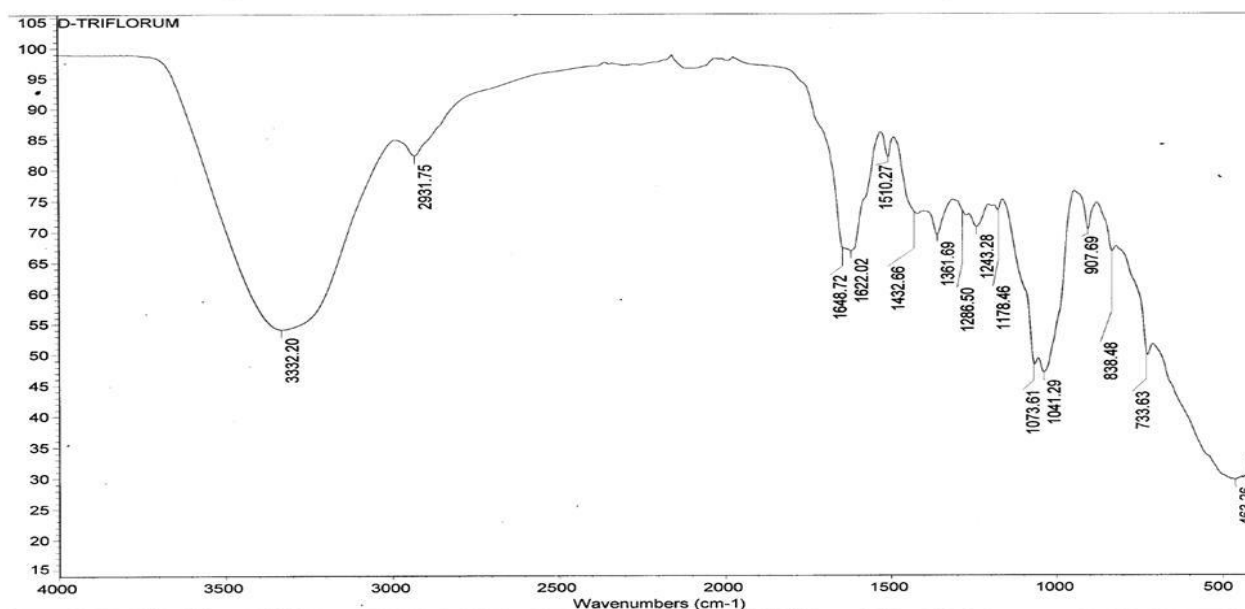


Figure 1: IR Spectrum of the Compound DT-1 from ETDT

### <sup>13</sup>C-NMR Studies with DT -1

From the spectra it was observed that the spectrum showed various signals. It revealed that the chemical signals were at  $\delta$  115.93 ,  $\delta$  117.12,  $\delta$  118.27,  $\delta$  120.92,  $\delta$  122.99,  $\delta$  125.24,  $\delta$  127.86,  $\delta$  139.12,  $\delta$  141.66 and  $\delta$

144.98 showed the presence of at least five C=C groups. The chemical signals at  $\delta$  157.14 and  $\delta$  161.73 showed the presence of two phenolic carbons. The spectrum of the compound is given in Figure-2.

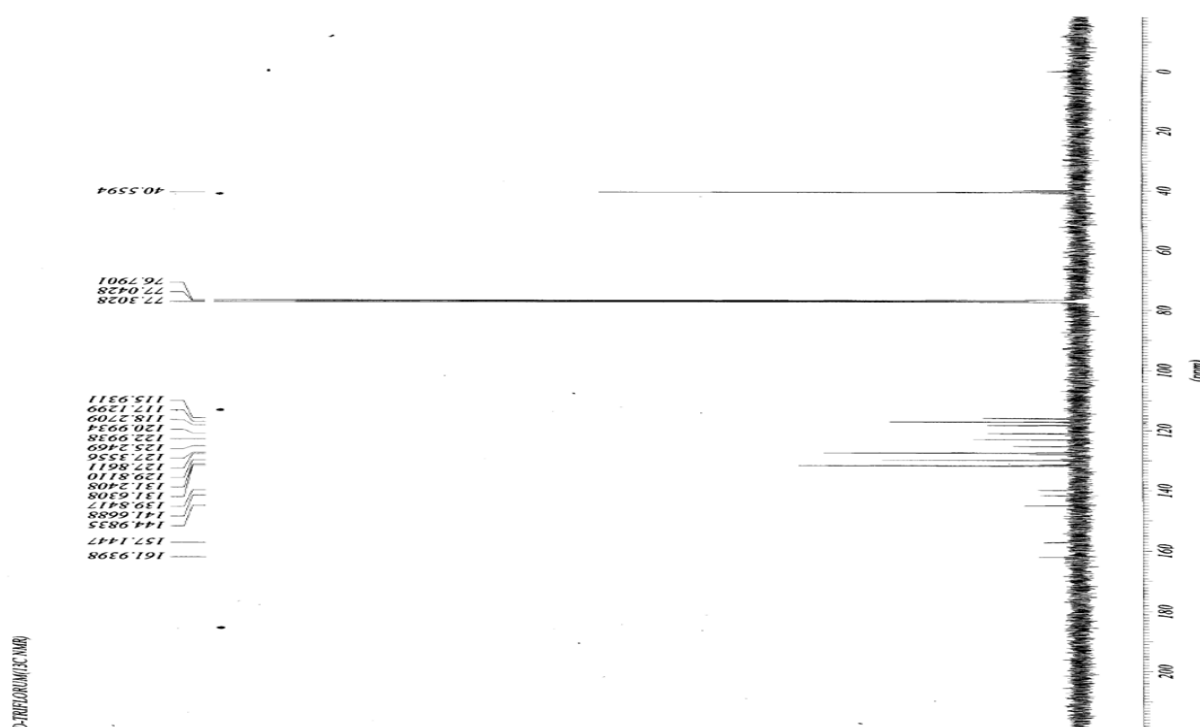


Figure 2: <sup>13</sup>C-NMR Spectrum of the Compound DT-1 from ETDT

### <sup>1</sup>H - NMR Studies with DT- 1

From the spectra it was observed that the compound exhibited signal at  $\delta$  0.86 ppm and  $\delta$  0.90 ppm for a methyl groups, a broad singlet at  $\delta$  1.27 ppm with a signal

at  $\delta$  1.86 ppm for a long chain of methylene groups and signal at  $\delta$  3.51 ppm for protons under oxygen function. The signal at  $\delta$  4.69 ppm for the unsaturated protons. The spectrum of the compound is given in Figure-3.



Figure 3: <sup>1</sup>H-NMR Spectrum of the Compound DT-1 from ETDT

**Mass Spectrum Studies with DT – 1**

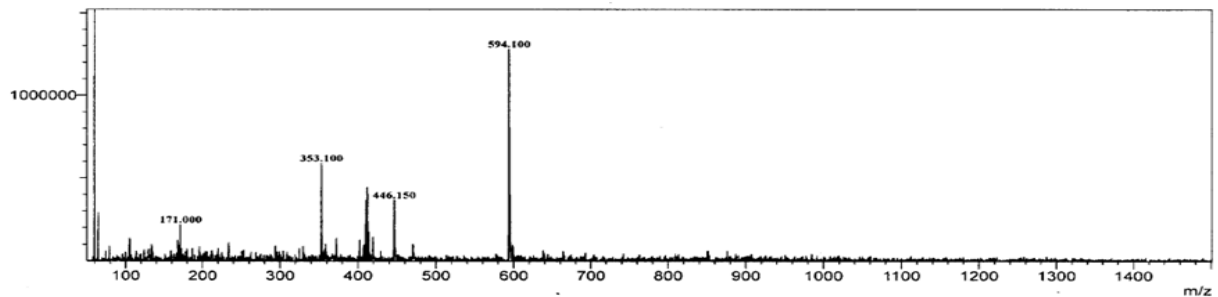
From the mass spectrum of DT - 1, it was observed that a molecular ion peak at signal m/z 353.1, m/z 446.1 and m/z 594.1 (ESI MS positive mode) and a peak at m/z

164.7, m/z 263.1 and 400.5 (ESI MS negative mode). The observed fragmentation pattern shows that the similarity of a compound having an aromatic origin. The spectrum of the compound is given in Figure-4.

Sample Name : D-TRIFLORIUM001  
 Injection Volume : 0.3  
 Method File : TAG-070919-Q1.lcm  
 Data File : D:\PRSDATA FILE\070919-SCAN-D-TRIFLIRIUM001.lcd

**Sample Information**

R. Time:----(Scan#:----)  
 MassPeaks:10 BasePeak:59.100(3015717)  
 Spectrum Mode:Averaged 0.126-0.326(25-63)  
 BG Mode:Averaged 1.134-1.166(217-223) Polarity:Positive Segment 1 - Event 1



R. Time:----(Scan#:----)  
 MassPeaks:40 BasePeak:58.600(642882)  
 Spectrum Mode:Averaged 0.131-0.331(26-64)  
 BG Mode:Averaged 1.139-1.171(218-224) Polarity:Negative Segment 1 - Event 2

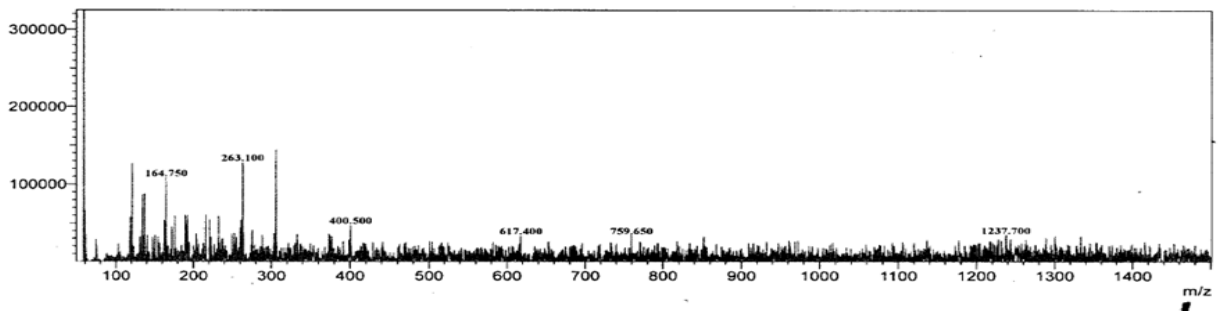


Figure 4: Mass Spectrum of the Compound DT-1 from ETDT

## DISCUSSION

Now a day, the interest in the study of natural product is growing rapidly, especially as a part of drug discovery programs. In our previous studies proved that the anti-diabetic activities are associated with the active constituents of ETDT.<sup>10</sup> In continuation to the previous study, we have shown interest to isolate the pure constituents responsible for the above mentioned pharmacological action. An attempt was made to isolate the purified compounds responsible for anti-diabetic activity using column chromatography technique with ETDT. The fraction F from ETDT showed strong anti-diabetic activity on a par with the standard drug metformin. To ensure the compound responsible for anti-diabetic activities associated with F respectively, In addition a column chromatographic analysis was carried out with 'F' using various solvent systems. We isolated one compound named as DT-1 from the column which was amorphous powders with decomposition point; however DT-1 is Polyphenolic compound nature confirmed by spectral analysis. Antidiabetic action of polyphenolic compound was found that non-covalent interactions occur between polyphenols and enzymes (proteins).<sup>11</sup> Hydroxyl groups and galloyl groups are present in the molecular structure of polyphenols.<sup>12</sup> The phenolic groups can form hydrogen bonds with the polar groups of enzyme. In contrast, there are many hydrophobic amino acids found in enzymes (protein). Galloyl groups in polyphenols show hydrophobicity<sup>13</sup> and, therefore, polyphenols can bind enzymes through hydrophobic association.<sup>12</sup> The galloyl moiety may play an important role to interact with mammalian  $\alpha$ -amylase and  $\alpha$ -glucosidase and their positions mostly affect the effectiveness.<sup>14</sup> The cooperative effects of hydrophobic association and hydrogen bond formation between polyphenols and the starch digestive enzymes could contribute to control postprandial hyperglycemia in type 2 diabetic patients.

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