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Antioxidant capacities of *Gundelia tournefortii* L. extracts and inhibition on glutathione-S-transferase activity

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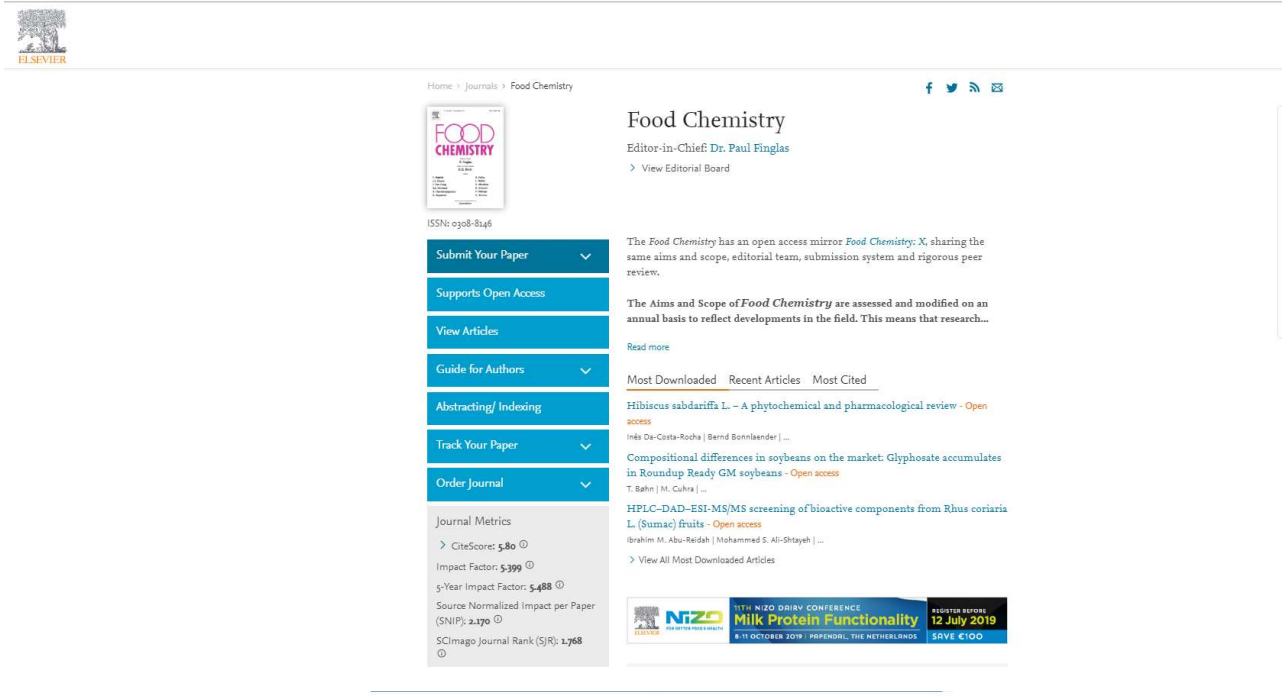
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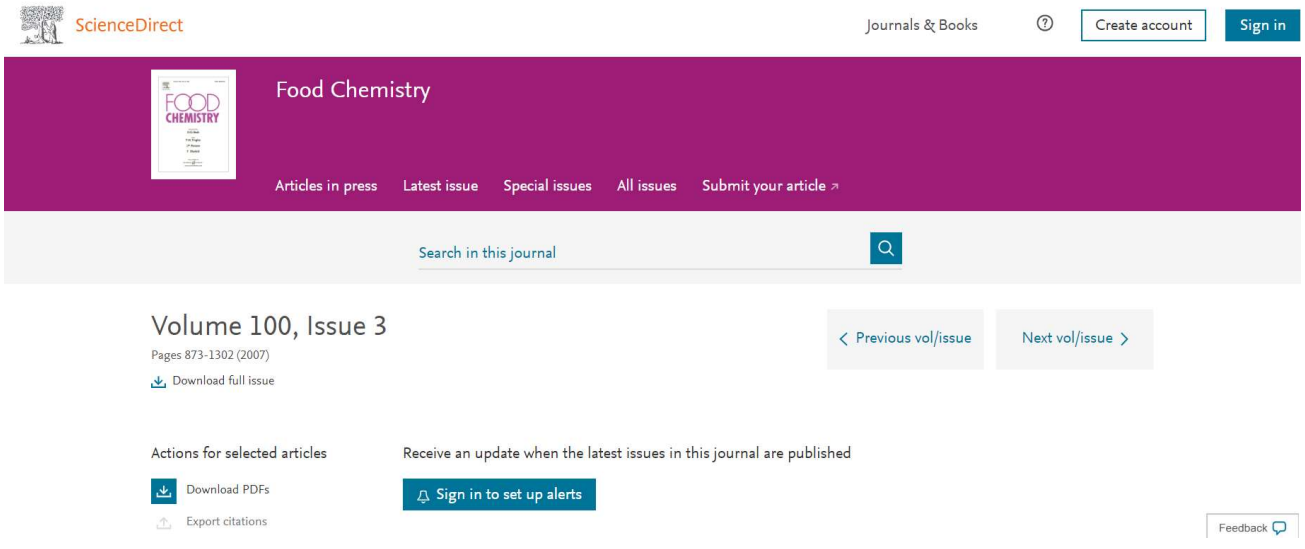
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Antioxidant capacities of *Gundelia tournefortii* L. extracts and inhibition on glutathione-S-transferase activity

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Abstract

Gundelia tournefortii L. is an important food source and a well-known medicinal plant in Eastern Anatolia. Therapeutic effects of medicinal plants are known to be closely related to their antioxidant capacities. Antioxidant activities of *G. tournefortii*, both for the aerial parts and seeds, were investigated by using both 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and lipid peroxidation inhibition methods. The seeds were found to have higher antioxidant potential than the aerial, with IC_{50} values of 0.073 mg/mL for DPPH scavenging and 0.146 mg/mL for lipid peroxidation inhibition capacities. In addition, total phenolic contents of the *Gundelia tournefortii* L. extracts, especially the seed extracts correlates to its high antioxidant activity with 105.1 ± 8.7 μg gallic acid equivalents (GAEs) per mg of seed extract. Plant extracts with high phenolics content are known to have important effects on various enzymes, as well as glutathione-S-transferases, which are important detoxification enzymes in phase II systems with an important role in developing multi-drug resistance to chemotherapy in tumour cells. Consequently, the effects of *G. tournefortii* extracts on crude cytosolic glutathione-S-transferase was also studied and the seed extracts have shown effective inhibition of cytosolic GST activity, with an IC_{50} of 97.51 μg/mL.
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Keywords: *Gundelia tournefortii* L.; Antioxidant; DPPH; Lipid peroxidation; Total phenolics; Glutathione-S-transferase

1. Introduction

Gundelia tournefortii L. var. *armata* Freyn. and Sint. from the Asteraceae (Compositae) family, is a medicinal plant, native to Asian-temperate zones of Western Asia, namely Cyprus, Egypt, Iran, Israel, Jordan, Turkey, Azerbaijan and Turkmenistan. It is recorded that the flowers, leaves, seeds and stems of *G. tournefortii* are used as food sources (Ertuğ, 2000). In the Middle East, the young and still undeveloped flower buds are sold in the local markets just like artichoke hearts; it is a highly sought item (Hedrick, 1972; Kunkel, 1984). Dry seeds of *G. tournefortii* are also known to be effective for the treatment of vitiligo disease, in Eastern Anatolia folk medicine. Fresh seeds of *G. tournefortii* are used in pickles and also are effective diuretics.

In this study, the antioxidant activities of *G. tournefortii* extracts were evaluated for their DPPH radical scavenging and lipid peroxidation inhibition capacities and for their total phenolics contents. The effects of *G. tournefortii* extracts on glutathione-S-transferase (GST) activities were also investigated along with their antioxidant capacities. GST enzymes are important for the detoxification of by-products of lipid peroxidation or DNA hydroperoxides in biological systems (Hayes & Pulford, 1995; Nordberg & Arn'er, 2001). Resistance developed, against antitumor drugs used in chemotherapy, is due to the ability of tumor cells to promote GSH conjugation catalyzed by glutathione-S-transferase (Guadiano et al., 2000; Zanden et al.,

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2004). Therefore, investigation of novel antioxidants with inhibitory effects on GST activity is another important reason for this research (Gyamfi, Ohtani, Shinno, & Aniya, 2004).

2. Materials and methods

2.1. Materials

Preparative chromatography grade methanol used in the preparation of extracts was purchased from Merck (Darmstadt, Germany). Potassium chloride (KCl), ethylenediaminetetraacetic acid (EDTA), glycerol, bovine serum albumin (BSA), copper sulphate ($CuSO_4$), sodium potassium tartarate, sodium hydroxide (NaOH), sodium carbonate (Na_2CO_3), Tris–HCL, ferrous sulphate ($FeSO_4 \cdot 7H_2O$), thiobarbituric acid (TBA), ortho-phosphoric acid (H_3PO_4), α-tocopherol, quercetin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, 1-chloro-2,4-dinitrobenzene (CDNB), and reduced glutathione (GSH), were purchased from Sigma Chemical Company, (St. Louis, MO, USA). Dimethyl sulphoxide (DMSO) and Folin-Ciocalteu's phenol reagent were purchased from Merck (Darmstadt, Germany). All other chemicals were analytical grade and were obtained from commercial sources at the highest purity available.

2.2. Plant materials

Plant samples of *Gundelia tournefortii* L. var. *armata* Freyn. & Sint. were collected at various periods in 2003, from Van in Turkey. The plant specimens with their localities and the necessary field records were written and enumerated as voucher specimen number, F 11174. They were press-dried according to herbarium techniques and identified by "Flora of Turkey" (Davis, 1975), and stored in the herbarium at Yüzüncü Yıl University (VANF).

2.3. Preparation of plant extracts

Plant samples were ground using a Waring type stainless steel commercial blender. Chemical extraction was carried out in a Heidolph Laborota 4000 rotary evaporator, overnight at 50 °C. For each extraction, 10 g of the sample were extracted in methanol with a sample to solvent ratio of 1:10 (w/v). Extracts were then filtered through a double cheesecloth and dried under vacuum using a rotary evaporator.

2.4. Isolation of sheep liver microsomes

Sheep livers, bought from the slaughterhouse of Sincan, Ankara Turkey, were homogenized using a glass-teflon homogenizer and centrifuged by a Sorvall RC 5C Plus with SS-34 rotor and Sorvall Combi Plus Ultracentrifuge with 10,800*g* for 25 min. The microsomes in the suspension, containing 25–30 mg protein/mL, were stored in an Sanyo ultra-low freezer (−84 °C) for up to 3 months (Iscan et al., 1998) and their protein content was measured by the Lowry's Method (Lowry, Rosebrough, Farr, & Randall, 1951).

2.5. Lipid peroxidation inhibition evaluated by thiobarbituric acid test

Lipid peroxidation of the microsomes was induced using a 100 μM ferrous sulphate solution. The end products of lipid peroxidation were quantified by determining the formed malondialdehyde (MDA), and related products. In acidic medium, MDA reacts with thiobarbituric acid (TBA) upon boiling and the resultant MDA-TBA adduct as other TBA reactive substances (TBARS) absorbs at 532 nm. Formation of MDA-TBA adduct was monitored by a Cary 50 Bio UV–vis spectrophotometer in both the presence and absence of the extracts, and was expressed as nmoles/mg of protein/minute. The TBARS values were calculated using the extinction coefficient of 1.56×10^{-5} $M^{-1}\,cm^{-1}$ (Heath & Packer, 1968). Percent inhibition in lipid peroxidation was calculated using the equation

$$\%\ \text{Inhibition} = [(A_0 - A_1)/A_0 \times 100],$$

where, A_0 is the absorbance of control with methanol and A_1 is the absorbance with the extract samples. Fifty percent inhibitory concentration (IC_{50}) values were calculated after constructing the percent inhibition versus log (extract concentration) curve.

2.6. Free radical scavenging activity by DPPH method

The free radical scavenging activities were determined by the DPPH method of Blois (1958) with some modifications. A 0.05 mg/mL of DPPH ethanol solution which absorbs at 517 nm, produces approximately 1.3 unit of absorbance. A series of extract solutions with varying concentrations were prepared by dissolving the dried extracts in ethanol. A solution of each extract (0.1 mL) was added to 1.4 mL of DPPH solution. The absorbance at 517 nm was recorded after 5 min of incubation at room temperature. The radical scavenging capacity of each extract was calculated as the percent DPPH radical scavenging affect

$$\text{DPPH Scavenging Effect}\ (\%) = [(A_0 - A_1)/A_0 \times 100],$$

where, A_0 is the absorbance of the control with ethanol and A_1 is the absorbance of the sample in the presence of the extracts. IC_{50} concentrations were also calculated as explained above.

2.7. Determination of total phenolics content

The total concentration of phenolic compounds in the extracts was determined using a series of gallic acid standard solutions (0.05–0.3 mg/mL) as described by Singleton and Rossi (1965) but with some modifications. Each extract solution (0.1 mL) was mixed with 2 mL of a 2% (w/v) sodium carbonate solution and vortexed vigorously. The same pro-

cedure was also applied to the standard solutions of gallic acid. After 3 min, 0.1 mL of 50% Folin-Ciocalteu's phenol reagent was added and each mixture was vortexed again. The absorbance at 750 nm of each mixture was measured, after incubation for 30 min at room temperature. Results were expressed as milligrams of total phenolics content per milligrams of extract as gallic acid equivalents (GAE).

2.8. Determination of sheep liver glutathione-S-transferase activity toward CDNB

GST activities were determined spectrophotometrically by monitoring the thioether formation at 340 nm using 1-chloro-2,4-dinitrobenzene (CDNB) as the substrate (Habig, Pabst, & Jakoby, 1974). Sheep liver cytosolic fractions were prepared and used as the enzyme source to measure GST activity toward CDNB. Potassium phosphate buffer (0.2 M) and final 1 mM GSH were used throughout the experiments. Enzyme activity determinations were carried out at 25 °C. Sheep liver microsomes (21 mg/mL) were used after 1/100 dilution with 10 mM potassium phosphate buffer (pH 6.0). Concentrations ranging between 0.2 and 2 mM CDNB were used as the substrates. The reactions started by the addition of the enzyme. Incubation mixtures without an enzyme source were used as the blanks (non-enzymatic reactions). Enzyme activities with 10 μL of DMSO were regarded as control. The enzyme activities were expressed in terms of percent specific activity. One unit of GSTs activity is defined as the amount of enzyme producing 1 μM thioether per min. The enzyme activities were expressed in terms of percent specific activity. The extinction coefficient for the CDNB conjugate at 340 nm is 0.0096 μM^{-1} cm^{-1} (Habig et al., 1974).

Each data point was obtained by making at least 6 independent measurements. The results were expressed as mean ± SD and levels of significance were assessed using ANOVA test (Analysis of Variance).

3. Results and discussion

Each extract was prepared by dissolving 10 g of dry samples in 100 mL methanol. The methanol extraction of the aerial parts and the seeds of *G. tournefortii* yielded 6.3% and 2.18% of dry weight, respectively. In order to identify the antioxidant capacity of plants extracts, DPPH and lipid peroxidation inhibition tests were selected as the methods of choice. The DPPH method, is the direct test used to evaluate the real-time free radical scavenging. On the other hand, lipid peroxidation inhibition test was used to mimic a biomembrane medium.

3.1. Lipid peroxidation inhibition by thiobarbituric acid test

Iron induced microsomal lipid peroxidation inhibition was used as one of the methods to evaluate antioxidant capacity. α-Tocopherol, a well-known antioxidant, was used as the standard in this study. During the measurements, the absorbance of each sample was corrected for the methanol background. The lipid peroxidation inhibition capacities of each methanol extract were calculated as percent inhibiton as shown in Fig. 1. Results are also listed in Table 1 as the concentration of each extract necessary to inhibit microsomal lipid peroxidation by 50 percent, (IC_{50}). As a result IC_{50} values for inhibition of lipid peroxidation were found to be 0.255 and 0.146 mg/mL, for aerial parts and seeds extracts of *Gundelia tournefortii* L., respectively. The IC_{50} value of α-tocopherol was 0.23 mg/mL.

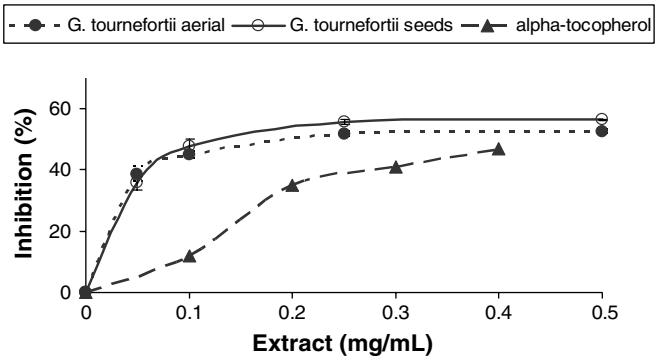


Fig. 1. Inhibition of iron induced lipid peroxidation (%) by *Gundelia tournefortii* L. aerial and seed extracts compared with α-tocopherol.

Table 1
Comparison of 50% inhibitory concentration for lipid peroxidation, % DPPH radical scavenging activity, and total phenolic contents of *Gundelia tournefortii* L. extracts and their effects on GST activity

Plants	DPPH scavenging IC_{50} (mg/mL)	LP inhibition IC_{50} (mg/mL)	TP (μg GAE/mg extract)	GST inhibition IC_{50} (μg/mL)
Gundelia tournefortii aerial parts	0.442	0.255	64.4 ± 4.8	155.2
Gundelia tournefortii seeds	0.073	0.146	105.1 ± 8.7	97.5
Quercetin	0.0061	ND	NA	ND
α-Tocopherol	ND	0.23	NA	ND

DPPH scavenging IC_{50}: concentration of plant extracts for 50 percent of DPPH scavenging. LP inhibition IC_{50}: concentrations of plant extracts for 50 percent inhibition of lipid peroxidation. TP: total phenolics content μg equivalents of Gallic acid/mg of plant extract. GST inhibition IC_{50}: concentration of plant extracts for 50 percent inhibition of GST activity. ND: not determined. NA: not applicable.

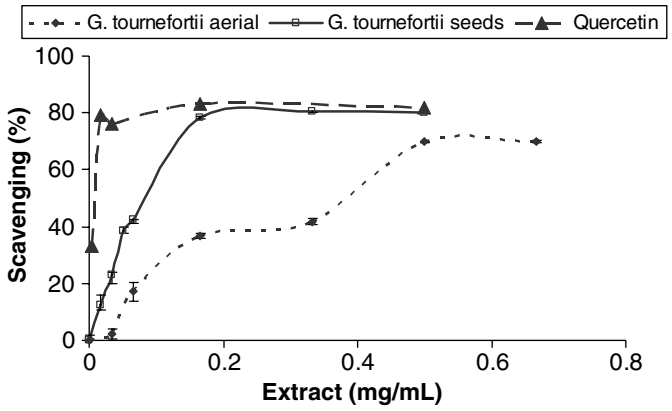


Fig. 2. DPPH radical scavenging activity (%) of *Gundelia tournefortii* L. aerial and seed extracts compared with quercetin.

3.2. Free radical scavenging activity by DPPH method

Free radical scavenging activity of the extracts was evaluated by using the DPPH radical scavenging method. Dry extracts were dissolved in ethanol in concentrations ranging between 0.1 and 0.8 mg/mL. DPPH radical scavenging activity of the extracts are expressed as % radical scavenging, by measuring the absorbance decrease at 517 nm due to disappearance of the DPPH radical. Results are presented as % DPPH radical scavenging activity of the Gundelia extracts in mg/mL concentrations and shown in Fig. 2.

The IC_{50} values for DPPH percent scavenging were determined from the percent inhibition versus log (extract concentration) curve, and for the aerial parts and the seeds of *G. tournefortii* extracts, IC_{50} values were found to be 0.442 and 0.073 mg/mL, respectively. IC_{50} for quercetin, which was used as a standard was 0.0061 mg/mL, as listed in Table 1.

3.3. Total phenolics content of G. tournefortii extracts

The total phenolics content of the *G. tournefortii* extracts was determined by using the method of Singleton and Rossi (1965) and the results are expressed in Table 1 as μg per mg of gallic acid equivalents (GAE).

The amount of total phenolic compounds found in the extract from aerial parts of *G. tournefortii* was 64.4 ± 4.8 μg in 1 mg of methanolic crude extract. The total phenolics content in the seeds, however, was 105.1 ± 8.7 μg in 1 mg of methanolic crude extract, gallic acid equivalents. The results are consistent with the respective antioxidant capacities of each extract.

Phenolic constituents have been studied extensively as important contributers to the antioxidant activity in plants (Aruoma, Bahorun, & Jen, 2003; López, Martínez, Del Valle, Ferrit, & Luque, 2003; Rice-Evans, Miller, & Paganga, 1997; Skerget et al., 2005). There are reports in the literature which correlate the total phenolics content of a plant extract with its antioxidant activity (Cai, Luo, Sun, & Corke, 2004; Kaur & Kapoor, 2002; Velioglu, Mazza, Gao, & Oomah, 1998; Yen & Duh, 1993) and this was also the case in the present study, as shown in Table 1. The seed extracts with high phenolics content, also has high DPPH radical scavenging and lipid peroxidation inhibition capacities.

3.4. Effects of G. tournefortii extracts on glutathione-S-transferase acitivity

GSTs activity was determined spectroscopically by monitoring the thioether formation at 340 nm using CDNB as the substrate (Habig et al., 1974). The extracts were used within 0.01–0.15 mg/mL final concentrations in order to calculate the percent inhibition of GST activity and respective IC_{50} values. The inhibitory effect of extracts (100 μg/mL concentration) on GST activity was compared with their phenolics content in μg equivalent of GAE/mg of extract (Fig. 3). Concentrations of the extacts for 50% inhibition of GST activity were also listed in Table 1.

Phenolic content of the seed extracts of *G. tournefortii* was higher (105.1 μg/mg extract) than the aerial parts of the plant (64.4 μg/mg extract). Plant extracts with high polyphenols are known to have important inhibitory effects on glutathione-S-transferases in the literature. Consequently, *G. tournefortii* seed extracts which have higher phenolics content, are also expected to be more effective in GST inhibition, as given in Fig. 3 and Table 1, coinciding with literature (Gyamfi et al., 2004). Furthermore, GST activity of cancer cells was inhibited by polyphenols (Zhang & Wong, 1997). Hence, the search for novel antioxidants with GST inhibitory activity has become an important issue because of their role in multi-drug resistance.

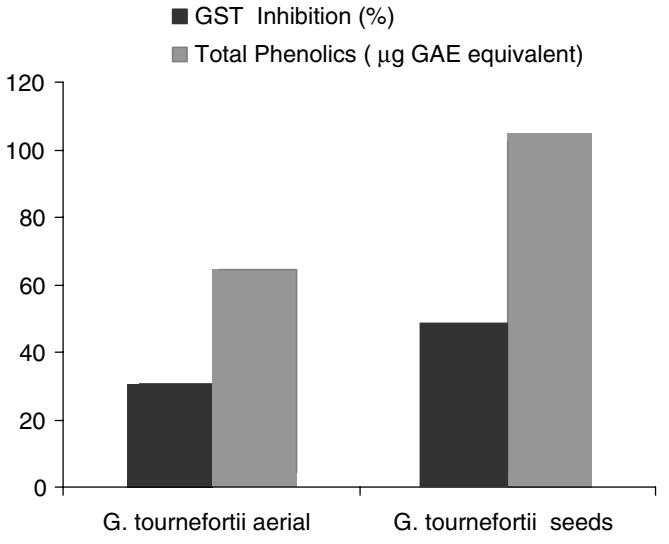


Fig. 3. Comparison of the GST inhibition of *Gundelia tournefortii* L. extracts (100 μg/mL), with their phenolics content in μg equivalent of GAE/mg of each extract from aerial parts and the seeds.

4. Conclusions

This is the first study in the literature, on the antioxidant capacity, content of total phenolics and the GST inhibitory activities of *G. tournefortii* plant. Methanol extracts of *G. tournefortii*, especially the seed extracts, have considerable antioxidant capacity compared with α-tocopherol. The seed extracts, having higher phenolic content, were also more effective GST inhibitors, with an IC_{50} of 97.5 μg/mL.

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