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# Isolation, Identification and Antioxidant Potential of Major Flavonoids from Ethyl Acetate Fraction of *Torreya grandis*

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There is increasing interest in plant extracts as potential therapeutic agents and the use of a mixture of natural products to treat disease has a number of interesting outcomes, most notably their synergistic effects and polypharmacological action. Some flavonoids with antioxidant properties from the aerial parts of the plant species *Torreya grandis* (family Taxaceae) were identified. Upon chromatography, the ethyl acetate extract afforded flavonoids, such as: 4',5,7-trihydroxy flavone-5-O-[α-L-rhamnopyranosyl-(1-4)-6-O-acetyl-β-D-glucopyranoside] (**1**), apigenin-7-O-β-glucopyranoside (**2**), apigenin (**3**), luteolin (**4**) and chrysin (**5**). The structures of the isolated compounds were determined by interpretation of their physical and spectral data. The antioxidant activities of water and 80 % ethanol extracts were measured by FRAP, 2,2'-diphenylpicrylhydrazyl (DPPH$^{\bullet}$) and reducing power activity methods, while the antioxidant activities of isolated compounds were estimated by TEAC assay. The 80 % ethanol extract and isolated compound luteolin showed a strong concentration-dependent antioxidant effect than commercial antioxidant α-tocopherol. This investigation demonstrated that the plant species *T. grandis* is a new and favourable source of natural antioxidants and the potency of these compounds could provide a chemical basis for some of the health benefits claimed for *T. grandis* in folk medicine and warrant further studies to assess their potential as effective natural remedies.

**Key Words: *Torreya grandis*, Isolation, Antioxidant activity, Polyphenols, Flavonoids.**

## INTRODUCTION

Plants are sources of natural antioxidants and some of the compounds have significant antioxidative properties and health benefits. Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions. The potential of the antioxidant constituents of plant materials for the maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists and food manufacturers as consumers move toward functional foods with specific health effects[1].

Flavonoids have been of particular interest because many of these flavonoids exhibit a broad spectrum of biological activity, including antiinflammatory, anticarcinogenic, antiviral, antioxidant, antithrombogenic and antiatherogenic properties[2]. Antioxidant components are microconstituents present in the diet that can delay or inhibit lipid oxidation, by inhibiting the intiation or propagation of oxidizing chain reactions and are also involved in scavenging free radicals. Some epidemiological studies indicate a negative correlation between the intake of flavonoids and coronary heart disease[3], cancer[4] and stroke[5].

It has been reported that dietary administration of synthetic antioxidants such as butylated hydroxytoluene (BHT) to rats can result in fatal hemorrhages[6]. In recent years, evaluation of antioxidative activity of naturally occurring substances has been our focus of interest. However, the use of natural antioxidants is limited by lack of knowledge about their molecular composition, amount of active ingredients in the source material and the availability of relevant toxicity data[7]. Several natural antioxidants have already been isolated from plant materials, such as oil seeds, cereal crops, vegetables, fruits, leaves, roots, spices and herbs.

*Torreya grandis* Fort ex. Lindl (Taxaceae), common name nutmeg yew tree, ornamental plant common in China and Japan is a large sized ever-green coniferous tree with dioecious flowers (occasionally monoecious); branches whirled; branchlets subopposite or subwhirled, base with bud scales not persistent; winter buds with several pairs of decussate bud scales. Leaves decussate or subopposite and drup-like fruits

with nut seeds[8]. It is an indigenous medicinal plant due to its anthelmintic, antitussive, carminative, laxative, antifungal, antibacterial and antitumor activity[9].

The isolation and biological activities of flavonoids have been of particular interest. During the course of our biological activity studies of flavonoids from *T. grandis* five main flavonoids were isolated (Fig. 1) and their structures were elucidated by HPLC-DAD, liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS) and $^1$H NMR and were found to be compatible with available known structures of the same compounds. This paper reports the isolation and characterization of these compounds existing in *Torreya grandis* and to evaluate their antioxidant capacities by the 2,2'-diphenylpicrylhydrazyl (DPPH$^\bullet$) free radical-scavenging assay and reducing power activity.

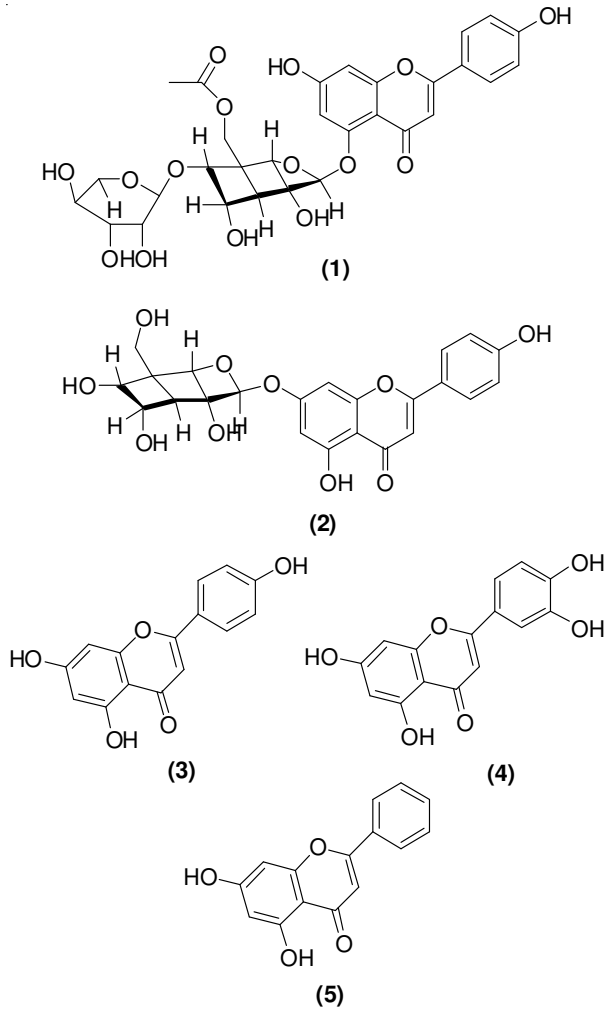


Fig. 1. Structures of isolated compounds **1-5**; apigenin-5-O-[α-L-rhamnopyranosyl-(1-4)-6-O-acetyl-β-D-glucopyranoside] (**1**), apigenin-7-O-β-glucopyranoside (**2**), apigenin (**3**), luteolin (**4**) and chrysin (**5**)

## EXPERIMENTAL

The leaves of *Torreya grandis* plant were collected from southern area of China. The plant was taxonomically identified with the help of a botanist using taxonomic rules and a specimen has been deposited in the herbarium of school for future references.

$^1$H NMR (nuclear magnetic resonance) spectra were taken on a Bruker ARX-400 spectrometer (MHz) in DMSO-$d_6$, using tetramethyl silane (TMS), as a reference marker. The chemical shifts are given in ppm as δ values and coupling constants in Hz. The UV spectra were recorded on a UV-VIS 2550 spectrophotometer (Shimadzu). When necessary, the UV spectra ($\lambda_{max}$ in nm) were measured after the addition of different reagents. The mass spectra were measured on a LC-MSD-Trap-SL Agilent 1100. HPLC analysis was performed using HPLC DAD-230 Elite, separation was performed on a Scienhome kromasil C18, 5μ column (250 mm × 4.6 mm). The mobile phase was ACN-$H_2O$ (5-100 %, gradient, 40 min) and the flow rate was 1.0 mL/min. Column chromatography was performed on a polyamide (Merck, 100-200 μm), Sephadex LH-20 (Pharmacia) and silica gel 60 $F_{254}$ (Merk) were used for TLC.

**Extractions and isolations of the chemically pure compounds:** The air-dried and ground plant material (50 g) was extracted three times with 2 L of solvent mixture ethanol/water (8:2, v/v) for 3 h at 80 ºC. Ethanol was distilled under reduced pressure in a rotary evaporator to yield the total extract (16 g). This extract was then suspended in distilled water and partitioned sequentially with petroleum ether, chloroform, ethyl acetate and *n*-butanol.

The (5 g) extract of ethyl acetate were subjected to polyamide (mesh size 100-200 μm) column chromatography and washed with 1000 mL of 95 % (v/v) aqueous ethanol and water (both at a rate of 40 mL/min). Then, the ethyl acetate extract was eluted with 10-95 % (v/v) aq. ethanol (gradient elution) and 20 fractions were collected and were concentrated to dryness at 70 ºC under a reduced pressure. The relevant fractions (No. 2-6) (0.12 g) containing flavonoids (1, 2) were rechromatographed with sephadex LH-20 (MeOH-$H_2O$ = 8:2) to provide 14 fractions (sub fractions No. 4-7) to give compound **1** (13 mg) and (sub fractions No. 8-11) providing compound **2** (27 mg). Fractions (No. 8-10) (0.15 g) were loaded on to Sephadex LH-20 (Pharmacia) column and eluted with 250 mL of 80 % methanol provided compound **3** (65 mg). Fractions (No. 12-17) (0.09 g) were combined and further purified on semi preparative RP-HPLC (Shimdazu 4 μm, 250 × 10 mm, acetonitrile-water = 1:1), providing compound **4** (15 mg) and compound **5** (20mg). All the chemical, reagents and solvents used in this study were purchased from Sigma Chemical (St. Louis, MO, USA) unless specified.

**Identifications of flavonoids:** Fractionation of ethyl acetate extract of *Torreya grandis* aerial parts resulted in the isolation of five pure flavonoid compounds, using column chromatography and preparative HPLC. The structures of these compounds were elucidated by spectroscopic analyses, notably UV, LC-MS and NMR whose structures are presented in Fig. 1.

Compound **1** identified as apigenin-5-O-[α-L-rhamnopyranosyl-(1-4)-6-O-acetyl-β-D-glucopyranoside: EIMS, m/z 620 $[M]^+$; EI-MS m/z: 270 (aglycone, 100 %); UV, $\lambda_{max}$ (nm) (MeOH 332, 263; m.p. 188 ºC; $^1$H NMR (DMSO-$d_6$, 400 MHz): δ 7.87 (2H, d, $J$ = 8.8 Hz, H-2' and H-6'), 6.91 (2H, d, $J$ = 8.76 Hz, H-3' and H-5'), 6.60 (1H, d, $J$ = 1.96 Hz, H-8), 6.48 (1H, d, $J$ = 1.88 Hz, H-3,6). The β-D-glucopyranosyl moiety signals were found at 5.06 (H-1''), 3.57 (H-2''), 3.51

(H-3''), 3.43 (H-4''), 3.78 (H-5''), 4.02 (H-6''a) and 3.75 (H-6''b). Moreover, the α-L-rhamnopyranosyl moiety signals appeared at 4.72 (H-1'''), 5.16 (H-2'''), 5.01 (H-3'''), 3.43 (H-4'''), 3.76 (H-5''') and 1.75 (H3-6'''). By comparison of $^1$H NMR data with those given in the literature, the structure of this compound **1** was identified as 4',5,7-trihydroxy flavone-5-O-[α-L-rhamnopyranosyl-(1-4)-6-O-acetyl-β-D-glucopyranoside][10,11].

Apigenin-7-O-β-glucopyranoside, compound 2: EIMS, m/z 433 $[M]^+$; EI-MS m/z: 270 (aglycone, 100 %); UV, $\lambda_{max}$ (nm) (MeOH 336, 265; m.p. 226 ºC; $^1$H NMR (DMSO-$d_6$, 400 MHz): δ 7.83 (2H, d, *J* = 8.8 Hz, H-2' and H-6'), 6.92 (2H, d, *J* = 8.8 Hz, H-3' and H-5'), 6.83 (1H, d, *J* = 2.1 Hz, H-6), 6.71 (1H, d, *J* = 2.1 Hz, H-8), 6.5 (1H, d, *J* = 2.04 Hz, H-3,6), β-D-glucopyranosyl moiety signals were found at 5.04 (H-1''), 3.55 (H-2''), 3.50 (H-3''), 3.41 (H- 4''), 3.76 (H-5''), 4.01 (H-6''a) and 3.74 (H-6''b). Data of the compound **2** matched with the reported NMR data for apigenin-7-O-β-glucopyranoside from literature[12,13].

Compound **3** was pale yellow crystals identified as apigenin: EIMS, m/z 270 $[M]^+$; UV, $\lambda_{max}$ (nm) (MeOH 335, 295, 266; m.p. 348 ºC; $^1$H NMR (DMSO-$d_6$, 400 MHz): δ 7.94 (2H, d, *J* = 8.76 Hz, H-2' and H-6'), 6.93 (2H, d, *J* = 8.76 Hz, H-3' and H-5'), 6.48 (1H, d, *J* = 2.12 Hz, H-8), 6.19 (1H, d, *J* = 2.04 Hz, H-3,6). Data was identical with the literature[14,15].

Compound **4** was identified as chrysin: EIMS, m/z 254.2 $[M]^+$; UV, $\lambda_{max}$ (nm) (MeOH 312, 265, 286; m.p. 285 ºC. Its $^1$H NMR (DMSO-$d_6$, 400 MHz) δ ppm: 8.07 (2H, d, *J* = 6.76 Hz, H-2', H-6'); 7.61 (3H, d, *J* = 3.0 Hz, H-3', H-4', H-5'); 6.52 (1H, d, *J* = 1.4 Hz, H-3 and H-8); 6.22 (1H, d, *J* = 1.92 Hz, H-6). Its instrumental analysis data corresponded with the reference data for chrysin[16,17] and the data of an authentic sample.

Compound **5** luteolin: EIMS, m/z 286.1 $[M]^+$; UV, $\lambda_{max}$ (nm) (MeOH) 325, 290, 252; m.p. 329 ºC; $^1$H NMR (DMSO-$d_6$, 400 MHz) δ ppm: 7.39 (1H, d, *J* = 1.4 and 8.2 Hz, H-6'); 7.38 (1H, d, *J* = 1.4 Hz, H-2'); 6.87 (1H, d, *J* = 8.2 Hz, H-5'); 6.43 (1H, d, *J* = 1.2 Hz, H-8); 6.65 (1H, d, *J* = 1.2 Hz, H-3); 6.18 (1H, d, *J* = 1.2 Hz, H-6). All of these data are consistent with a 5,7,3',4'-tetrahydroxyflavone[18,19]. Thus, compound **5** was identified as luteolin and its structure was confirmed by chromatographic comparisons with an authentic marker.

**Determination of total phenolic contents:** The total phenolic contents of *T. grandis* fractions were determined using a modified Folin-Ciocalteu method[20]. A 125 µL aliquot of a known dilution of the extract was added to the test tube and combined with 0.5 mL of Folin- Ciocalteu's reagent. The tubes were vortexed for 15 s and then allowed to stand for 6 min at 20 ºC. About 1.25 mL of 7 % sodium carbonate solution was then added to the test tubes and the mixture was diluted to 3.0 mL with distilled, de-ionized water. Colour was developed after 90 min and absorbance was measured at 760 nm using the UV-VIS 2550 spectrophotometer (Shimadzu). The measurement was compared to a standard curve of prepared gallic acid solutions and expressed as gallic acid equivalents in milligrams. Triplicate determinations were performed on each sample; data shown later represent the means of three measurements.

**Determination of flavonoids:** Total flavonoid contents in extract were determined by the method described by Zhishen *et al.*[21] with minor modifications. To 0.5 mL of the extract solution, 0.5 mL of 20 mg/mL $AlCl_3$ ethanol solution was added. After 1 h at room temperature, the absorbance was measured at 420 nm using the UV-VIS 2550 spectrophotometer (Shimadzu). Total flavonoid contents were calculated as quercetin (mg/g) from a calibration curve.

**FRAP assay:** Total antioxidant activity of investigated *T. grandis* extracts by FRAP assay were carried out by the method of Benzie and Strain[22]. Prior to analysis, the extracts (1.0 mL) were transferred into 10 mL volumetric flasks and diluted with the same solvent. Diluted extracts (0.1 mL) were transferred into test tubes and 3 mL of freshly prepared FRAP-reagent (25 mL acetate buffer, 300 mmol/L, pH 3.6 + 2.5 mL 10 mmol/L TPTZ in 40 mmol/L HCl + 2.5 mL 20 mmol/L $FeCl_3 \cdot 6H_2O$) were added. The absorbance was recorded after 5 min at 593 nm against a blank containing 0.1 mL of solvent. Relative activities were calculated from the calibration curve of L-ascorbic acid standard solutions (0.1-1 mmol/L) under the same experimental conditions and expressed as ascorbic acid equivalents (AAE) per mg of dry plant material. Ascorbic acid equivalents is defined as the reducing power of a 1 mg sample that is equivalent to that of 1 nmol ascorbic acid[23]. All measurements were done in triplicate.

**DPPH radical scavenging activities:** The hydrogen atom or electron donation abilities of the corresponding extracts were measured from the bleaching of the purple-coloured methanol solution of DPPH. This spectrophotometric assay uses the stable radical, 2,2'-diphenylpicrylhydrazyl ($DPPH^{\bullet}$), as a reagent[24,25]. 100 µL of 0.2 mg/mL of the extracts and various fractions in methanol were added to 3 mL of a 0.004 % methanol solution of DPPH. After a 0.5 h incubation period at room temperature, the absorbance as read against a blank at 517 nm in a spectrophotometer (UV 2550 spectrophotometer, Shimadzu). Inhibition of free radical DPPH in percent (% Inhibition) was calculated in following way:

$$\text{Inhibition (\%)} = \frac{(A_{blank} - A_{sample})}{A_{blank}} \times 100$$

where $A_{blank}$ is the absorbance of the control reaction (containing all reagents except the test sample) and $A_{sample}$ is the absorbance of the test sample. The values of inhibition were calculated for the various concentrations of *T. grandis* extract. Tests were carried out in triplicate.

**Reducing power activity:** The reducing power of *T. grandis* extracts was determined according to the method of Oyaizu[26] with some minor modification. The five concentrations of *Torreya grandis* (0.2, 0.4, 0.6, 0.8 and 1.0 mg/mL) in 1.0 mL of distilled water were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide $[K_3Fe(CN)_6]$ (2.5 mL, 1 %). The mixture was incubated at 50 ºC for 20 min. A portion (2.5 mL) of trichloroacetic acid (10 %) was added to the mixture, which was then centrifuged for 10 min at 1000 g (Biofuge 22 R Heraeus, Germany). The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%) and the absorbance was measured at 700 nm in a spectrophotometer (UV 2550

spectrophotometer, Shimadzu). Higher absorbance of the reaction mixture indicated greater reducing power.

**Trolox equivalent antioxidant capacity method:** A common method described by Miller and Rice-Evans[27] was used for testing the antioxidative potential of antioxidants as hydrogen-donating agents is to measure their ability to scavenge $ABTS^{\bullet+}$. This method is reliant on the generation of a long-lived specific $ABTS^{\bullet+}$ chromophore and its quenching (or suppression) by an antioxidant. Trolox (6-hydroxy-2,5,7,8-trimethyl-chroman-2-carboxylic acid), a water-soluble vitamin E analogue is taken as a standard. Thus, Trolox equivalent antioxidant capability (TEAC), which is defined as the concentration of the Trolox with the same antioxidant activity as a 1 mM concentration of the substance under investigation, is taken as an "index" to evaluate antioxidative activity of an antioxidant. Briefly, 2,2-azino-*bis*(3-ethyl-benzothiazoline-6-sulfonic acid) diamonium salt ($ABTS^{\bullet+}$) radical cation was generated by the interaction of ABTS (250 μM) and $K_2S_2O_8$ (40 μM). After addition of 1.0 mL of the $ABTS^{\bullet+}$ solution was added to 100 μL of standard or sample and vortexed for 10 s. The decolourization caused by reduction of the cation by antioxidants from the sample was measured by UV-visible recording spectrophotometer (Shimadzu UV2550,) exactly 3 and 6 min after the initial mixing. The percentage decrease of the absorbance at 734 nm was calculated and plotted as a function of the concentration of the test samples and of Trolox for the standard reference data[28].

**Statistical analysis:** All experiments were conducted with three independent replicates. The data are expressed in terms of mean and standard deviation. The experimental data were analyzed using Microsoft Excel software (Microsoft Software Inc.).

## RESULTS AND DISCUSSION

**Yield of extract, total polyphenol, flavonoids and FRAP value:** Several investigations have indicated that the phenolic compounds in herbal medicines can scavenge free radicals[29]. The total polyphenol, flavonoids contents in water and 80 % ethanol extract were measured (Table-1). The 80 % ethanol produced the highest yield of extract (345 mg/g), polyphenols (49.50 mg/g), flavonoids (32.50 mg/g), than the water extract 200, 16.0 and 6 mg/ dry *T. grandis* plant, respectively. Similarly the FRAP value of 80 % ethanol extract was 155 AAE/mg and for water extract it was 98 AAE/mg dry plant material. FRAP assay treats the antioxidants in the samples as reductants in a redox-linked colorimetric reaction and FRAP value increases proportionally to the polyphenol content and flavonoids[30].

**$DPPH^{\bullet}$ scavenging activity:** The antioxidant activities of extracts and purified compounds were tested by utilizing the free radical scavenging activity of ($DPPH^{\bullet}$). The DPPH radical is a stable organic free radical with an absorption wavelength in the range of 515-528 nm. The radical loses this absorption capacity when accepting an electron or a free radical species, resulting in a visually noticeable discolouration from purple to yellow. Because the DPPH radical assay can accommodate many samples in a short period and is sensitive enough to detect active ingredients at low concentrations, it has been extensively used to screen antiradical activities of extracts and flavonoids[31]. Fig. 2 shows the $DPPH^{\bullet}$ scavenging activity of extracts of *T. grandis*. We found that the 80 % ethanol extract (EE 80%) showed the greatest antiradical activity than α-tocopherol and water extract. Notably, the scavenging activity of the 80 % ethanol extract of the *T. grandis* was better than that of α-tocopherol. The antioxidant activity of many plants extracts rises with the rising polyphenol content of the extract[32]. This study indicating that increasing the polyphenol content strengthens the antioxidant activity. This finding is similar to that reported by Spiteller *et al.*[33].

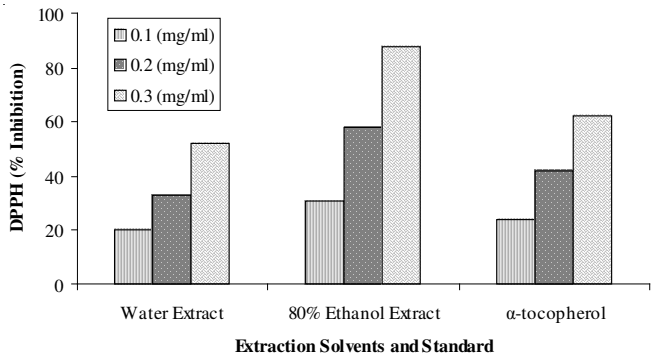


Fig. 2. Free radical scavenging activity of extracts and α-tocopherol

**Reducing power ability:** The antioxidant activity of extracts and purified compounds was also measured by reducing power ability and it is considered to be related to its reductive activity. Thus, to assess the electron-donating properties of the extracts, their ability to reduce iron (III) was assessed[34].

Fig. 3 shows the reducing ability of *T. grandis* extract. The results of this assay show the same trend as in DPPH assay. The reducing ability was found to decline in the order of EE 80 % > α-tocopherol > water extract. Again, the reducing ability increased with increased concentration of samples. Thus, *T. grandis* are good electron donors and may terminate the radical chain reaction by converting free radicals to more stable products. Yu *et al.*[35] noted that the antioxidant property is concomitant with the development of reducing ability. The findings suggested that both ethanol and water extract exhibited remarkable reducing abilities, which may be attributed to reductones present in *T. grandis* plant and reacted with free radicals to stabilize and terminate radical chain reactions.

TABLE-1
EFFECTS OF EXTRACTION SOLVENTS ON EXTRACT YIELD, TOTAL PHENOLS, FLAVONOIDS AND FRAP VALUE OF *T. grandis* EXTRACT

| Solvents | Yield of extract (mg/g) | Total phenols (mg GAE/g dry wt of plant) | Total flavonoids (mg QE/g dry wt of plant) | FRAP values (AAE/mg plant material |
|---|---|---|---|---|
| Water | 200 ± 2.8 | 16.0 ± 0.8 | 6.0 ± 0.4 | 98 |
| Ethanol 80 % | 345 ± 3.5 | 49.30 ± 1.7 | 32.5 ± 1.1 | 155 |

Data are presented as means ± standard deviation (n=3); GAE: Gallic acid equivalents; QE: Quercetin equivalents; AAE: Ascorbic acid equivalents

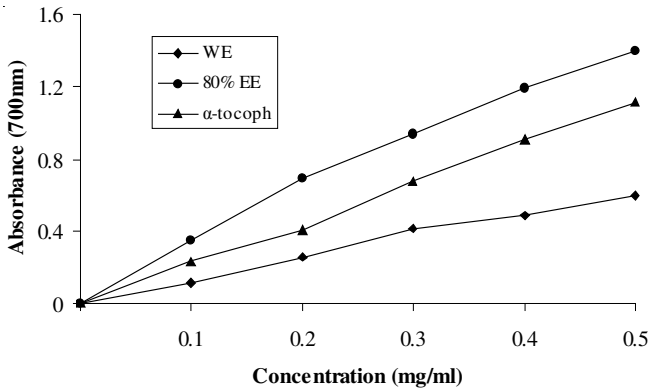


Fig. 3. Reducing power activity of extracts and α-tocopherol

**TEAC assay:** The scavenging potential of isolated pure compounds as assayed by TEAC method and ranged from 2.21-1.04 mM at 6min. The TEAC values were found to decline in the order luteolin > apigenin > apigenin-7-O-β-glucopyranoside > chrysin > 4',5,7-trihydroxy flavone-5-O-[α-L-rhamnopyranosyl-(1-4)-6-O-acetyl-β-D-glucopyranoside].

TABLE-2
TEAC VALUES OF ISOLATED COMPOUNDS AND STANDARD

| Compounds | m.f. | m.w. | TEAC (mM) (3 min) | TEAC (mM) (6 min) |
|---|---|---|---|---|
| 1 | $C_{29}H_{32}O_{15}$ | 620 | 0.98 ± 0.10 | 1.04 ± 0.11 |
| 2 | $C_{21}H_{21}O_{10}$ | 433 | 1.02 ± 0.11 | 1.08 ± 0.12 |
| 3 | $C_{15}H_{10}O_5$ | 270 | 1.19 ± 0.12 | 1.42 ± 0.13 |
| 4 | $C_{15}H_{10}O_4$ | 254 | 1.00 ± 0.10 | 1.06 ± 0.11 |
| 5 | $C_{15}H_{10}O_6$ | 286 | 2.01 ± 0.13 | 2.21 ± 0.13 |
| Quercetin (St) | $C_{15}H_{10}O_7$ | 302 | 3.18 ± 0.14 | 3.70 ± 0.15 |

Data are presented as means ± standard deviation (n = 3).

The antioxidant activities of flavonoids are largely determined by the number of hydroxyl groups on the aromatic ring. The higher the number of hydroxyl groups, the greater the expected antioxidant activity[36]. The presence of four hydroxyl group in luteolin (**4**) significantly boosts the radical-scavenging activity 2.21 ± 0.13 mM than apigenin (1.42 ± 0.13 mM) and chrysin (1.06 ± 0.11 mM), while the flavonoids glycosides exhibited low antioxidant activities. The weaker radical-scavenging activities of the flavonoid glycosides were apparently due to the inclusion of non-participating structures, such as sugars and the weakly active phloroglucinol A-ring in their molecules. When compared to standard quercetin (3.70 ± 0.15 mM) these compounds showed less radical-scavenging activities, probably because, of the three criteria for radical-scavenging capacity suggested by Pietta[37]. First, presence of a catechol group in the B ring, has better electron-donating properties. Second, the 2,3-double bond conjugated with the 4-oxo group, which is responsible for electron delocalization. Third, it could be due to the presence of a 3-hydroxyl group in the heterocyclic ring. These finding are also in accordance with the results reported[38-40].

## Conclusion

Fractionation of ethyl acetate extract of *Torreya grandis* aerial parts revealed the presence of flavonoids like, 5,7,4'-trihydroxyflavone-5-O-[α-L-Rhamnopyranosyl-(1-4)-6-O-acetyl-β-D-Glucopyranoside] (**1**) apigenin 7-O-β-glucopyranoside (**2**), apigenin (**3**), luteolin (**4**) and chrysin (**5**). The structures of these compounds were elucidated by spectroscopic analysis, *viz.*, UV, LC-MS and NMR. The antioxidant activities of water and 80 % ethanol extracts were measured by FRAP assay, 2,2'-diphenylpicrylhydrazyl (DPPH$^{\bullet}$) and reducing power activity methods, while the antioxidant activities of isolated compounds were estimated by TEAC assay. The 80 % ethanol extract and isolated compound luteolin showed strong antioxidant activities. In the present study, we demonstrate that *T. grandis* contain phenolic compounds which can serve as natural sources to develop free radical scavengers. Natural antioxidants may responsible for the protective effects against the risk of many physiological and pathological processes. Therefore, it is suggested that next work should focus on further isolation and identification of more radical scavenging components using bio-organic chemical methods to study these active extracts.

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