Isolation and Identification of Flavonoids Components from *Trichilia emetica* Whole Seeds

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- *Trichilia emetica*
- Naringenin
- Elephantorrhizol

**Abstract**

Five known flavonoids were isolated from the ethyl acetate soluble fraction of aqueous extract of *T. emetica* whole seeds. On the basis of 1D and 2D-NMR experiments and MS data analyses, these compounds were identified as naringenin (B), taxifolin 4′-0-D-glucopyranoside (C), elephantorrhizol (D), catechin 3-0-P-D-glucopyranoside (E) and eriodictyol 3-0-P-D-glucopyranoside (F). DPPH radical scavenging activity was used to estimate the antioxidant capacity of each of these compounds. The result shows that elephantorrhizol has stronger DPPH scavenging activity than other isolated flavonoids.

1. Introduction

The genus *Trichilia* belongs to the Meliaceae (Mahogany family), it consist of 260 species that are widely distributed in America, Middle East and Africa. In Africa, about 20 species has been identified [1], and only two of these species, *Trichilia emetica* and *Trichilia drageana*, produce seeds with high oil contents [1, 2]. Many *Trichilia* species are grown for their ornamental qualities, and derived plants products for traditional cosmetic formulations and other medicinal values [2]. *T. emetica* are evergreen tree reaching 20-35 m in height [3]. The seed leaves, root and stem bark of this plant has been used in African folk medicine for the treatment of pneumonia, jaundice, malaria, asthma, fracture and as poison antidote. The seeds of this plant together with other indigenous plants have been used to produce multivitamin juice to control malnutrition, a major challenge faced by children and mothers in rural areas in Africa [4, 5]. *T. emetica* seeds, leaves, root and stem bark have emetic properties, and the ether extract of the seeds of this plant is also very bitter. Thus for the oil to be used for edible purposes, the whole nuts are boiled in water for 20 minutes, dried in the sun and then milled for oil extraction upon which, a yellow edible oil is obtained [2, 6].

In our continuous studies of the boiled aqueous extract of the whole seeds, resulted in the isolation of five known compounds. To the best of our knowledge, this study reports for the first time the isolation of these compounds in *T. emetica*.

2. Experimental Methods

1H and 13C NMR spectra were recorded on a Bruker spectrometer at 400.13 and 100.62 MHz using tetramethylsilane (TMS) as an internal standard. Thermo Instruments HPLC system mass spectrometer with electron spray ionization (ESI) source was used for recording of the mass spectra. Flash chromatography (Reveleris™ Flash Chromatographic System) fitted with Reveleris® SRC column with silica flash cartridges of 18 g (Reveleris™ SRC Cartridges); detection, ELSD (Evaporation Light Scattering Detection) and photodiode array detector (254-280 nm); mobile phase, linear gradient of methanol/water (containing 0.1% formic acid) at a flow rate of 18 mL/min was used for the isolation of the compound. Thin layer chromatography (TLC) and preparative thin layer chromatography (PTLC) was performed on (silica gel 60 F254, 25 Glass plates 20 × 20 cm, E. Merck, Germany). Visualization of the compound was done using UV lamp UVL-14 EL, hand held 220 V 50 Hz 4 W 254 nm white light by UVP.

2.1 Collection of Plant Materials

*T. emetica* seeds was collected from Kumasi, Ghana, in February 2014 and identified by botanist Mr. Martin A. Arkoh of Kwame Nkruma University of Science and Technology, Kumasi, Ghana. A voucher specimen TBG-2014-1 was deposited at the herbarium of Treborth Botanical Garden Bangor, UK.

2.2 Extraction and Isolation

*T. emetica* whole seeds (1.0 kg) was boiled in water three times under reflux for 20 minutes at 100 °C each, the filtrates were combined and filtered. After the solvent was removed under vacuum, the concentrate was suspended in water, acidified with 2 M HCl to pH 2 and successively partition with CHCl3 and EtOAc respectively. The EtOAc extract (20 g) was subjected to silica gel column chromatography eluted with 100% hexane, chloroform and methanol gradient elution mixtures (1:0:0 to 0:1:0) to obtain 6 fractions, after it was pooled based on their TLC profiles. Fraction 3 (4.74 g) was subjected to silica gel column chromatography eluted with gradient hexane-methanol (100:1:10:1) and finally Prep-TLC hexane-methanol (95:5) to afford compound C (19.93 mg) and E (14.18 mg). Fraction 4 (4.22 g) was subjected to repeated silica gel column chromatography eluted with gradient hexane-methanol (100:1-10:1) and finally Prep-TLC hexane-methanol (95:5) to afford compound D (19.93 mg) and E (14.18 mg). The HPLC chromatogram of EtOAc extract of decocted *T. emetica* whole seeds is shown in Fig. 1. Also, the structure of the isolated compounds are shown in Fig. 2.

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2.3 Radical Scavenging Activity

The study of the stable DPPH free radical scavenging activity of the isolates was performed according to the method described by Brahmachari et al. [7]. Each test isolate sample (0.2 mL) of various concentrations (0.20-2.00 µg/mL in methanol) was added to 3.8 mL of freshly prepared DPPH solution (40 µg/mL in MeOH). Synthetic butylated hydroxytoluene (BHT) was used as a standard for the investigation of the antiradical activity and was prepared in a similar manner. The mixtures were vortexed for 20 s. The blank sample consisted of 0.2 mL of methanol added to 3.8 mL DPPH. The mixtures were then incubated in the dark at room temperature for 1 hour and the absorbance measured at 517 nm. The inhibition percentage of radical scavenging activity was calculated as follows:

\[
\text{Percentage inhibition} = \left( 1 - \frac{A_d}{A_o} \right) \times 100
\]

where, \(A_d\) is the absorbance of test isolate at different concentrations and \(A_o\) is the absorbance of the blank.

3. Results and Discussion

Naringenin (B): ESI-HRMS at m/z 273.0759 [M + H]+. \(^1\)H-NMR (400 MHz, CDOD) \(\delta\) 3.43 (H-2, d, J=8.04 Hz), 3.52 (H-3, d, J=8.04 Hz), 3.91 (H-4, d, J=1.65 Hz), 4.02 (H-5, d, J=7.69 Hz), 4.20 (H-6, d, J=7.69 Hz), 6.76 (H-7, d, J=8.04 Hz). \(^13\)C-NMR data (Table 1) of this compound are consistent with the reported literature values [8].

Taxifolin 4’-O-β-D-glucopyranoside (C): EIMS, m/z 466 [M]+. \(^1\)H-NMR (400 MHz, CDOD) \(\delta\) 3.30 (H-2, d, J=1.65 Hz), 3.39 (H-3, d, J=1.65 Hz), 3.46 (H-4, d, J=1.65 Hz), 4.02 (H-5, d, J=1.65 Hz), 4.19 (H-6, d, J=1.65 Hz), 4.23 (H-7, d, J=1.65 Hz). \(^13\)C-NMR data (Table 1) of this compound are consistent with the reported literature values [8].

Elephantorrhizol (D): EIMS, m/z 522 [M]+. \(^1\)H-NMR (400 MHz, CDOD) \(\delta\) 4.55 (H-2, d, J=7.55 Hz), 3.94 (H-3, d, J=6.25 Hz), 3.97 (H-4, d, J=6.25 Hz), 3.83 (H-5, d, J=5.93 Hz), 4.28 (H-6, d, J=5.93 Hz), 6.97 (H-7, d, J=7.55 Hz). \(^13\)C-NMR data (Table 1) of this compound are consistent with the reported literature values [8].

(+)-Catechin 3-O-β-D-glucopyranoside (E): EIMS, m/z 452 [M]+. \(^1\)H-NMR (400 MHz, CDOD) \(\delta\) 4.95 (H-2, d, 5.85 Hz), 4.25 (H-3, m), 2.55 (H-4a, dd, J=8.30, 16.24 Hz), 2.87 (H-4e, dd, J=5.25, 16.24 Hz), 5.95 (H-5, dd, J=1.90 Hz), 5.91 (H-6, d, J=1.90 Hz), 6.84 (H-7, d, J=1.65 Hz), 6.76 (H-8, d, J=8.15 Hz), 6.71 (H-9, d, J=8.15 Hz), 6.71 (H-10, d, J=8.15 Hz), 6.71 (H-11, d, J=8.15 Hz), 3.33 (H-2’, d, J=8.15 Hz), 3.35 (H-3’, d, J=8.15 Hz), 3.25 (H-4’, d, J=8.15 Hz), 3.18 (H-5’, m, J=8.15 Hz), 3.65 (H-6’, d, J=3.24 Hz, 11.76 Hz) and 3.85 (H-7’, d, J=1.90 Hz). The \(^1\)H and \(^13\)C-NMR data (Table 1) of this compound are consistent with the reported literature values [11].

Eriodictyol 3-O-β-D-glucopyranoside (F): EIMS, m/z 449 [M + H]+. \(^1\)H-NMR (400 MHz, CDOD) \(\delta\) 5.78 (H-1, d, J=7.58 Hz), 3.94 (H-2, d, J=8.50 Hz), 6.83 (H-7’, m, J=8.39 Hz), 3.50 (H-3’, m, J=3.94 Hz, m, J=6.83 Hz, dd, J=1.95, 12.01 Hz). The \(^1\)H and \(^13\)C-NMR data (Table 1) of this compound are consistent with the reported literature values [12].

### Table 1

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<th>Compound</th>
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<th>C</th>
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All the compounds tested were found to have a pronounced scavenging activity (Table 2). Compound D has the highest activity than the others. This is primarily due to the high number and position of hydroxyl substitutions in its flavonoids structure, and this in turn has made it to have a stronger scavenging activity against DPPH radical. In contrast, compound B and F, containing less hydroxyl groups, performed less well in the DPPH assay [13]. In comparison with standard BHT, the scavenging activity exhibited by compounds D and E was significant.

4. Conclusion

Phytochemical screening of *T. emetica* whole seeds was carried out. Five known flavonoids were obtained and identified as naringenin (B), taxifolin 4’-O-β-D-glucopyranoside (C), elephantorrhizol (D), catechin 3-O-β-D-glucopyranoside (E) and eriodictyol 3-O-β-D-glucopyranoside (F) respectively. The use of high-performance liquid chromatographic system in place of silica gel column chromatography can lead to the isolation of more phytochemicals from this plant sample. These compounds when subjected to antioxidative assay, they show moderate to very high antioxidative activity.

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### References


