



### **Isolation of Catechin from *Trichilla Emetica* Whole Seeds**

<sup>1,2\*</sup>A. Usman, <sup>1</sup>V. Thoss and <sup>3</sup>A.U. Itodo

<sup>1</sup>School of Chemistry, Bangor University, LL57 2UW, UK

<sup>2</sup>Basic Science Unit, Faculty of Agriculture, Nasarawa State University Keffi, PMB 135 Lafia.

<sup>3</sup>Department of Chemistry, University of Agriculture Makurdi, Benue State.

\*Corresponding author: [ausman2015@yahoo.com](mailto:ausman2015@yahoo.com)

Received: March 2016

Accepted: May 2016

---

#### **Abstract**

*The purification of boiled water extract of T. emetica seeds resulted in the isolation of catechin, a known flavonoid. The structure was established on the basis of spectroscopic methods 1D (<sup>1</sup>H and <sup>13</sup>C) NMR and 2D (COSY, HSQC and HMBC) NMR in addition to the mass spectrometry and comparison with literature data. The isolation of this compound is been reported for the first time in T. emetica.*

**Keywords:** *Trichilia emetic; chromatography; traditional medicine; spectroscopy; catechin.*

---

#### **1.0 INTRODUCTION**

*T. emetica* (Vahl) (family Mahogany) is an evergreen tree reaching 35 metres in height and are widely distributed in sub-Saharan Africa. They are propagated by cuttings and regenerate naturally by root suckers, and seeds (Orwa *et al.*, 2009). This plant has long history of use in African traditional medicine, and are used alone, mixed or in combination with other plant parts in treating different diseases. The seed oil is rubbed into cuts made on a fractured limb in order to hasten healing, and it is also taken internally to treat rheumatism (Grundy and Campbell, 1993). The seed oil is used in combination with *Cyathula natalensis* Sond to treat leprosy (Cunningham, 1996).

The oil is sometimes combined with coconut oil to provide an emollient and moisturizing effect on the skin (Vermaak *et al.*, 2011). In South Africa, the decoction of the root and bark is used as an emetic, purgative and also as a remedy for colds, pneumonia, and intestinal disorders such as hepatitis. In Mali, the powdered root is given to treat river blindness and ascaris, while its decoction is supposed to cure jaundice, infertility, induce labour in women, and to relief stomach and chest pain (Van der Vossen and Mkamilo, 2007). This paper describes the isolation and structure elucidation of catechin from *T. emetica* whole seed.

## 2.0 MATERIALS AND METHODS

**2.1. NMR spectra:**  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded on a Bruker Avance (400 MHz) spectrometer with internal references of  $\delta_{\text{H}}$  3.31 and  $\delta_{\text{C}}$  49.0 ppm for  $\text{CD}_3\text{OD}$  using TMS (Trimethylsilane) as an internal standard.

**2.2. Mass and Ultraviolet/Visible (UV) spectra:** Thermo Instruments HPLC system mass spectrometer with an electrospray ionization (ESI) source was used for recording of the mass and UV spectra.

**2.3. UV Lamp:** UV lamp UVL-14 EL hand held 220V 50Hz 4W 254nm white light by UVP.

**2.4. Thin layer chromatography (TLC):** TLC and preparative thin layer chromatography (PTLC) was performed on (silica gel 60 F<sub>254</sub>, 25 Glass plates 20 × 20 cm, E. Merck, Germany)

**2.5. Column chromatography (CC):** CC was performed using Fluorochem silica gel 60A.

**2.6. Collection of plant seeds:** The seeds of *T. emetica* was collected and identified from Kumasi- Ghana by a botanist Mr Martin Arkoh of Kwame Nkrumah University of Science and Technology Kumasi. The voucher specimen TBG-2014-1 was deposited at the herbarium of Treborth botanical garden, Bangor, United Kingdom.

## 3. PREPARATION OF THE MATERIALS

**3.1. Extraction.** *T. emetica* whole seeds (1.0 kg) were boiled in water under reflux for 20

minutes and filtered. The filtrate was rotary evaporated at 40°C to give 72.14 g dark viscous residue. The residue was used as the crude extract for the experiment.

### 3.2. Purification.

The crude extract (20 g) was subjected to column chromatography. A clean column (450 mm × 95 mm) was packed with 300g of silica gel 60A, using the wet packing method in hexane. The sample was prepared by adsorbing 20 g crude extract onto silica gel, dried down in vacuum and subsequently transferred to the top of the adsorbent layer in the column. The column was run using hexane, chloroform and methanol using gradient elution techniques (Stahl, 1969). Ninety six (96) fractions were collected, and were pooled based on their TLC profiles. All the combine fractions were dried down under vacuum at 40 °C. Fractions 31 to 34 (458 mg) was further subjected to column chromatography and finally, fractions 14 and 15 (57 mg) containing catechin substrate was obtained and purified using preparative thin layer chromatography. The plate was developed using Hexane – EtOAc – MeOH (5:4:1, v/v). The main band with reddish-brown colour visualized under UV lamp was scrapped from the plate and eluted with the same developing solvents yielding 3,3',4',5,7-pentahydroxyflavan (22 mg).

## 4.0 RESULTS AND DISCUSSION

Catechin (22 mg) was obtained as a yellow needle.  $[\alpha]_D^{24.7} +15.7$  (MeOH: c 0.5) and is comparable to +15.5 (Seto et al., 1997). It has a molecular formula of  $\text{C}_{15}\text{H}_{15}\text{O}_6$  which was established on the basis of ESI-HRMS at  $m/z$  291.0863[M + H]<sup>+</sup>(Calcd for 291.0866) (Fig.

1). The mass at  $m/z$  291 corresponded to the protonated molecule [M + H]<sup>+</sup> of catechin whose exact mass is 290.0790. The spectrum also shows the main fragment ions at  $m/z$  139 [M – C<sub>8</sub>H<sub>7</sub>O<sub>3</sub>]<sup>+</sup> which is due to retro Diels Alder reaction (Shen et al., 2006; Wu et al., 2003).

au1 #313-328 RT: 5.35-5.56 AV: 5 NL: 1.00E6  
 F: FTMS + p ESI Full ms [100.00-2000.00]

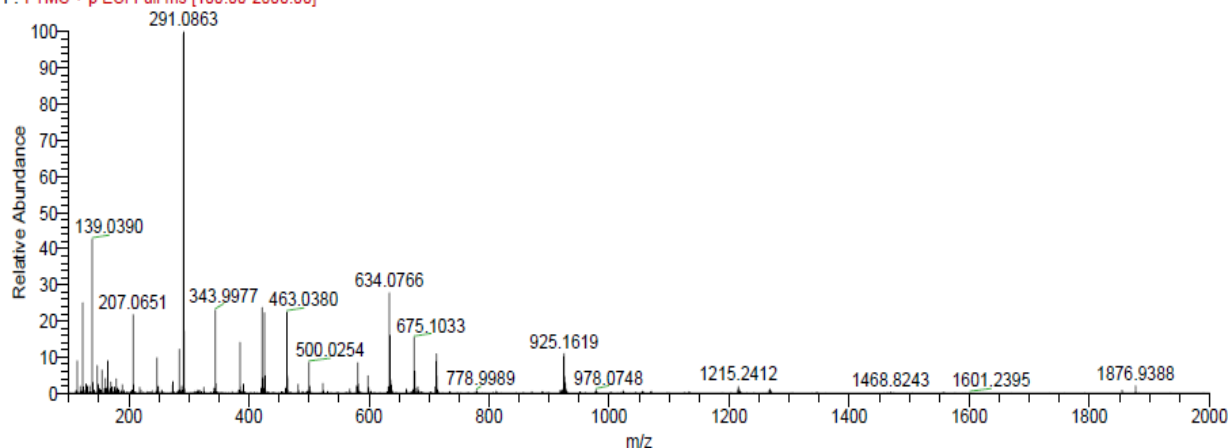


Figure 1. Mass spectrum of catechin.

The compound showed absorption band at 230 and 279 nm in the ultraviolet spectrum (Fig. 2). The IR spectrum also showed a band at 3306 (broad, O-H stretching), 2930 (saturated C-H stretching), 1608 (C=C stretching), 1519

(C-H deformation), 1141, 1032 (C-O stretching) and 819 (C-H out of plane deformation)  $\text{cm}^{-1}$ . These values are comparable to that reported in the literature (Hye *et al.*, 2009).

au1 #151-154 RT: 5.00-5.10 AV: 4 NL: 3.31E5 microAU

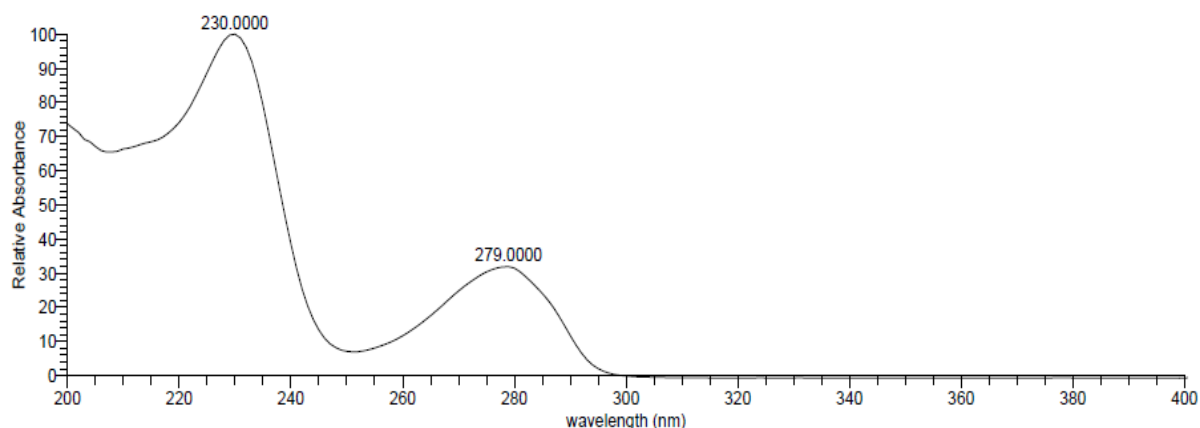


Figure 2. UV spectrum of catechin.

The DEPT spectrum (Fig. 3) showed fifteen carbon signals, which consist of one methylene, seven methine and seven quaternary carbons. The  $^1\text{H}$ NMR spectrum (Fig. 4), showed proton H-3 on ring C to be split by H-2 and H-4<sub>a&b</sub>, resulting in a multiplet at  $\delta_{\text{H}}$  3.96. The Protons at H-4<sub>a&b</sub> was split by H-3 resulting in a doublet of doublet at  $\delta_{\text{H}}$  2.49 (dd, 8.3, 16.2 Hz) and  $\delta_{\text{H}}$  2.84 (dd, 5.8, 16.2 Hz), and H-2 was split by H-3 resulting in a doublet at  $\delta_{\text{H}}$  4.57 (d, 7.5 Hz). The characteristic signals due to B-ring aromatic

protons is of the ABX- type and it composed of  $\delta_{\text{H}}$  6.85 (d, 2.2 Hz) assigned to position 2', and it meta-coupled with  $\delta_{\text{H}}$  6.72 (dd, 2.2, 8.2 Hz) assigned to position 6', which also undergo ortho coupling with  $\delta_{\text{H}}$  6.77 (d, 8.2 Hz) assigned to position 5'. Similarly, the meta-coupled aromatic proton signals at  $\delta_{\text{H}}$  5.87 (d, 2.3 Hz) and  $\delta_{\text{H}}$  5.94 (d, 2.3 Hz) were assigned to position H-6 and H-8 and it corresponded to the A-ring (Fig. 5).

Table 1.  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of catechin in deuterated methanol ( $\text{CD}_3\text{OD}$ ).

Position	$\delta_{\text{H}}$	$\delta_{\text{C}}$
2	4.57(1H, d, J=7.5 Hz)	82.9
3	3.96(1H, m)	68.8
4a	2.84(2H, dd, J=5.8, 16.2 Hz)	28.5
4b	2.49(2H, dd, J=8.3, 16.2 Hz)	28.5
5		156.9
6	5.87(1H, d, J=2.3 Hz)	96.2
7		157.9
8	5.94(1H, d, J=2.3 Hz)	95.5
9		157.6
10		100.8
1'		132.2
2'	6.85(1H, J=2.0 Hz)	115.2
3'		146.2
4'		146.3
5'	6.77(1H, d, J=8.2 Hz)	116.1
6'	6.72(1H, J=2.0, 8.2 Hz)	120.0

The signals of the  $^{13}\text{C}$ -NMR spectrum appeared at 82.9 (C-2), 68.8 (C-3), 28.5 (C-4), 156.9 (C-5), 96.2 (C-6), 157.9 (C-7), 95.5 (C-8), 157.6 (C-9), 100.8 (C-10), 132.2 (C-1'), 115.2 (C-2'), 146.2 (C-3'), 146.3 (C-4'), 116.1 (C-5') and 120.0 (C-6') (Table 1). The down field position of the C-2 chemical shifts ( $\delta_{\text{C}}$  82.9) and the appearance of a doublet at 4.57 (d, 7.5 Hz) attributed to position 2, indicated that the flavan moieties possessed the 2,3-trans stereochemistry (Foo and Karchesy, 1989; Ishimaru *et al.*, 1987; Takahama *et al.*, 2013).

Catechin are known flavonoids that has been isolated from the leaves and wood of *Acacia catechu* (Hye *et al.*, 2009; Shen *et al.*, 2006), bark of *Quercus marilandica* Muenchh (Bae *et al.*, 1994) and the leaves of green tea (Dalluge and Nelson, 2000; Si *et al.*, 2006). This compound has been reported to showed various pharmacological activities, such as antioxidant (Gadow *et al.*, 1997), anti-inflammatory (Monga *et al.*, 2014) and antimicrobial (Kumar *et al.*, 2015).

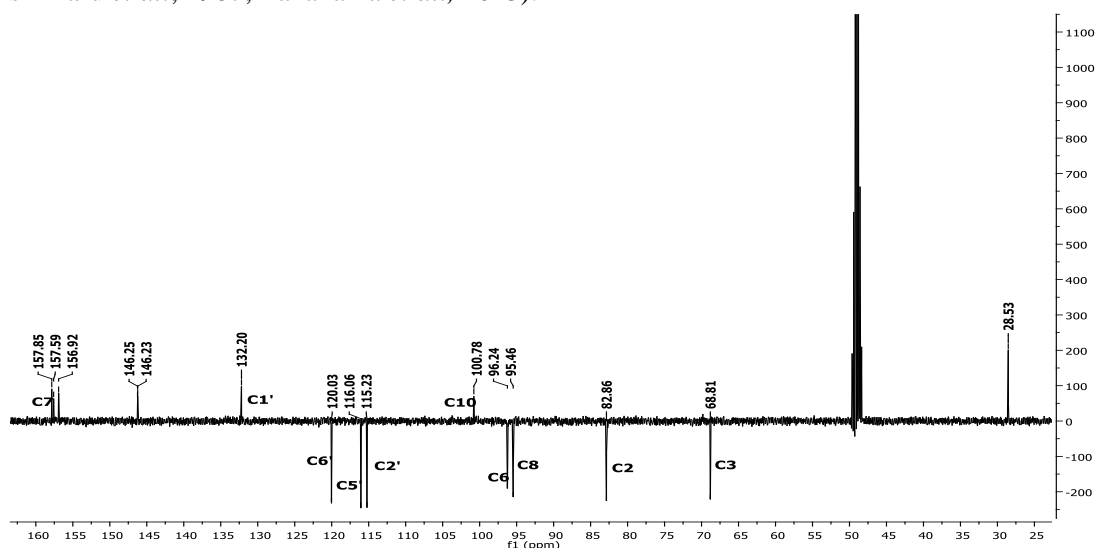


Figure 3. DEPT spectrum of catechin.

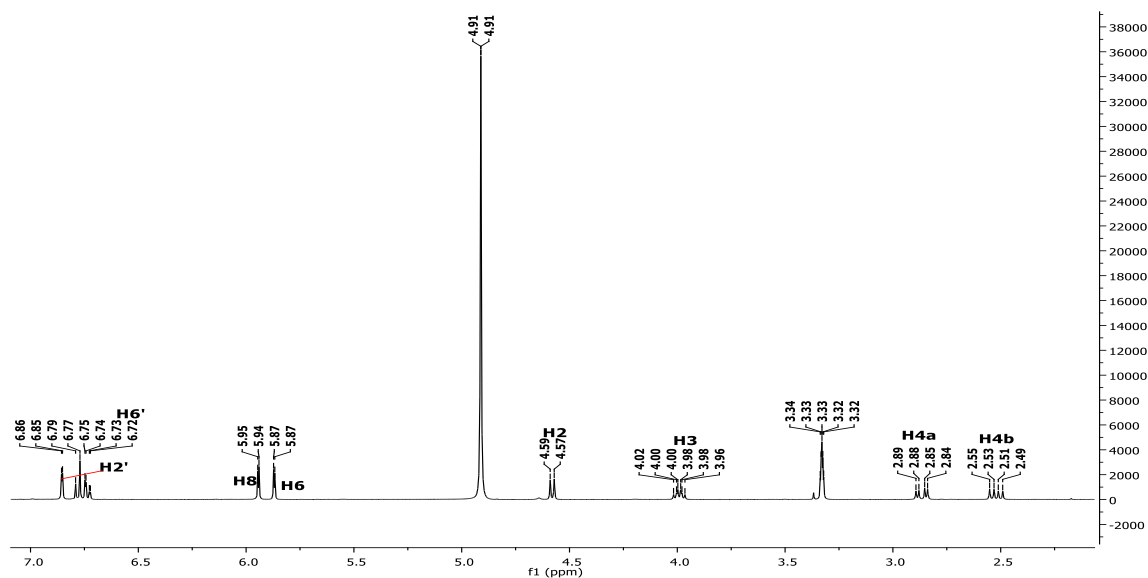
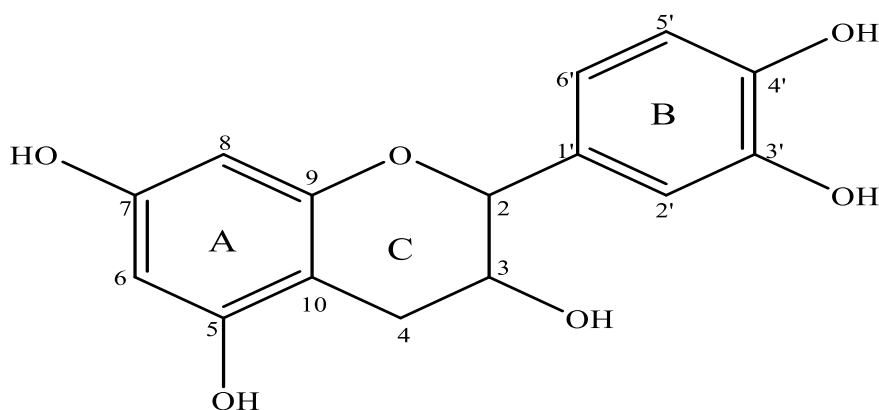
Figure 4.  $^1\text{H}$  NMR spectrum of compound AU 4.

Figure 5. The structure of catechin.

## 5.0 CONCLUSION

This is the first report of the isolation and identification of catechin from *T. emetica*. The work was carried out by using different chromatographic separation and spectroscopic techniques. The isolation of catechin from this plant seems to support some folkloric use of

## 7.0 REFERENCES

Bae, Y.S., Burger, J.F.W., Steynberg, J.P., Ferreira, D., Hemingway, R.W., (1994). Flavan and procyanidin glycosides from the bark of blackjack oak. *Phytochemistry* 35, 473–478.

the plant in African traditional medicine.

## 6.0 ACKNOWLEDGEMENT

A.U. would like to thank Nigerian Tertiary Education Trust Fund (TETFUND) for the sponsorship.

Cunningham, A.B.H.A.S.A.H.L.G., (1996). *Zulu Medicinal Plants*. University of Natal Press, Pietermaritzburg., KwaZulu-Natal.  
Dalluge, J.J., Nelson, B.C., (2000). Determination of tea catechins. *Journal of Chromatography*, 881, 411–424.

- Foo, L.Y., Karchesy, J.J., (1989). Polyphenolic glycosides from *Douglas fir* inner bark. *Phytochemistry* 28, 1237–1240.
- Gadow, A.V., Joubert, E., Hansmann, C.F., (1997). Comparison of the antioxidant activity of rooibos tea (*Aspalathus linearis*) with green, oolong and black tea. *Food Chem.* 60, 73–77.
- Grundy, I.M., Campbell, B.M., (1993). Potential production and utilisation of oil from *Trichilia SPP.* (Meliaceae). *Econ. Bot.* 47, 148–153.
- Hye, M.A., Taher, M.A., Ali, M.Y., Ali, M.U., Zaman, S., (2009). Isolation of (+)-Catechin from *Acacia catechu* (Cutch tree) by a convenient method. *Journal of Scientific Research* 2, 300–305.
- Ishimaru, K., Nonaka, G., Nishioka, I., (1987). Flavan-3-ol and procyanidin glycosides from *Quercus miyagii*. *Phytochemistry* 26, 1167–1170.
- Jang, K., Shin, D.-S., (2015). Antimicrobial and docking studies of (–)-catechin derivatives. *J. Korean Soc. Appl. Biol. Chem.* 58, 581–585.
- Kumar, D., Poornima, M., Kushwaha, R.N., Won, T.-J., Ahn, C., Ganesh Kumar, C., Seto, R., Nakamura, H., Nanjo, F., Hara, Y., (1997). Preparation of epimers of tea catechins by heat treatment. *Biosci. Biotech. Biochem.* 9, 1434–1439.
- Monga, J., Aggarwal, V., Suthar, S.K., Monika, M., Nongalleima, K., Sharma, M., (2014). Topical (+)-catechin emulsified gel prevents DMBA/TPA-induced squamous cell carcinoma of the skin by modulating antioxidants and inflammatory biomarkers in BALB/c mice. *Food Funct.* 5, 3197–3207.
- Orwa, C., Mutua, A., Kindt, R., J., R., Simons, A., (2009). *Trichilia emetica*. Agroforestry database: a tree reference and selection guide version 4.0. Available at <http://www.worldagroforestry.org/af/tree db/>. [Accessed: Jan 19, 2013].
- Stahl, E., (1969). Thin layer chromatography: A Laboratory Handbook. 2<sup>nd</sup> Edition, Springer, New York.
- Shen, D., Wu, Q., Wang, M., Yang, Y., Lavoie, E.J., Simon, J.E., (2006). Determination of the Predominant Catechins in *Acacia catechu* by Liquid Chromatography/Electrospray Ionization–Mass Spectrometry. *Journal Agricultural and Food Chemistry* 54, 3219–3224.
- Si, W., Gong, J., Tsao, R., Kalab, M., Yin, Y., (2006). Bioassay-guided purification and identification of antimicrobial components in Chinese green tea extract. *Journal of Chromatography* 1125, 204–210.
- Takahama, U., Yamauchi, R., Hirota, S., (2013). Isolation and characterization of a cyanidin-catechin pigment from adzuki bean (*Vigna angularis*). *Food Chem.* 141, 282–288.
- Vermaak, I., Kamatou, G.P.P., Komane-Mofokeng, B., Viljoen, a. M., Beckett, K., (2011). African seed oils of commercial importance - Cosmetic applications. *South African Journal of Botany*, 77, 920–933.
- Van der Vossen, H.A., Mkamilo, G.S., (2007). Vegetable oils of tropical Africa, conclusion and recommendations based on PROTA 14: “Vegetable oils”. Available on: PROTA (Plant Resources of Tropical Africa/Resources vegetales de l’Afrique tropicale), Wageningen, Netherlands <http://www.prota.co.ke/en/p. PROTA>.
- Wu, Q., Wang, M., Simon, J.E., (2003). Determination of proanthocyanidins in grape products by liquid chromatography/mass spectrometric detection under low collision energy. *Anal. Chem.* 75, 2440–2444.