



## Simplified Isolation of Catechin-3-o-beta-d-glucopyranoside from *Trichilia emetica* Seeds

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### ABSTRACT

The conventional method for the isolation of *catechin-3-o-beta-d-glucopyranoside* from *Trichilia emetica* whole seeds is laborious, costly and low yielding (3 - 5%). The isolation technique was developed to reduce the isolation cost and time as well as to get a better yield (20 - 30%). The structure of the isolated compound was confirmed on the basis of nuclear magnetic resonance (NMR), Infrared (IR) spectroscopy and ultraviolet-visible (UV-vis) spectrophotometry as well as by the comparison of the data reported in the literature. The method of isolation of (+)-Catechin 3-O-β-D-glucopyranoside presented here is very simple, economical and gives better yield than the conventional method.

**Keywords:** *Trichilia emetica*, catechin, methylation, spectral analysis, hydrolysis

### INTRODUCTION

*T. emetica* is commonly known as Natal mahogany, Ethiopian mahogany, Cape mahogany, Christmas bells, red ash, thunder tree or woodland mahogany. This species is native to Africa and it grows widely throughout sub-Saharan African extending from Ghana to the Red Sea, throughout East and Central Africa to Zambia and KwaZulu-Natal in South Africa (Iwu, 2014; Orwa et al., 2009; Van der Vossen and Mkamilo, 2007). *T. emetica* seeds have a seed weight between 0.35-1.0g and made up of 21 – 29% oily shell-like husk called sarcotesta as well as 71 – 79% kernel which contains 55-65 % of a brownish fat that melts between 35-41°C (Fupi and Mork, 1982). The multivitamin juice produced from a mixture of *T. emetica* seeds and other edible indigenous African plants is used to control malnutrition, a major challenge faced by children and mothers in rural areas in Africa (Komane et al., 2011; Saka and Msonthi, 1994).

Previous qualitative phytochemical studies on *T. emetica* whole seeds have implicated to with a substantive number of flavonoids (Usman et al., 2018). The boiled water extract of *T. emetica* whole seeds is dark brown coloured materials with bitter test. The most abundant flavonoids in this extract is catechin-

3-o-beta-d-glucopyranoside, which has been reported to be potent antioxidant at an IC<sub>50</sub> value of 39 µg/ml (Khallouki et al., 2007). The conventional method of isolating this compound is as shown in Figure 1.

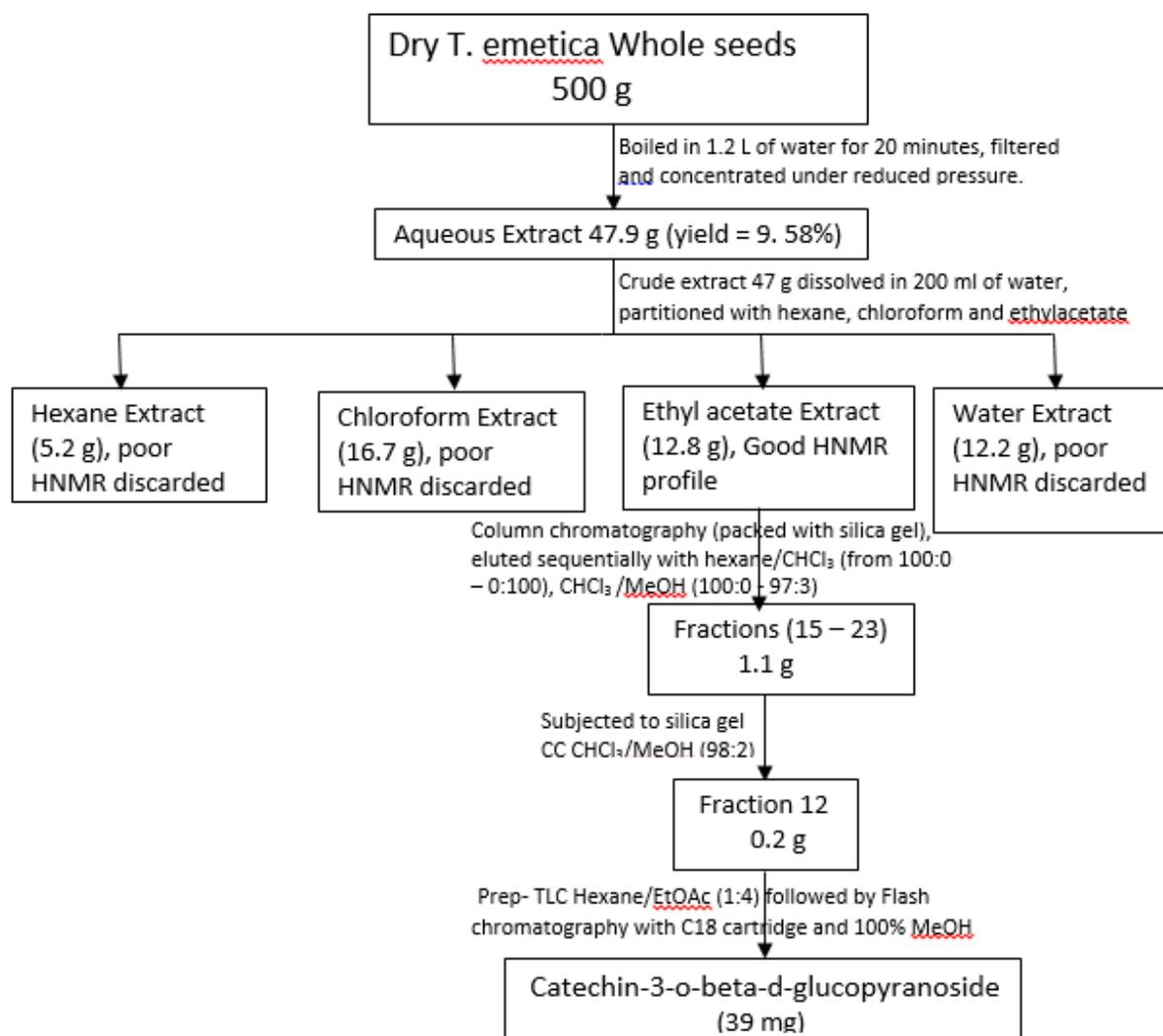
Received 25 July, 2019

Accepted 29 September, 2019

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Figure. 1. Scheme for the isolation of catechin-3-o-beta-d-glucopyranoside from *T. emetica* whole seeds. Usman *et al.*, 2018.



## METHODOLOGY

**Solvent purification:** Solvents used during the work were of analytical grade supplied by Fisher scientific and there was no further purification. The purity of the compound isolated was tested by thin layer chromatography (TLC) on silica gel 60 GF<sub>254</sub> glass plate and the spot was made visible by exposure to UV lamp.

**Melting point:** A Stuart instrument was used for recording the melting points. Care was taken to ensure that the heating was done steadily.

**<sup>1</sup>H-NMR spectra:** The <sup>1</sup>H-NMR spectra were recorded on a Bruker Avance (400 MHz) spectrometer with internal reference of δ<sub>H</sub> 3.31

ppm for CD<sub>3</sub>OD using TMS (Trimethylsilane) as an internal standard.

**<sup>13</sup>C-NMR spectra:** The <sup>13</sup>C-NMR spectra were recorded on a Bruker Avance (400 MHz) spectrometers with internal reference of δ<sub>C</sub> 49.0 ppm for CD<sub>3</sub>OD using TMS (Trimethylsilane) as an internal standard.

**Infrared spectra:** Infrared spectra were recorded on a Perkin Elmer 100 FT-IR spectrophotometer. Spectra were taken in KBr pellets.

**Optical rotation:** Optical rotations were measured in methanol solution on an ADP 440+

polarimeter.

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

**Silylation of the sugar:** A Thermo Scientific 1300 gas chromatograph equipped with a TRIPLUS RSH autosampler and an ITQ 900 MS detector was used for the analysis of the silylated sugars. The conditions of the GC were as follows: column, Rxi®-ms (length: 30 m, ID: 0.25 mm, df: 0.25 µm). Inlet temperature 250 °C, split flow 30 ml/min, split ratio 20 and carrier flow rate 1.5 ml/min. The temperature program start at 172 °C, hold for 1 min, then increased at 10 °C/min up to 210 °C, hold for 1 min then 20 °C/min up to 220 °C, hold for 1 min, then a final ramp of 10 °C/min to 280 °C and hold for 1.5 min. Total run time of 15 min.

**Collection of raw materials:** *T. emetica* seeds was collected from the Botanical garden of Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

#### Isolation Procedures

The method described by Hye *et al.*, (2009) with modification was adopted. Dried *Trichilia emetica* whole seeds (500 g) was refluxed in 1L of distilled water at 100 °C for 2 hours and allowed to stand for 3 hours. The extract was decanted off, filtered and evaporated in vacuum at 50 °C. The residue obtained was air dried and weighed (97.2 g). 95 g of the residue was taken in a 2L glass beaker containing 500 ml distilled water. It was boiled with constant stirring for complete dissolution and filtered while hot. The filtrate was concentrated to about 200 ml and allowed to stand for 3 hours. The obtained precipitate was filtered, the aqueous filtrate was discarded while the residue was dissolved in ethanol 200 ml and filtered. The ethanolic solution was concentrated in vacuum, the resulting residue was dissolved in hot water (200 ml) and allowed to stand for 6 hours. The precipitate formed was filtered, air dried and weighed (19.2g, 20%). The process of re-crystallization from water was repeated two times.

#### Thin layer chromatography of the compound

The isolated compound and authentic samples were applied on a silica gel 60 F<sub>254</sub> glass plate

using ethyl acetate and developed with Hexane, ethyl acetate, methanol (3:2:1). Spots were visualised using phosphor molybdic acid. Both the compounds showed single spot (*R<sub>f</sub>* =0.24).

#### Spectral analysis of (+)-Catechin 3-O-β-D-glucopyranoside

**IR spectra:** IR spectra [ν<sub>max</sub> (KBr)] showed band at 2700-3340 (broad), 1609, 1520, 1464, 1373, 1285, 1254, 1142, 1103, 1072, 1028, 821 cm<sup>-1</sup>.

**UV spectra:** UV spectra (λ<sub>max</sub> [methanol]) showed maxima at 279nm and 232 nm.

**Optical rotation:** -11.73 (c 0.33 in methanol)

**Mass spectra:** The mass spectrum showed a base peak at 453.

**<sup>1</sup>H-NMR** (400 MHz, CD<sub>3</sub>OD) δ 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), 5.95 (H-6, d, J=1.90 Hz), 5.91 (H-8, d, J=1.90 Hz), 6.84 (H-2', d, J=1.65 Hz), 6.76 (H-5', d, J=8.15 Hz), 6.71 (H-6', dd, J=1.65, 8.17 Hz), 4.20 (H-1'', d, J=7.43 Hz), 3.33 (H-2'', m), 3.30 (H-3'', m), 3.25 (H-4'', m), 3.18 (H-5'', m), 3.65 (H-6'', dd, J=5.24, 11.76 Hz) and 3.85 (H-6'', dd, J=1.52, 11.76 Hz).

**<sup>13</sup>C-NMR** (400 MHz CD<sub>3</sub>OD): 80.1 (C-2), 76.1 (C-3a), 26.1 (C-4), 157.9 (C-5), 96.4 (C-6), 157.5 (C-7), 95.6 (C-8), 156.6 (C-9), 100.7 (C-10'), 132.3 (C-1'), 114.8 (C-2'), 146.4 (C-3'), 146.3 (C-4'), 116.1 (C-5'), 119.6 (6'). Glucose moiety peaks appeared at 103.8 (C-1''), 75.2 (C-2''), 77.8 (C-3''), 71.6 (C-4''), 78.1 (C-5'') and 62.9 (C-6'').

#### Acid hydrolysis

Sample (3 mg) was dissolved in methanol (10 ml), 10 ml of 5% H<sub>2</sub>SO<sub>4</sub> was added and refluxed for 5 hours. On cooling, the methanol was evaporated under vacuum and the H<sub>2</sub>O washed with EtOAc (3 × 5 ml). The aqueous portion was concentrated and compared to standard sugars using the TLC systems CH<sub>2</sub>Cl<sub>2</sub>/MeOH/H<sub>2</sub>O (17:6:1) while the EtOAc portion contained the aglycon (a catechin). The sugar was identified as D-glucose (Chaturvedula and Prakash, 2011).

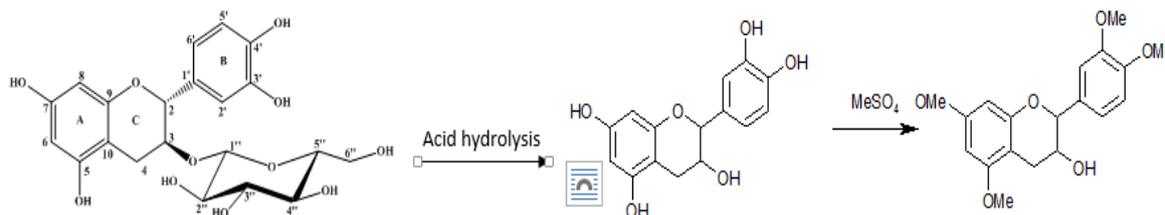
#### Methylation of (+)-Catechin 3-O-β-D-glucopyranoside

The method described by Vogel, 1969 was adopted with modification. The attempt to methylate the catechin glycoside was not possible. After acid hydrolysis of the catechin glycoside, the aglycon (catechin) was

methylated with dimethyl sulphate. The yield of the methylated aglycon was 79%. The melting point of the methylated aglycon is

between 144-145°C which corresponds to 5,7,3',4'-tetra methyl ether of (+)-catechin.

Fig. 2. Methylation of (+)-catechin after acid hydrolysis



## RESULTS AND DISCUSSION

The conventional method of isolating catechin-3-o-beta-d-glucopyranoside involves several steps which is time consuming and economically not viable. The method of isolation described in this report was found more suitable, because it involve lesser steps and gives better yield. The thin layer chromatographic behaviour of the isolated compound ( $R_f = 0.24$ ) was in agreement with

the authentic compound (Raab *et al.*, 2010). The UV spectrum showed absorption bands at 232 and 279 nm, consistent with conjugated  $\pi-\pi^*$  transition arising from the aromatic ring. The IR spectrum showed a broad band at 3340  $\text{cm}^{-1}$  corresponding to aliphatic and aromatic C-H, phenolic and alcoholic O-H stretching. A band observed at 1609  $\text{cm}^{-1}$  is due to aromatic C=C stretching, 1142, 1072 (C-O stretching) and 821  $\text{cm}^{-1}$  out of plane deformation.

Figure 3. The mass spectrum of (+)-Catechin 3-O-β-D-glucopyranoside

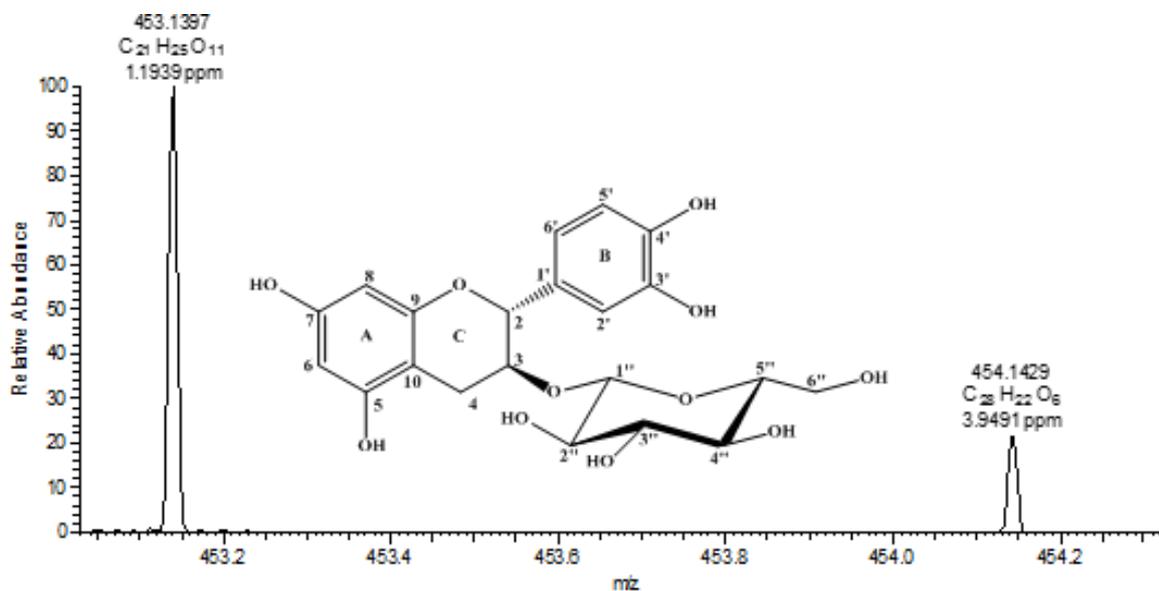


Figure 4. The <sup>1</sup>H NMR spectrum of (+)-Catechin 3-*O*-β-D-glucopyranoside

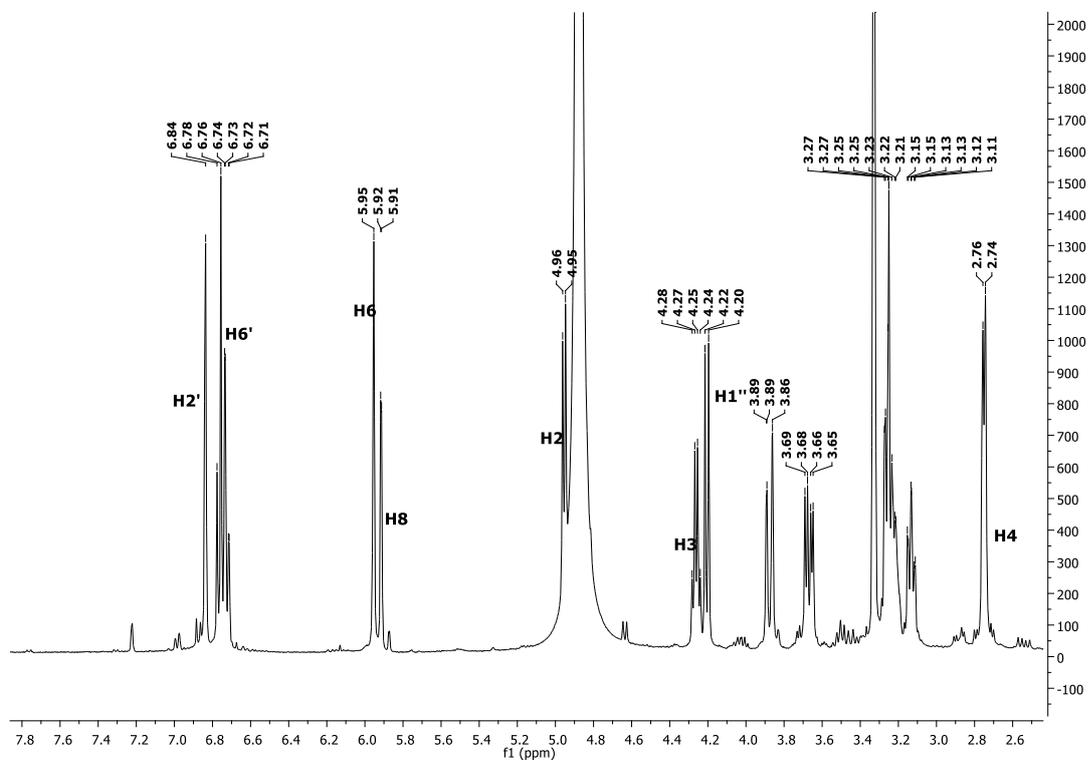
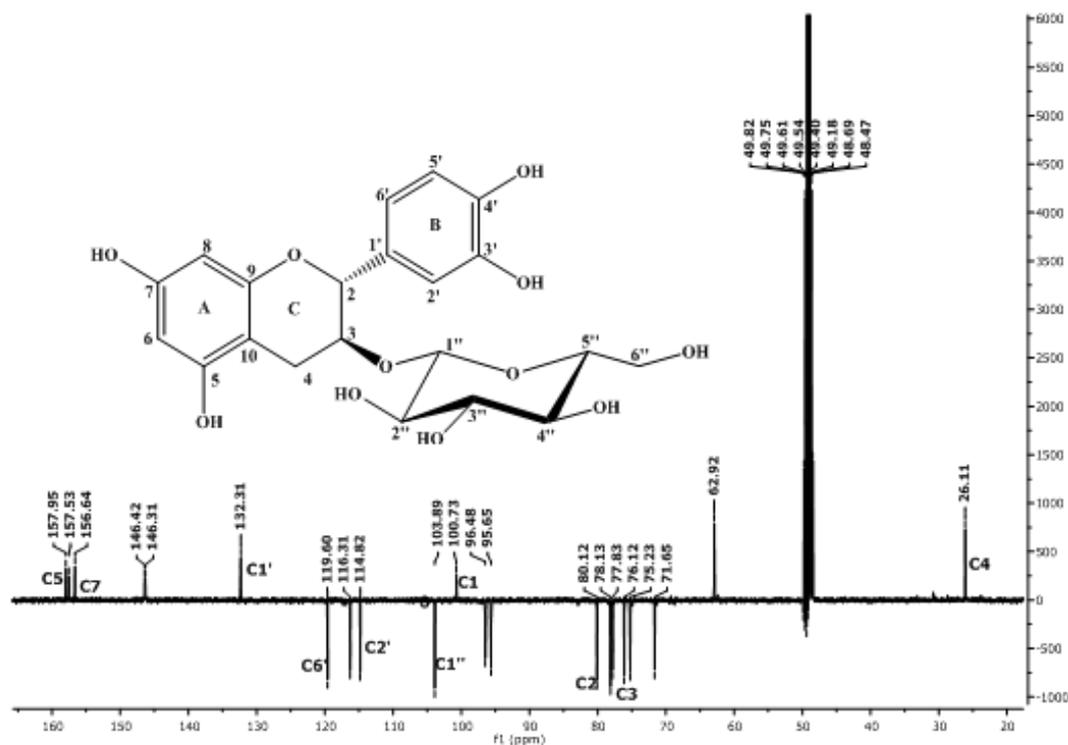


Figure 5. The DEPT spectrum of (+)-Catechin 3-*O*-β-D-glucopyranoside



The compound's molecular formula of  $C_{21}H_{24}O_{11}$  was established on the basis of ESI-HRMS at  $m/z$  453.1397  $[M + H]^+$  (Calcd for 453.1398) (Figure 3). The optical rotation is  $-12.10$  (MeOH:  $c$  0.33) which is comparable to the literature value of  $-11.73$  (Usman *et al.*, 2018). The  $^1H$  NMR spectrum of this compound is consistent with the literature value (Ishimaru *et al.*, 1987; Hwang *et al.*, 1989). The spectrum also shows one anomeric proton at  $\delta_H$  4.20 (d, 7.5 Hz, H-1''), based on this coupling constant, the configuration of the sugar moiety is determined to be  $\beta$ -oriented indicating a  $\beta$ -glucosyl moiety (Figure 4).

The DEPT spectrum (Figure 5) revealed twenty one signals equivalent to two methylene, twelve methine and seven quaternary carbons. The spectrum also show one anomeric carbon at 103.89 (C-1'') and other signals for glucose were observed at  $\delta_H$  75.2 (C-2''), 77.8 (C-3''), 71.6 (C-4''), 78.1 (C-5'') and 62.9 (C-6''). Additionally, the C-3 site of glycosides resonated at higher field  $\delta_C$  76.10, thus exhibiting a shielding effect and this indicated that the attachment of the sugar was at C-3 via a C-O-C linkage. The melting point of the compound also agree well with the literature values (Bae *et al.*, 1994; Raab *et al.*, 2010). Acid hydrolysis of catechin-3-o-beta-d-

glucopyranoside yielded catechin and on methylation gave 5,7,3',4'-tetra methyl ether of (+)-catechin.

## CONCLUSION

The method of isolation of (+)-Catechin 3-O- $\beta$ -D-glucopyranoside presented here is very simple, economical and gives better yield than the conventional method. This compound can be isolated commercially for research and medicinal purposes employing the same techniques.

## Acknowledgement

The authors are grateful to Nigerian Tertiary Education Trust Fund (TETFUND) for financing the research work.

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