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Isolation and Characterization of Naturally Occurring Butylated Hydroxytoluene from *Trichilia emetica* Whole Seeds

A. Usman[1,2,*], R.H. Mohammad[1], D. Raheem[1]

[1]*School of Chemistry, Bangor University, Bangor LL57 2UW, United Kingdom.*
[2]*Department of Chemistry, Nasarawa State University, Keffi, Nigeria.*

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ABSTRACT

Trichilia emetica whole seeds were refluxed in water for twenty minutes and the extract subjected to column chromatography. The mass spectrum of the isolated compound in negative ion mode showed a parent molecular ion peak at m/z 219 which corresponds to the molecular formula of $C_{15}H_{24}O$. The structure was characterized by means of Infrared (IR), nuclear magnetic resonance (NMR) and mass spectroscopy. And the comparison of the ^{1}H and ^{13}C NMR data with that reported in the literature. Finally, the structure of the compound was confirmed by X-ray crystallography.

1. Introduction

2,6-di-t-butyl-4-methylphenol popularly called butylated hydroxyltoluene (BHT) is a synthetic antioxidant extensively used in food industry to manufactured food containing fats [1], petroleum products and rubber [2]. It is also use as food preservatives [3]. The US Food and Drug Administration (FDA) have given it GRAS (Generally Recognized as Safe) status, but has restricted it use because of the findings that it may be toxic at higher concentration [4]. Due to the importance of BHT, several studies have been conducted with the sole aim of finding new natural antioxidant biomolecules present in plants, which will served as a substitutes for synthetic ones. Naturally, BHT has been reported from the aerial parts of *Cytisus triflorus* L'Hérit. (Fabaceae) [5], in *Mesembryanthemum crystallinum* L. (Aizoaceae) [6], and in volatile fractions of *Azadirachta indica* (Meliaceae) [7] and *Camellia sinensis* (Theaceae) [8]. Similarly, it has also been synthesized in green algae (*Botryococcus braunii* Kütz.) and three Cyanobacteria [*Cylindrospermopsis raciborskii* (Wolloszynska), *Mycrocystis aeruginosa* (Kütz.) and *Oscillatoria sp*.] [9].

T. emetica (Meliaceae), commonly called natal mahogany, is an evergreen tree that is widely distributed through-out tropical and subtropical region of Africa [10]. All parts of this plant such as the seeds, fruit, leaves, root and stem bark are used in African traditional medicine. 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 [11]. The decocted powdered root is given to cure jaundice and to relief stomach and chest pain [12]. Also, a decoction of the leaves is used against fever, cough and bronchial trouble [13]. This study reports for the first time the isolation of naturally occurring BHT from *T. emetica*.

2. Experimental Methods

2.1 Collection of Plant Materials

*T. emetic*a seeds were collected from Kumasi, Ghana, in February 2013 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

T. emetica whole seeds 1.0 kg was 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.

2.3 Purification

The crude extract (20 g) was subjected to column chromatography. A clean column (450 mm × 95 mm) was packed with 300 g of silica gel 60 Å, 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 [14]. 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 4 to 21 (1.31 g) obtained with gradient elution mixture of 100 % hexane: 0 % chloroform to 30 % hexane: 70 % chloroform was found to be pure butylated hydroxyltoluene (BHT).

2.4 Radical Scavenging Activity

The analysis of the DPPH radical scavenging activity was performed according to the method described by Brand-William et al [15]. A 0.5 mL aliquot of the isolated compound (100 mg/mL) and 0.3 mL of DPPH (0.5 mM) were added to 3 mL of absolute ethanol. Synthetic butylated hydroxytoluene (BHT) was used as a standard for the investigation of the antiradical activity and was prepared in a similar manner. The reaction mixtures were vigorously shaken for 30 s in a Vortex apparatus and allowed to stand in the dark at room temperature for 30 minutes. The absorbance was measured spectrophotometrically at 517 nm. The blank was prepared by mixing 0.5 mL of isolated compound or standard (BHT) with 3.3 mL of ethanol. Similarly, the control solution was prepared by mixing 3.5 mL of absolute ethanol and 0.3 mL of DPPH radical solution.

*Corresponding Author
Email Address: ausman2015@yahoo.com (Abdullahi Usman)

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The percentage of scavenging activity (Z%) was calculated according to the equation below:

$$Z\% = 100 - [(Abs_{sample} - Abs_{blank}) \times 100/Abs_{control}]$$

3. Results and Discussion

The compound was obtained as pale yellow needles (Fig. 1), it has a molecular formula of $C_{15}H_{24}O$ which was established on the basis of ESI-HRMS/MS at *m/z* 219.1754 [M – H]$^-$,(Calculated for 219.1748). The IR spectrum of this compound has a band at 3647 (O-H stretching), 2958 (C-H stretching), 1431(C-H deformation) and 860 (C-H out of plane deformation) cm^{-1}.

The DEPT and ^{13}C NMR spectra (Fig. 2) showed seven carbon signals, which consist of two methyl, one methine and four quaternary carbons. The carbon signals were as follows: δ= 151.5 (C-1), 135.8 (C-2/6), 125.3 (C-3/5), 128.3 (C-4), 21.2 (C-7) and 30.3 (34.33) [2'/6' (2''/6'')] for t-butyl. In the ^{1}H NMR spectrum, the methine protons signals on the ring appeared at δ_H 7.03 (2H, s) assigned to positions 3/5 are magnetically equivalent and have no spin-spin interaction [16]. The OH-signal lies at δ_H 5.05 (s) and the signals at δ_H 2.31 is attributable to aromatic CH_3 on account of the ring effect while the signal at δ_H 1.48 is assigned to 18 equivalent protons in the two tert-butyl substituent.

The HSQC spectrum (Fig. 3) show that the carbon resonances of the ring at δ_C 21.2 (C-7) correlated with proton at δ_H 2.31 (H-7) and the side chains (tert-butyl methyl) C (2/6) at δ_C 31.3 correlated with protons at δ_H 1.47 (H-2/6). Similarly, the COSY spectrum displayed correlations between δ_H 7.02 (H-3/5) and δ_H 2.31 (H-7); and also between δ_H 7.02 (H-3/5) and δ_H 1.47 assigned to position (H-2''/6''). These correlations were further confirmed by long-range couplings observed in the HMBC spectrum (Table 1 and Fig. 4), where the methine protons at H-3/5 (δ_H 4.55) correlate with C-1, C-2/6, and C(2'/6') (δc 34.3); and H(2''/6'') (δ_H 1.47) correlate with C(2'/6'), C(2/6) and C(3/5), while the methyl protons at H-7 (δ_H 21.2) correlate with C-1, C-3/5 and C-2/6 (δc 135.5) respectively. The structure was therefore deduced as 2,6-di-t-butyl-4-methylphenol (BHT).

The NMR data (Table 1) thus showed signals typical of 2,6-di-t-butyl-4-methylphenol (BHT), and it is comparable to the ^{1}H and ^{13}C NMR data reported in the literature [16,17]. Finally, the structure of the compound was confirmed by X-ray crystallography and it is identical to a known crystal structure (CSD: MBPHOL).

Table 2 showed a high antioxidant level in the isolated compound (97% of DPPH inhibition) than that in the synthetic BHT.

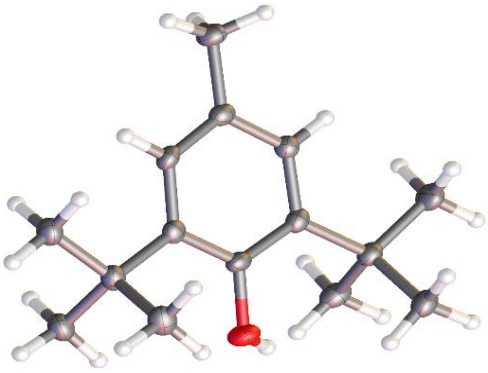

Fig. 1 The X - ray crystal structure of BHT

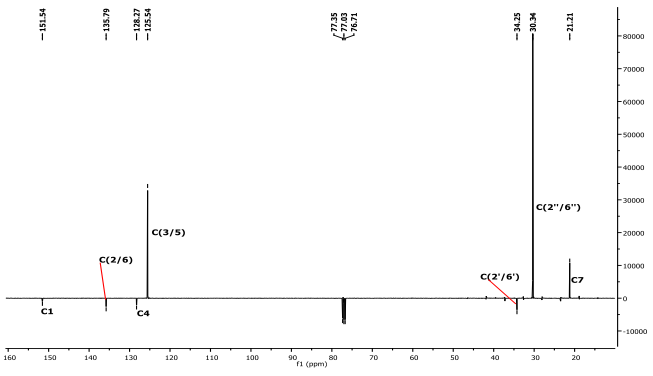

Fig. 2 The DEPT spectrum of BHT in $CDCl_3$

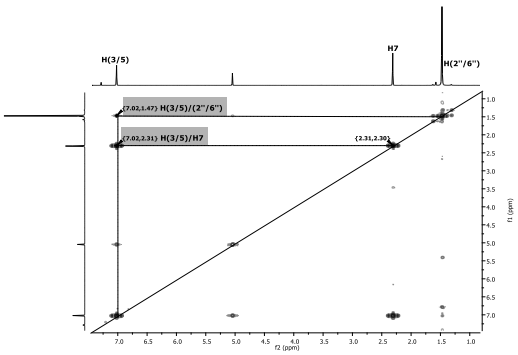

Fig. 3 The HSQC spectrum of BHT in $CDCl_3$

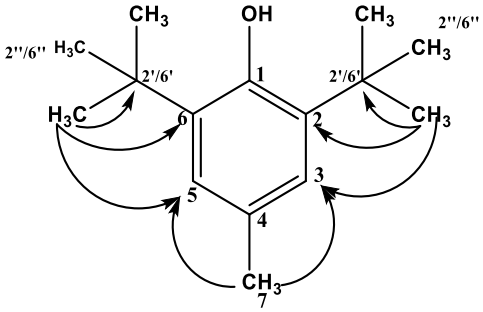

Fig. 4 COSY and HMBC correlations of BHT

Table 1 The ^{1}H and ^{13}C NMR data of BHT

Atom No.	Benjamin 1978 $CDCl_3$ (δc)	This study $CDCl_3$ (δc)	This study $CDCl_3$ δ_H (m, *J* in Hz)	Pliev 1970 $CDCl_3$ δ_H (m, *J* in Hz)	HMBC [H→C]
1	151.3	151.5			
2/6	135.5	135.8			
3/5	125.3	125.5	7.02 (s)	6.85 (s)	C-1, C-4, C-7, Ct, C-2&6, C-A
4	128.0	128.3			
7	21.2	21.2	2.31 (s)	2.22 (s)	C-4, C-2&6, C-3&5
t-butyl [2'/6' (2''/6'')]	30.4 (34.2)	30.3 (34.33)	1.47 (s)	1.41 (s)	C-A, C-2&6, C-3&5

Table 2 Radical scavenging activity of isolated compound and synthetic BHT

Extract	Isolated compound	Standard BHT
% of DPPH inhibition Quantity	97 %	89 %

4. Conclusion

2,6-di-t-butyl-4-methylphenol (BHT) is a synthetic antioxidant that has been reported in some plant species. The isolation of this compound in *T. emetica* agreed with the finding of other researchers. The work was carried out by utilizing several kinds of separation methods (solvent extraction, column chromatography and thin layer chromatography) and spectroscopic techniques such as IR, 1D and 2D NMR spectroscopy.

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