

## Isolation, identification and characterization of gallic acid derivatives from leaves of *Tapinanthus bangwensis*

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### ABSTRACT

Gallic acid and its derivatives are widely present in the plant kingdom and are known to display antioxidant activity by the ability to counteract the damaging effects of free radicals in tissues and thus are believed to protect against cancer, arteriosclerosis, heart disease, and several other diseases. Hence, the present work describes the isolation of gallic acid derivatives from the ethyl acetate fraction of leaves of *T. bangwensis*. The structures of three phenolic acids of gallic acid derivatives namely; Methyl syringate (**1**), 3,4,5- trimethyl gallic acid (**2**) and 3, 4- dimethoxy-5- hydroxyl benzoic acid (**3**) were determined using data obtained from FAB-MS, <sup>1</sup>H and <sup>13</sup>C NMR spectra as well as by various correlation experiments (COSY, NOESY, HMQC and HMBC). The result corroborates the therapeutic potential of *T. bangwensis*. The possible mechanisms of action may be attributed to the presence of these polyphenolic compounds isolated from the leaves of *T. bangwensis*.

**Keyword:** Gallic acid derivatives; *T. bangwensis*; Fractionation; Phenolic compounds.

### INTRODUCTION

The search for antioxidants from natural products is on the increase as against antioxidants of synthetic origin. It has been reported that reactive oxygen species (ROS) readily attack and induce oxidative damage to various biomolecules including proteins, lipids, lipoproteins and DNA (Faber, 1994; Heikal, et al., 2012). Therefore, the need to develop natural antioxidants that will aid in the slowdown of the oxidative damage caused by oxidation is of primary concern. Antioxidant has been defined as any substance that delays, prevents or removes oxidative damage to a target molecule'' (Halliwell, 2007). Halliwell and Gutteridge (1995) also defined antioxidants as any substance that when present at low concentrations compared with that of an oxidizable substrate significantly delays or inhibits oxidation of that substrate.

Gallic acid (GA, 3, 4, 5-trihydroxybenzoic acid) and its derivatives are widely present in the plant kingdom and represent a large family of plant secondary

polyphenolic metabolites and hence natural antioxidants. They are present in the forms of either methylated gallic acids (for example, syringic acid). Gallic acid derivatives have been reported in many phytomedicines with a number of biological and pharmacological activities, including scavenging on free radicals (Kanai and Okano, 1998), apoptosis of cancer cells (Sakagami, et al., 1997; Serrano, et al., 1998; Saeki, et al., 2000), inhibiting squalene epoxidase (Abe, et al., 2000) and interfering with the signal pathways involving  $\text{Ca}^{2+}$  and oxygen free radicals (Sakaguchi, et al., 1998, 1999; Inoue, et al., 2000; Sohi, et al., 2003).

Mistletoe is a semi-parasitic evergreen plant found growing on a host of evergreen and deciduous trees all year round, around the branches of the tree. It is an obligate parasite, obtaining part of its food from the host plant. It depends on its host for minerals and water only, but photosynthesizes its carbohydrate by means of its green leathery, oblong leaves (Osadebe and Uzochukwu, 2006). In Nigeria and some other parts of Africa, the leaves of African mistletoe have been used traditionally as antihypertensive and antidiabetic plant (Bikomo, 1992; Obatomi and Bikomo, 1994). It is generally used as anticancer agents (Grossarth-Maticek and Ziegler, 2007) and in the management of diabetes mellitus (Kafaru, 1993; Obatomi, et al., 1994; Osadebe, et al., 2004) as well as antihypertensive agent (Kafaru, 1993). Kafaru (1993) described the mistletoe plant as “an all purpose herb” because of its rich folkloric uses. Some of these uses have been reported (Obatomi, et al., 1994; Fischer, et al., 1997; Osadebe, et al., 2004).

There were several reports on the phytochemical and antimicrobial properties of African mistletoe *Loranthus micranthus* (Osadebe and Ukweze, 2004). It has also been reported that flavonoids, lectins, polypeptides, triterpenes and polyphenolic compounds are present in the plant (Duong, et al., 2003). The presence of phlobotannins, alkaloids, anthraquinones as well as cardiac and steroidal glycosides have also been reported (Wahab, et al., 2010). Previous studies have shown that the ethyl acetate fraction of leaves of *T. bangwensis* possesses anti-inflammatory property in a carrageenan-induced hind paw oedema in rats (Patrick-Iwuanyanwu, et al., 2010a); hepatoprotective property in a  $\text{CCl}_4$  induced hepatotoxicity (Patrick-Iwuanyanwu, et al., 2010b), in-vitro cytotoxic and antioxidant activities (Bassey, et al., 2012). All these properties were attributed to phenolic compounds present in the ethyl acetate fraction of *T. bangwensis*. Bassey, et al., (2012) also reported that the leaves of the plant contain an appreciable amount of fiber, carbohydrate, protein and mineral elements. However, information is scanty on the isolation and structural elucidation of active compounds present in the leaves of the plant investigated. The present study however, reports for the first time the isolation and structural elucidation of gallic acid derivatives from the ethyl acetate (EtOAc) fraction of leaves of *T. bangwensis*.

## MATERIALS AND METHODS

**General Experimental:** NMR spectroscopy experiments on the compounds were performed on a Bruker<sup>®</sup> Avance 300, 400 and 500 respectively at 400 MHz (for  $^1\text{H}$  NMR) and 100 MHz (for  $^{13}\text{C}$  NMR) with  $\text{CDCl}_3$  as solvent. FAB-MS (negative-ion mode, glycerol matrix) was recorded on an R210C (VG Instruments, Altrincham, UK) spectrometer equipped with an IPC (P2A) MSCAN uWALLIS computer system. COSY, HMQC, and HMBC spectra were obtained using the usual pulse sequences.

**Plant Materials:** Leaves of *Tapinanthus bangwensis* were collected from the Vice Chancellor's Orchard, Delta Park, University of Port Harcourt, Port Harcourt, Rivers

State, Nigeria, identified by Dr. Edwin Wosu of Plant Science and Biotechnology Department, University of Port Harcourt, Port Harcourt, Rivers State, Nigeria.

**Extraction and Isolation:** The sun-dried leaves of *T. bangwensis* were pulverized into a fluffy mass. One kilogramme of the powdered leaves of *T. bangwensis* was extracted with 8L of MeOH using Soxhlet extractor for 24h. The extract was evaporated to dryness under reduced pressure (below 40°C) to yield crude methanolic extract. Forty grams of the dried crude methanolic extract was dissolved in distilled water and made up to 200ml. It was further fractionated by successive solvent extraction with n-hexane, ethyl acetate (EtOAc) (2 x 100ml) and n-butanol (BuOH) saturated with H<sub>2</sub>O (3 x 100ml). Each extract was evaporated to dryness under reduced pressure to yield n-hexane, ethyl acetate, n-butanol and aqueous fractions respectively (Patrick-Iwuanyanwu, et al., 2010a; Patrick-Iwuanyanwu, et al., 2010b). The EtOAc fraction of *T. bangwensis* (10.5g) was subjected to a silica gel chromatography column (3.0 x 50cm). The column was developed with a solvent gradient of n-hexane: ethyl acetate, in order of increasing polarity (100:0 → 0: 100) and the fractions were collected. Out of 10 fractions A-J, fraction D, obtained from 30% EtOAc/hexane, were further subjected to column chromatography in same solvent system, which yielded compound **1** (29mg) at polarity 10% EtOAc/hexane. Fraction E obtained from 40% EtOAc/hexane, were subjected to LH-20 column chromatography with MeOH as an eluting solvent yielded compounds **2** (22mg) and **3** (41mg).

## RESULTS

The structures of compounds **1-3**, isolated from *T. bangwensis* leaves was elucidated using data obtained from FAB-MS, 1D- and 2D-NMR spectra (Fig. 1). <sup>1</sup>H and <sup>13</sup>C-NMR spectra of compound **1** indicated that compound is gallic acid derivative, showing resonances for two proton methine at  $\delta_H/\delta_C$  [7.31 d (2H,  $J = 2.0$  Hz)/108.1, CH-2/6], three methoxy groups at  $\delta_H/\delta_C$  [3.87 s (6H)/56.8, CH<sub>3</sub>-3/5-OMe; 3.86 s (3H)/52.5, Me-8], and five quaternary carbons at  $\delta_C$  168.8 (C-7), 148.9 (C-3 and 5), 142.0 (C-4), 121.3 (C-1). Position of methoxy groups was determined with the help of <sup>13</sup>C-NMR value and HMBC spectrum. Compound **2**, was similar to compound **1**. The only difference observed was the presence of methoxy group at C-4 instead of hydroxyl in compound **1**. Compound **3** was also similar to compound **1**. However, there was a slight difference in the position of methoxy and hydroxyl groups.

## DISCUSSION

The structures obtained from spectroscopic data are in consonance with previous reports (Lu, et al., 2005). The antioxidant activity of phenolic products is generally ascribed to the redox properties of their hydroxyl groups, which can play an important role in absorbing and neutralizing free composing peroxides. The antioxidant capacity of phenolic products relies on their ability to transfer the phenolic hydrogen atom to chain-carrying peroxy radical, at a rate faster than that of the chain-propagating lipid peroxidation step (Pratt, et al., 2001). It is also known that electron-donating groups located at the *ortho* or *para* positions to the phenolic hydroxyl group lower the O-H bond dissociation enthalpy and increase the transfer rate of the hydrogen-atom to the peroxy radical (Ching-Chuan, 2002). The significant antioxidant and hepatoprotective effects on CCl<sub>4</sub>-induced hepatic damage in rats by the methanolic extract and fractions of leaf of *T. bangwensis* as observed in our earlier study may be attributed to these gallic acid derivatives compounds isolated from the leaf of *T. bangwensis* (Patrick-Iwuanyanwu, et al., 2010a). The result of this study also corroborates our

report on the anti-inflammatory property of leaf extracts from *T. bangwensis* in Wistar albino rats (Patrick-Iwuanyanwu, et al., 2010b). However, data obtained from previous and present study suggest that EtOAc fractions of leaves of *T. bangwensis* may have significant antioxidant, anti-inflammatory and hepatoprotective effects on CCl<sub>4</sub>-induced hepatic damage in rats. These results show that the possible therapeutic potential of the leaves of the plant may depend on the antioxidant properties exerted by their flavonoid contents. These antioxidant properties could be attributed to the gallic acid derivatives (**compounds 1-3**) isolated from EtOAc fractions of *T. bangwensis*. The mechanism of action may be due to their ability to block the bioactivation of toxicant and their potent antioxidant activity, or by scavenging the free radicals and inhibiting lipid peroxidation.

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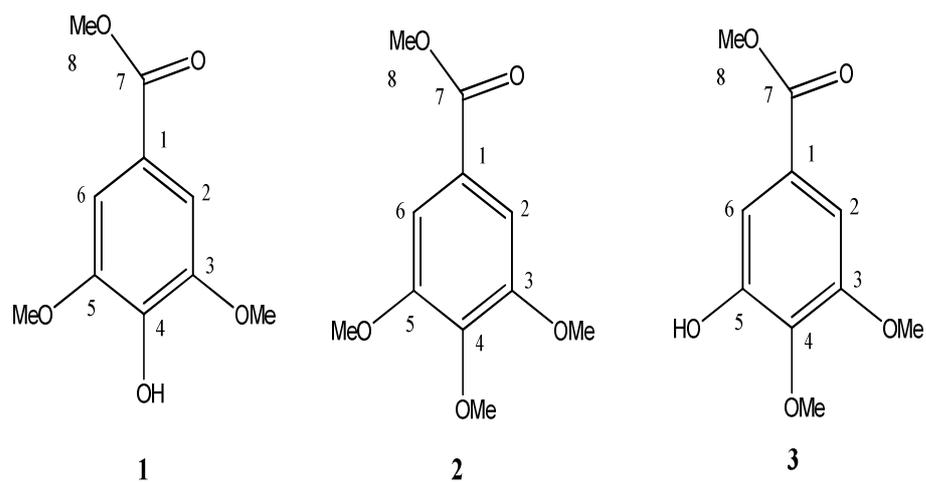


Figure- 1: Gallic acid derivatives isolated from leaves of *T. bangwensis* (compounds 1-3).