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CHEMICAL CONSTITUENTS FROM METHANOLIC STEM BARK EXTRACTS OF *Piliostigma thonningii* BELONGING TO FABACEAE.

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ABSTRACT

Piliostigma thonningii (Schum.) Milne-Redh is a legume plant belonging to the Fabaceae family. It is locally used in the treatment of various human diseases like microbial infections, dysentery, fever, skin diseases, snake bites, infections, respiratory ailments and hookworm. The presence of various phytochemicals in this plant may be responsible for the biological activities of this plant, hence the major objective of this work is to isolate and characterize the phytochemical constituents of the stem bark of *P. thonningii*. The methanol crude extract obtained was partitioned with ethyl acetate, butanol and distilled water and the resulting fractions were purified over silica gel column chromatography to isolate the respective pure compounds. The isolated compounds were structurally elucidated by means of ^{1}H, ^{13}C, DEPT 135, HSQC and HMBC-NMR spectroscopic technique and compared with published data. The result of phytochemical investigation of *P. thonningii* methanolic stem bark extract led to isolation of five compounds: Garcinielliptone Q (**1**), (20S, 24R)-Epoxydammarane-3β, 25-diol (**2**), Protocatechuic acid (**3**), Epicatechin (**4**) and β-Sitosterol (**5**). Garcinielliptone Q (**1**) was isolated for the first time from this plant. The compounds isolated could be part of the phytochemicals responsible for its various biological activities.

Keywords: Phytochemicals, Isolation, triterpenoid, Garcinielliptone Q, *Piliostigma thonningii*.

INTRODUCTION

Phytochemicals are bioorganic molecules found in plants. They are otherwise known as secondary metabolites and are biosynthesized during plant secondary metabolism. These include alkaloids, flavonoids, saponins, tannins, terpenoids, steroids etc. [1].

Piliostigma thonningii (Schum.) Milne-Redh is a legume plant belonging to the Fabaceae family. It is locally used in the treatment of various human diseases like microbial infections, dysentery, fever, skin diseases, snake bites, infections, respiratory ailments and hookworm. The various biological properties of this plant may be associated with its different phytochemical contents. The growing awareness of the significance of medicinal plants to human health has prompted the need for study on phytochemicals present in the various parts of different medicinal plants [3]. The phytochemicals constituents of the plants are largely responsible for the definite physiological

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action they exert on the human body [4]. This study on medicinal plants traditionally used in the treatment of microbial infections has attracted the attention by many research communities as a potential alternative to currently existing antibiotics to which microorganisms have developed resistance [2].

Piliostigma thonningii (Schum.) Milne-Redh is a legume plant belonging to the Fabaceae family. The tree is perennial in nature and the color of its petals varies from white to pink [5].

In African countries *P. thonningii* is used for various medicinal purposes [6]. The decoction of the leaves and bark is used for the treatment of diarrhea, toothache, gingivitis, cough and bronchitis, ulcers, malaria, pyrexia, leprosy, sore throat, wounds, heart pain, arthritis [7]. Its roots and branches are used to treat dysentery, fever, wound infections, cough, and skin diseases. The crude extract of *P. thonningii* was reported to possess antilipidemic [8], antibacterial [9], anthelminthic [10] and antiinflammatory [11] activities. In Nigeria, this plant is locally known as *Abafe* in Yoruba, *Kalgo* in Hausa and *Okpoatu* in Igbo language. The common names include monkey bread, camel's foot, monkey biscuit tree, Rhodesian bauhinia and wild bauhinia.

P. thonningii is one of the plants with diverse ethnomedical applications [57]. Different parts of this plant have been described to be useful medicinally. Its roots and branches have been used to treat dysentery, fever, skin diseases, snake bites, infectious diseases, respiratory diseases and hookworm [58], [48].

Previous phytochemical research on *P. thonningii* showed the presence of varied chemical classes of compounds that possibly are responsible for the various activities of this medicinal plant. Among the identified chemical classes are flavonoids, alkaloids, tannins, saponi ns, kaurene diterpenes, carbohydrates, terpenes a nd volatile oils [49]; [52]; [40]; [10]; [42]. An illustrative pivotal metabolite isolated from *P. thonningii* is D-3-*O*-methylchiroinosital, which possesses analgesic, antipyretic, antidiabetic, antioxidant and antilipidemic activities [47]; [44] and anthelmintic activity [38]; one more promising lead compound is C-methyl flavanols, which was identified from the same species and showed antibacterial and antiinflammatory activities [40] and recently, phytochemical investigation of methanolic leaf extract of *Piliostigma thonningii* yielded two compounds newly isolated from natural sources, 2β-methoxyclovan-9α-ol and methyl-ent-3β-hydroxylabd-8(17)-en-15-oate [6], along with 14 known compounds [6]: β-amyrin [45], quercetin [37], quercitrin [37], Afzelin [37], (3S,5R,6S)-trihydroxy-7E-megastigmen-9-one [46], anticopalic acid [55], quercetin, 3-hexenyl-1-O-β-D glucopyranoside [54] clovane-2β,9α-diol

ISSN 0795 - 22066

[51], piliostigmin [39], (+)-epicatechin [53], alepterolic acid [50], Vitamin E [43] including stigmasterol and β-sitosterol glucoside which have been reported from different parts of *P. thonningii* [6].

In this research, bioactive secondary metabolites from methanolic stem bark extract of *Pilostigma thonningii* was phytochemically investigated because based on literature review, there is little or no information in literature about bioactive compounds isolated from methanolic stem bark extract of this plant. Our team's goal is to isolate the phytoconstituents which may be responsible for the antimicrobial properties of *P. thonningii* in support of its ethnomedicinal uses in the treatment of infectious diseases.

MATERIALS AND METHODS

General

A Bruker model AMX 500 MHz and 400 MHz spectrometers operating on a standard pulse system collected ^{1}H and ^{13}C NMR spectra. The instrument operated at 500 and 400 MHz in ^{1}H and 125 to 100 MHz in ^{13}C. $CDCl_3$ and CD_3OD were utilized as solvents and TMS was adopted as an internal standard.

Plant material

Plant materials *P. thonningii* stem bark were collected in June 2017 from Kwara State University surroundings, Malete, Kwara State, Nigeria (GPS coordinates are 8^0 42' 35.9215" N, 4^0 27' 59.5904" E). The plant was identified and authenticated at the Herbarium, Department of Botany, University of Lagos, Lagos State, Nigeria by Dr. O.O. Oyebanji, where a voucher specimen was deposited with assigned voucher number LUH: 7027.

Phytochemical Studies:

The air-dried pulverized stem bark of *P. thonningii* (600g) was extracted with 95% methanol (3 × 5 L × 72 h) at room temperature. The extracts were filtered, combined and concentrated under reduced pressure at 40 ^{o}C to afford 64 g of crude extract. 50 g of the concentrated methanolic extract was suspended in distilled water (200 mL) and partitioned with ethyl acetate (3 x 1 L) and *n*-butanol (3 x 1 L) to afford 15 g, 8 g and 24 g water, ethyl acetate and n-butanol fractions respectively. The ethyl acetate fraction was subjected to fractionation through a Silica gel column using *n*-hexane: ethyl acetate gradient as mobile phase to give 5 subfractions (PITH1–PITH 6) (100%:0%–0%:100%). These subfractions were subjected to further purification using Sephadex LH-20 with methanol as the eluting solvent to give 5 compounds. Fraction PITH 2 yielded 5 mg of Garcinielliptone Q (**1**); fraction PITH 5 yielded 5 mg of (20S, 24R)-Epoxydammarane-3β, 25-diol (**2**) fraction PITH 4 yielded 7 mg of Protocatechuic acid (**3**) and 50 mg Epicatechin **(4)**; while fraction PITH 1 yielded 70 mg β-Sitosterol (**5**).

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RESULTS AND DISCUSSION

The Results of the Isolated Compounds

Compound **1** was isolated as white amorphous solid. Its ^{1}H-NMR spectrum (**Figure 2**) indicated the presence of nine methyl groups at α_H 1.25, 1.54 (s, H-11), 0.96 (s, H-18), 0.77 (s, H-19), 1.14 (s H-21), 1.64 (s, H-26), 1.65 (s, H-27), 0.85 (s, H-28), 0.99 (s, H-29) and 0.87(s, H-30). The signal at 3.66 ppm is assignable to oxymethine proton-3 of a typical lanostane triterpenoid. The appearance of a signal at 5.13 ppm is attributed to H-24 of lanostane triterpenoid. The ^{13}C-NMR spectrum (**Figure 3**) of Compound **1** displayed an oxygenated methine at 79.2 ppm for C-3 and a C=C double bond at 124.8 and 131.7 ppm for C-24 and C-25 respectively. The appearance of signal at 75.8 ppm can be assigned to C-20. The ^{13}C-NMR spectrum of **1** revealed 30 carbon atoms which were sorted by DEPT-135^{o} (**Figure 4**) and HSQC-NMR (**Figure 5**) into nine methyl carbons (CH_3), ten methylene groups, (CH_2), four methine groups, (CH) and seven quaternary carbons (C). The information from the above extensive NMR spectroscopic studies closely matches those from literature [6]**.** Thus**,** the structure of Compound **1** was established and identified as Garcinielliptone Q.

Compound 2 was isolated as a white amorphous solid and its molecular structure was deduced from the ^{1}H-NMR, ^{13}C-NMR, DEPT-135^{0}, HSQC and HMBC spectra. The ^{1}H and ^{13}C -NMR spectra (**Figures 6 and 7)** displayed 52 hydrogen and 30 carbon atoms respectively which were sorted by DEPT 135^{o} (**Figure 8**) and HSQC (**Figure 9**) into eight methyl (CH_3), ten methylene, (CH_2), six methine, (CH) and six quaternary carbon (C) carbon atoms. In ^{1}H-NMR spectrum (**Figure 6**), the signals at δ_H 0.96, 0.84, 1.14, 1.39, 1.14, 0.99, 0.79 and 0.87 ppm are assigned to the protons of eight groups α_H (H-18, H-19, H-21, H-26, H-27, H-28, H-29 and H-30) respectively. The signal at 3.23 ppm is assignable to oxymethine proton-3 of a typical triterpenoid. The appearance of signal resonating at 3.75 ppm is ascribable to proton of ocotillol at position 24, suggesting that ocotillol group is 20*S*, 24*R* isomer and this clearly distinguishes it from its existential isomer 20*S*, 24*S*.

The ^{13}C-NMR spectrum (**Figure 7**) exhibited signal for oxymethine carbon at 79.1 ppm assignable to C-3 of triterpenoid. The signals characteristic of ocotillol unit appeared at α_C 86.6, 35.8, 27.5 and 83.4 ppm for C-20, C-22, C-23 and C-24 respectively. The signal oscillates at 71.6 suggesting a carbon (C-25) bearing hydroxyl group. The ^{1}H and ^{13}C NMR spectra of **Compound 2** were similar to those of [5]. Based on the above results, the structure of **compound 2** was established as (20S, 24R)-Epoxydammarane-3β, 25-diol.

Compound 3 was isolated as reddish brown solid. Its ^{1}H-NMR spectrum (**Figure 10**) showed three signals at δ_H 7.44, δ 7.42 and δ 6.79 assigned to aromatic protons (H-2, H-5 and H-6). The ^{13}C

ISSN 0795 - 22066

NMR spectrum (**Figure 11**) of compound **3** exhibited signals for three methine aromatic carbons at δ_C 117.69, 124.0, 115.7ppm. Two oxygenated carbon atoms on the aromatic ring appeared at 146.04 (C-3) and 151.5 (C-4) respectively while the most deshielded carbon appears at δ 170.23 ascribed for carbonyl carbon of carboxylic acid. Based on the above results, the structure of **compound 3** was established as protocatechuic acid because ^{1}H and ^{13}C NMR spectra of **Compound 3** are in agreement with [29].

Compound **4** was isolated as colourless solid. The ^{1}H and ^{13}C NMR spectral data (**Figures 12 and 13**) showed the presence of two aromatic systems A and B. The signals resonating at δ_H 5.94 and 5.95 ppm assignable to H-6 and H-8 protons attached on carbons C-6 (96.4) and C-8 (95.9) on ring A. Three signals at 6.98, 6.77 and 6.81 ppm are ascribable to H-2', H-5' and H-6' protons attached to carbons C-2' (C 115.3), C-5' (δ_C 115.9) and C-6' (δ_C 119.4) of ring B respectively. Two oxygenated carbon atoms on the aromatic ring A appeared at 157.3 (C-5) and 157.9 (C-7) and also signals at 145.7(C-4') and 115.9 (C-5') assignable to oxygenated carbon atoms on aromatic ring B respectively. Other signals were assigned to C-1', C-2' and C-3' of aromatic ring B at 132.2, 115.3 and 1164.9 ppm respectively. The appearances of signals at 4.81, 4.18 and 2.85, 2.75 are assigned to H-2, H-3 and H-4 on carbons (C-2) 79.8, (C-3) 67.4 and (C-4) 29.2. Based on the above results, Compound **4** was established as (-) - Epicatechin [31].

Compound 5 was isolated as a white waxy substance. Its ^{1}H-NMR spectrum (**Figure 14)** showed the presence of six methyl signals at δ 0.91(d, *J* = 7.5, 3.8 Hz, 3H), 0.83(d, 3H, *J* = 6.4Hz), 0.80(d, 3H *J* = 6.4 Hz, H-27), 1.44(s, 3H, H-18), 1.47(s, 3H, H-19) and 1.01(t, *J* = 2.7 Hz, 3H, H-29). The doublet at δ 0.83 (d, 3H, *J* = 6.4Hz, H-26), and 0.80 (d, 3H *J* = 6.4 Hz, H-27) were assigned to the protons of two methyl groups at C-26 and C-27, respectively. The signals on δ 1.44(s, 3H, H-18) and 1.47(s, 3H, H-19) are assignable to the protons of two tertiary methyl groups at position C-18 and C-19 respectively. The doublet at δ 0.91 (*d*, J = 7.5, 3.8 Hz, 3H) was attributed to a methyl group proton at position C-21. The proton signal at δ_H 1.01 (t, *J* = 2.7 Hz, 3H) could be assigned to the proton of primary methyl group at C-29. More so, a broad singlet at down field region of δ 5. 39(m, 1H), assigned to H-6, is indicative of the presence of double bond functionality between C-5 and C-6. The signal at 3.51 was assignable to oxygenated methine proton at position C-3. The ^{13}C-NMR (**Figure 15)** spectra of Compound **5** indicated a total of twenty-nine carbon signals. The signal at δ_C71.9 corresponds to oxygenated methine carbon (C-3). The carbon signals at δ 140.9 and 121.8 ppm indicated the presence of olefinic carbons at

ISSN 0795 - 22066

position C-5 and C-6 respectively. Based on the above spectral analysis and comparison with literature data [19], established compound **5** as β-sitosterol.

DISCUSSION

Phytochemical investigation of the methanolic extract of dried stem bark of *P. thonningii* led to the isolation of five compounds (**1-5**, Fig. 1). The isolated Garcinielliptone Q was isolated for the first time from *Piliostigma thonningii*.

Compound 3, protocatechuic acid has been reported to possess antimicrobial [12], [14], [17], [59], antioxidant [28], [30], [27], antihyperglycemic [22], [23], [8] and anti-inflammatory [18], antiapoptosis versus proapoptotic [34], [36] activities. Compound 4, epicatechin has been reported to have antibacterial property against *Helicobacter pyrlori* [4], antimicrobial [5] anti-inflammatory [32] and anticancer [56] activities. While, β-sitosterol a commonly encountered compound in many plants has been reported to exhibit a wide range of activities including antibacterial [24], anti-inflammatory [20], antidiabetic [7], anticancer [25] and cholesterol lowering property [26]. Also, compound 2, (20S, 24R)-epoxydammarane-3β, 25-diol has been reported to possess anticancer activity [5]. While no pharmacological activity has been reported for Garcinielliptone Q.

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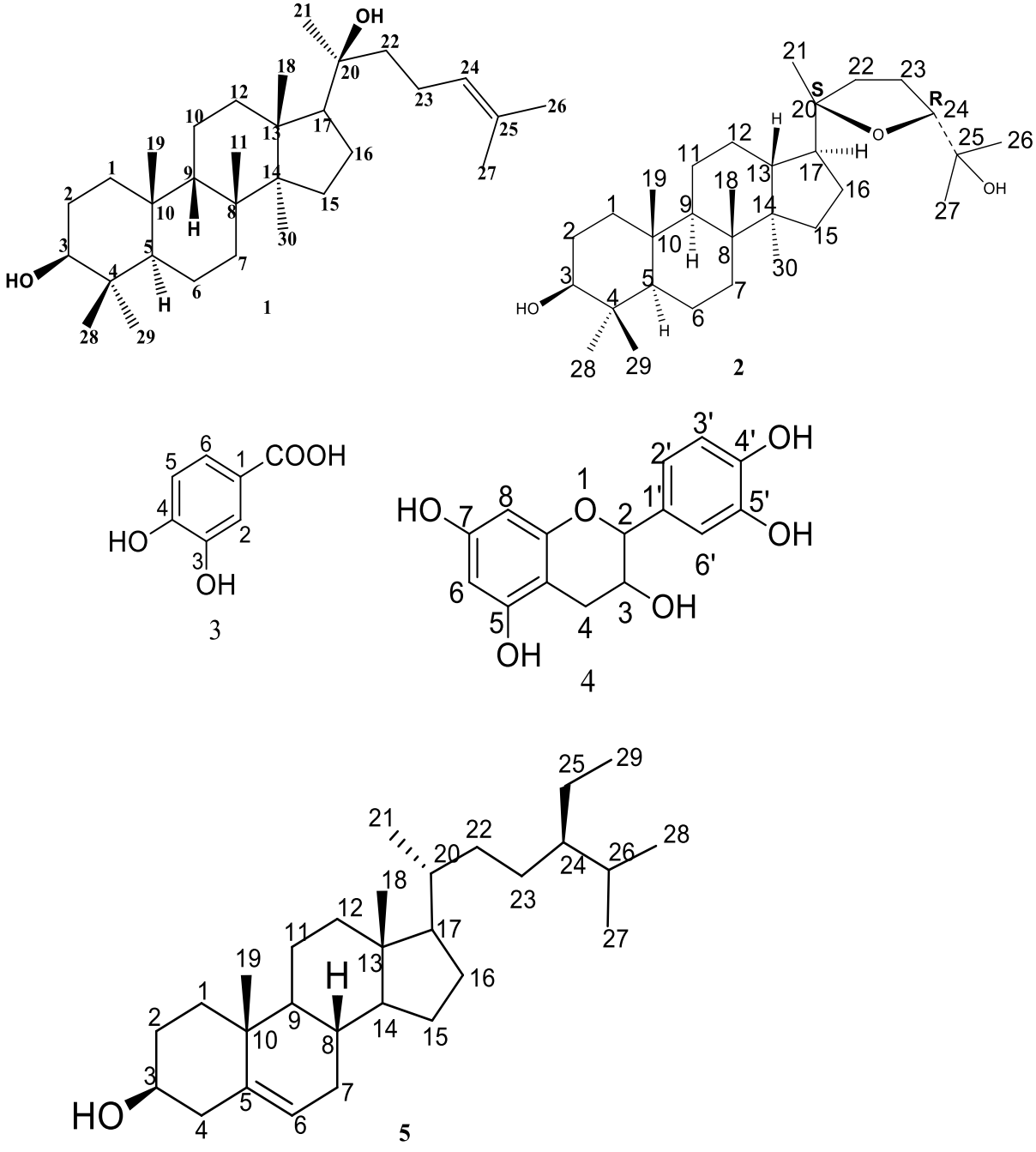

Figure 1: The structures of compounds isolated from stem bark extract of *P. thonningii*

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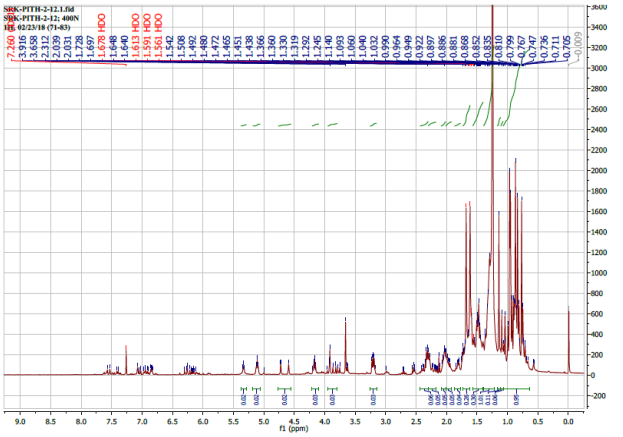

Figure 2: ^{1}H NMR spectrum of compound 1 Garcinielliptone O

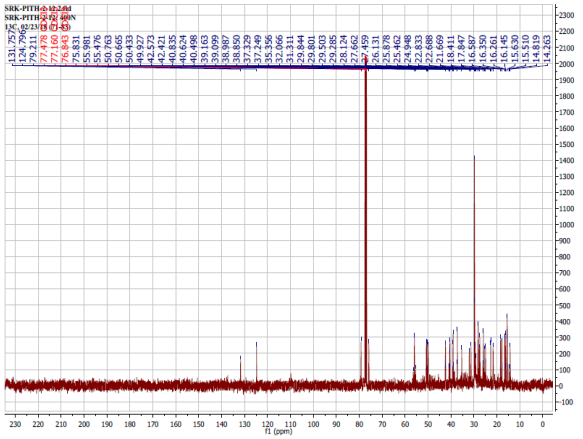

Figure 3: ^{13}C NMR spectrum of compound 1 Garcinielliptone O

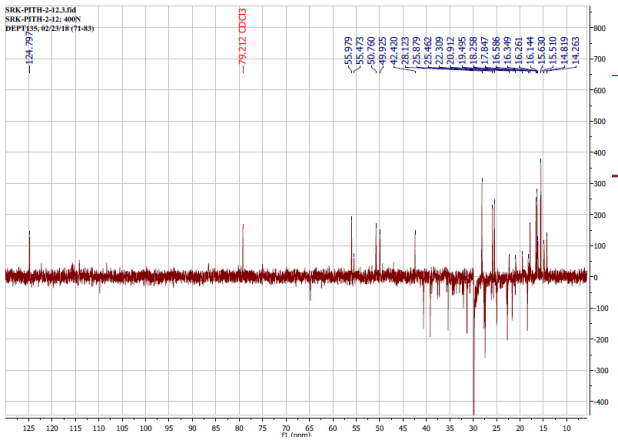

Figure 4: ^{13}DEPT-135^{o} NMR spectrum of compound 1**,** Garcinielliptone O

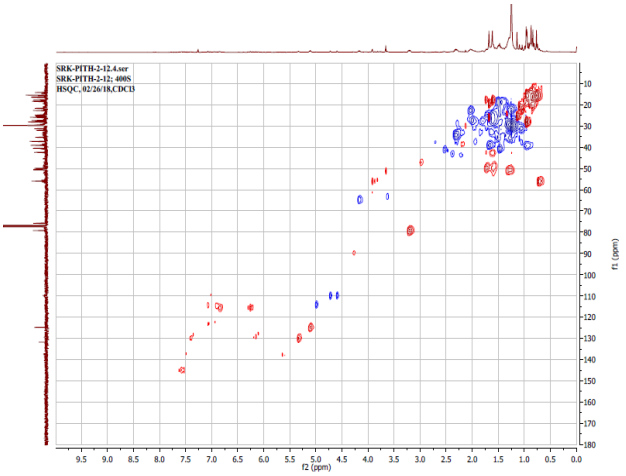

Figure 5: HSQC NMR spectrum of compound 1**,** Garcinielliptone Q

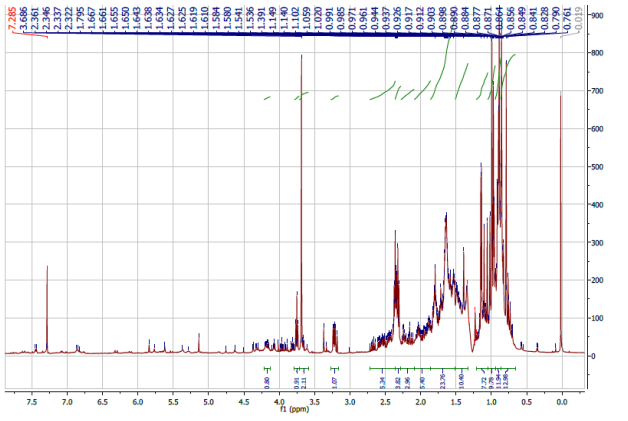

Figure 6: ^{1}H NMR spectrum of compound 2 (20S, 24R)-Epoxydammarane-3β, 25-diol.

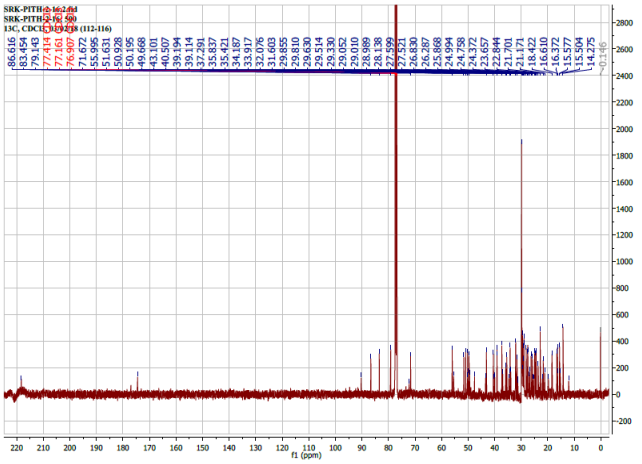

Figure 7: ^{13}C NMR spectrum of compound 2 (20S, 24R)-Epoxydammarane-3β, 25-diol.

ISSN 0795 - 22066

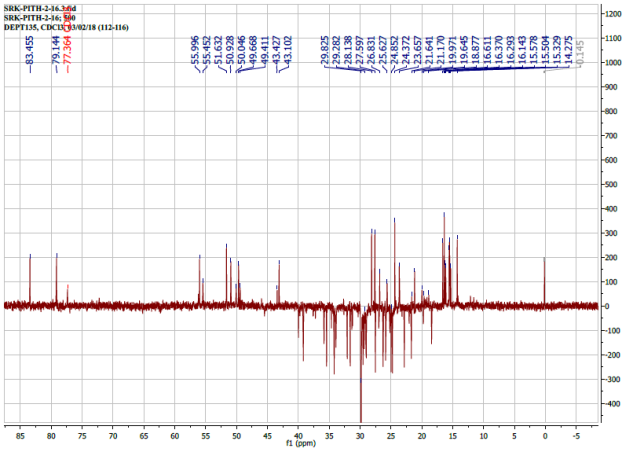

Figure 8: DEPT-135°-NMR spectrum of compound 2 (20S, 24R)-Epoxydammarane-3β, 25-diol.

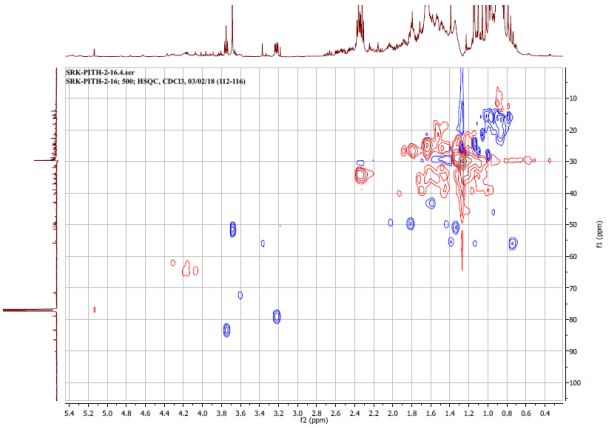

Figure 9: HSQC-NMR spectrum of compound 2 (20S, 24R)-Epoxydammarane-3β, 25-diol.

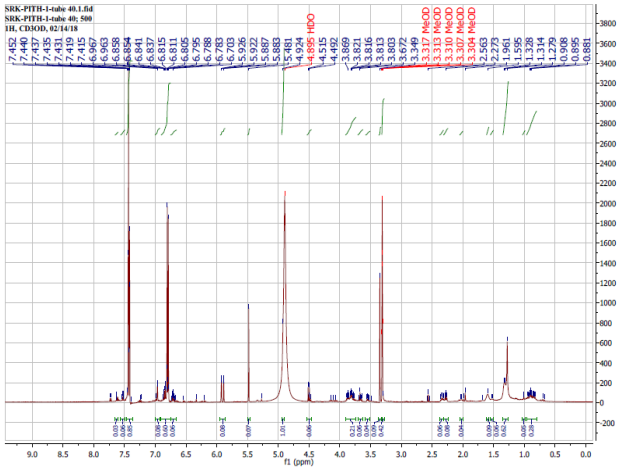

Figure 10: ^{1}H-NMR spectrum of compound 3 protocatechuic acid

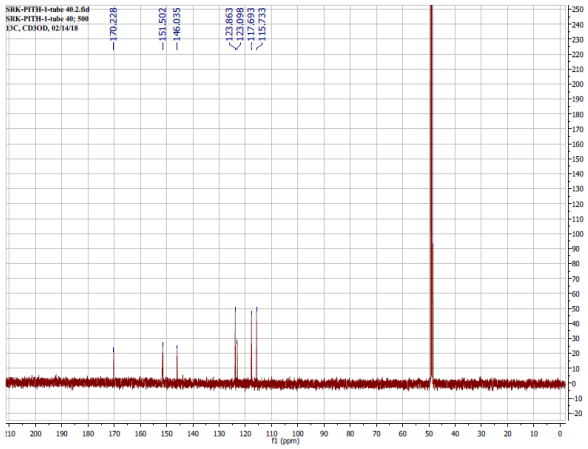

Figure 11: ^{13}C-NMR spectrum of compound 3 protocatechuic acid

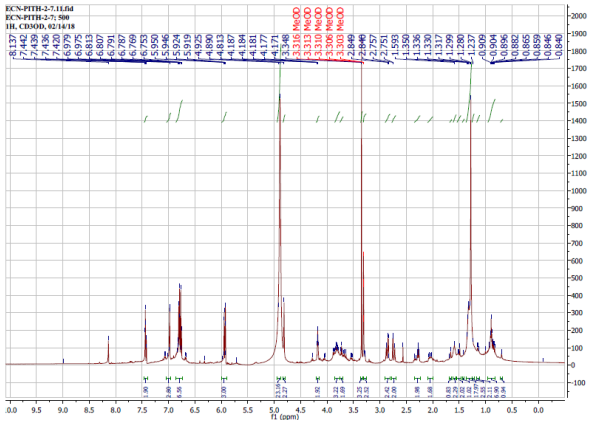

Figure 12: ^{1}H-NMR spectrum of compound 4 (-) - Epicatechin

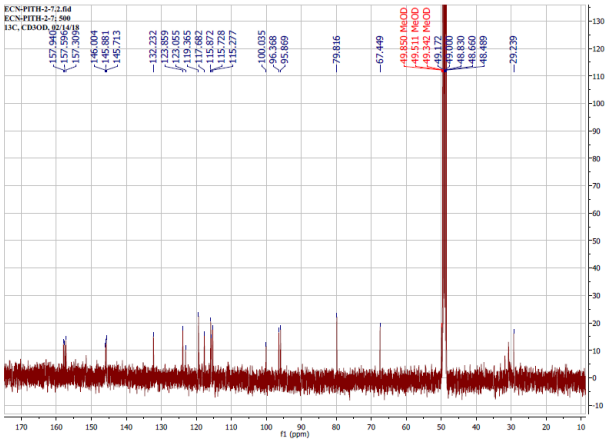

Figure 13: ^{13}C-NMR spectrum of compound 4 (-) – Epicatechin

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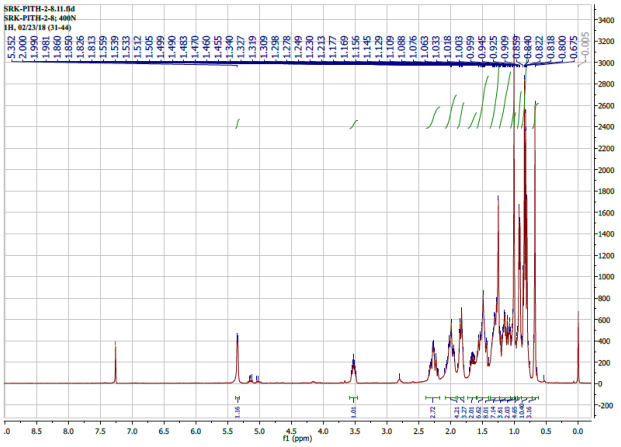

Figure 14: ^{1}H NMR spectrum of compound 5, β-sitosterol.

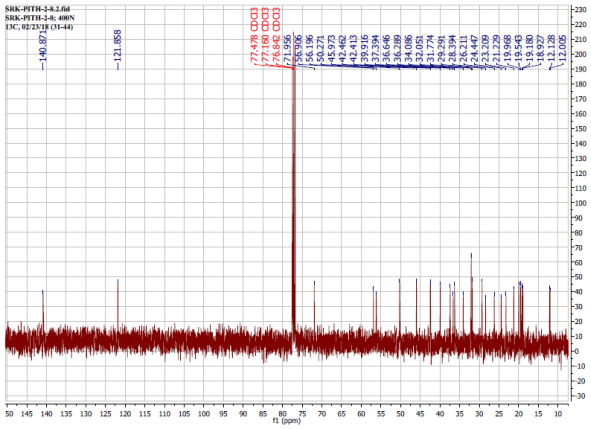

Figure 15: ^{13}C NMR spectrum of compound 5, β-sitosterol.

CONCLUSION

The present study isolated 5 phytochemicals from the crude methanol extract of stem-bark of *P. thonningii* and these compounds were structurally elucidated by spectroscopic analyses and compared with published data. The compounds could be responsible for the various biological activities of *Piliostigma thonningii*.

DECLARATION OF COMPETING INTEREST

The authors declare that there is no conflict of interest on the work reported in this paper.

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