(Research Article)

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Natural products with anticonvulsant potentials from *Syzygium aromaticum* (Myrtaceae)

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Abstract

Chemical investigation of the methanol crude extract of dried flower buds of *Syzygium aromaticum* (Myrtaceae) resulted in the isolation of seven known compounds; oleanolic acid (1), lignoceric acid (2), β-sitosterol (3), heneicosanoic acid (4), 5-hydroxy-7-methoxy-2-methylchromone (5), 5-hydroxy-7-methoxy-2,8-dimethylchromone (6) and vanillin (7). Their structures were elucidated using one-dimensional NMR spectroscopy and comparison with literature data. The crude extract, and compounds 1-6, revealed significant anticonvulsant activities against seizures induced by picrotoxin, pentylenetetrazole and bicuculline, respectively in mice. Compound 3 produced 100% protection, compound 2, and 5; on the other hand, each offered 83.33%, while the lowest protection came from compounds 1, 4, and 6 (16.66 – 50 00 %). This is the first report on the anticonvulsant activity of these isolated compounds, and the first report on the isolation of compounds 2, 4, 5, 6, and 7 from *Syzygium aromaticum*.

Keywords: *Syzygium aromaticum*; Anticonvulsant; Chemo-convulsants; Mice

1. Introduction

Plants have been used as medicines for several years [1, 2], either as crude drugs or other herbal formulations [3]. In developing countries, 80% of their population relies on herbal medicines as their first therapeutic strategy [4, 5]. Nowadays, in developed countries as well, herbal medicines have acquired some renewed interest [6. 7], as several plant extracts are being used as prescription drugs, especially in China, Germany, UK, and France [7]. Therefore natural products from plants continue to play an important role, as an endless source of drugs used to treat both communicable and non-communicable diseases [7, 8].

Epilepsy is a chronic brain disorder affecting over 50 million people globally [9]. Cameroon has one of the highest prevalence worldwide [10], and this disease accounts for a significant proportion of the world's disease burden *[11].* Anyone can develop epilepsy, as it affects people of all ages, social classes, races, and geographical locations [11, 12]. Several drugs have been used for the treatment of epilepsy. However, their limited supply, high cost, low efficacy, and severe side effects, such as; ischemia, hepatotoxicity, depression, sedation, cognitive impairment, and motor disability [13], have prompted the search for new drugs with better clinical profiles.

Faheem *et al*. 2022, stated that more than 200 plants have shown some anticonvulsant activity [14], and in Cameroon, several plants are used for treating epilepsy and convulsions [15, 16]. Nevertheless, very few of these plants have been studied scientifically [17]. Amongst these plants is *Syzygium aromaticum* (Myrtaceae family), also known as clove. It is

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a plant-derived spice used traditionally to treat a good number of diseases [18]. Previous phytochemical investigation revealed the presence of sesquiterpenes, monoterpenes, hydrocarbons, flavonoids, and phenolic acids [19] Furthermore, cloves possess antibacterial, antiviral, antifungal, antimicrobial, anticarcinogenic, and antioxidant activities [20]. The essential oil of *Syzygium aromaticum* [21], and one of its major constituents, eugenol, have shown interesting anticonvulsant properties [18]. Based on these findings, we embarked on the phytochemical investigation of this plant and seven known compounds, namely; oleanolic acid (**1**), lignoceric acid (**2**), β-sitosterol (**3**), heneicosanoic acid (**4**), 5-hydroxy-7-methoxy-2-methylchromone (**5**), 5-hydroxy-7-methoxy-2,8-dimethylchromone (**6**) and vanillin (**7**) were isolated and six of them showed very interesting anticonvulsant effects. Their structures are presented in Figure **1** below.
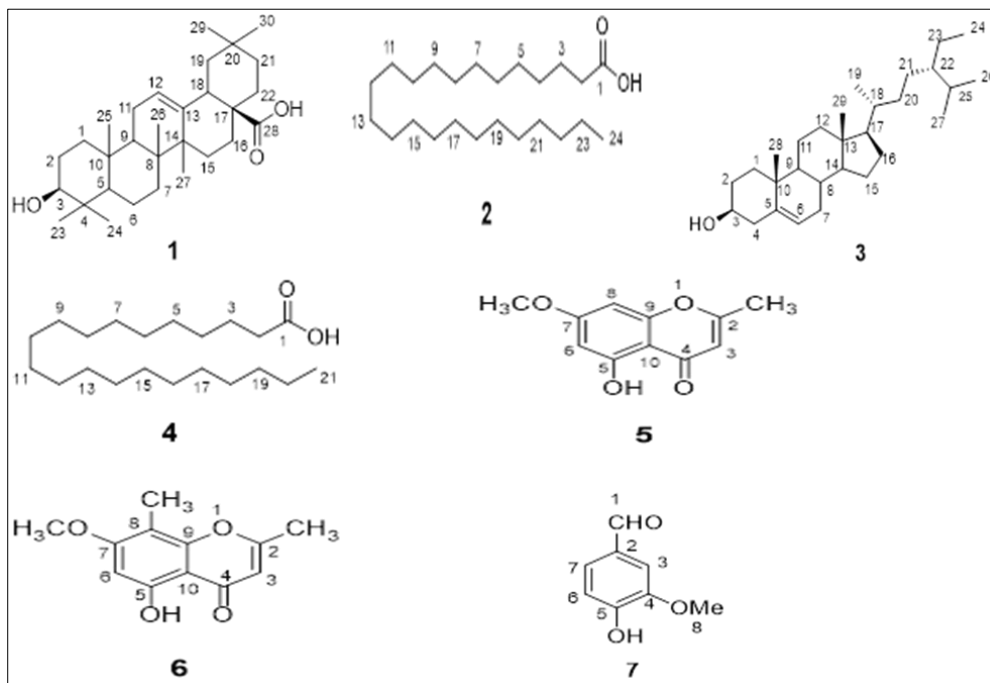

Figure 1 Structures of isolated compounds 1- 7 from *S. aromaticum*

2. Material and methods

2.1. General experimental procedures

The ^{1}H and ^{13}C NMR spectra of the isolated compounds were recorded at 400 and 175 MHz, respectively, using 5-mm sample tubes on a 400 MHz Varian Mercury NMR Spectrometer at the Center for Advanced Materials Characterization in the University of Oregon, USA. The chemical shift values were quoted on the scale in parts per million (ppm), with tetramethylsilane (TMS) as internal standard. The solvent used for measurement at room temperature was deuterated chloroform (CDCl$_3$). Melting points of the isolated compounds were recorded using an Automatic Digital Melting point apparatus (03012-90). Thin layer chromatographic (TLC) analysis, was done on pre-coated aluminium sheets with silica gel (Alugramsil/UV$_{254}$) purchased from Sigma-Aldrich, St. Louis, USA. Zones on these plates were either observed under UVGL-58 lamp at 254/365 nm, or exposed to iodine vapor in an iodine chamber. Open column chromatography was performed with a glass column using Merck silica gel 60 (particle size 60-200 μm) and alternatively Sephadex (LH-20).

For biological screening, Albino Swiss mice both male and female (18-22 g) were gotten from the Animal Centre, University of Buea, Cameroon. These mice were kept in plastic cages at room temperature and nourished with balanced rodent pellet diet and water *ad libitum* and were acclimatized for one week before using them for experiments. All the trials were carried out following the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (NIH publication No. 85-23, revised 1996) and received an approval from the University of Buea - Institutional Animal Care and Use Committee (UB-IACUC N° 008/2019). The following chemoconvulsants: picrotoxin (PIC), bicuculline (BICU) and pentylenetetrazole (PTZ) used, were purchased from Sigma-Aldrich, St. Louis, USA, while the drugs used; clonazepam (CLZ) and diazepam (DZP) were purchased from Roche, France.

2.2. Plant Material

Dried flower buds of the clove tree, *Syzygium aromaticum* were purchased from the Limbe main market, South West Region of Cameroon. It was authenticated by the botanist, Mr. Ndive Elias and a specimen with voucher number SCA786 was deposited at the Herbarium of the Limbe Botanical Garden.

2.3. Extraction and compound isolation

The dried flower buds of *Syzygium aromaticum* (3.70 kg) were pulverized and extracted with 15 L of methanol three times (15 L×3) with occasional shaking. The extract was filtered, and concentrated under reduced pressure until the MeOH dried up, using the rotary evaporator (BUCHI Rotavapor R-114) at 55 °C. This afforded a dark brown crude extract (150 g), which showed potential antiepileptic activity. About 140 g of this crude extract was suspended in 200 mL of methanol absorbed onto 200 g of silica gel 60 (particle size 60-200 μm), and dried under reduced pressure. The resulting brown extract was then subjected to gradient column chromatography packing with an open column filled with 900 g silica gel 60 (particle size 60-200 μm). Elution was done using a solvent mixture of increasing polarity starting from hexane, ethyl acetate/hexane (EA/Hex) and methanol (MeOH). Seventy (70) fractions of 150 mL each were obtained from this separation process. They were then combined into seven groups based on thin layer chromatography (TLC) profile. Group B (obtained from 10 % EA/Hex) was separated repeatedly using gel filtration chromatography (Sephadex LH-20) with 100% MeOH as eluent to afford compound **1** (620 mg), **2** (350 mg), **3** (800 mg) and **4** (200 mg). Group C (obtained from 20 % EA/Hex), was further chromatographed by silica gel column chromatography eluting with increasing polarity of ethyl acetate/ hexane (20:80 EA/Hex – 80:20 EA/Hex), to afford three sub-fractions: S-1 (14.0g), S-2 (10.4 g) and S-3 (22.1 g). Further separation of sub-fraction S-3 by Sephadex LH-20 with 100% MeOH as eluent yielded compound **5** (450 mg). Group D (obtained from 30% EA/Hex) was also purified repeatedly by silica gel column chromatography to afford compound **6**.Group A (obtained from 5% EA/Hex) was then subjected to repeated column chromatography on silica gel eluted with hexane to afford compound **7** (5 mg). Compounds **1**- **6** were all tested for their anticonvulsant activity.

2.3.1. Oleanolic acid (1)

White powder; mp: 305.6-306.6°C (literature mp 306-308°C);^{1}HNMR (400 MHz, CDCl$_3$): δ_H: 1.01 and 1.57 (H-1), 1.88 (H-2), 3.44 (dd, H-3), 0.85 (d, H-5), 1.57 and 1.29 (H-6), 1.53 and 1.36 (H-7), 1.71 (t, H-9), 1.96 (H-11), 5.30 (s, H-12), 1.22 and 2.19 (H-15), 1.96 and 2.12 (t, H-16), 3.20 (dd, H-18), 1.83 and 1.32 (H-19), 1.46 and 1.24 (H-21), 1.82 and 2.04 (H-22), 1.24 (s, H-23), 1.01 (s, H-24),0.97 (s,H-25), 1.04 (s, H-26), 1.29 (s, H-27), 0.97 (s. H-29), 1.01 (s. H-30) ppm;^{13}C-NMR (175 MHz, CDCl$_3$): δ_C 39.6 (C-1), 28.1 (C-2),78.0 (C-3), 38.5 (C-4), 54.9 (C-5), 18.0 (C-6), 32.9 (C-7), 40.8 (C-8), 47.4 (C-9), 36.8 (C-10), 23.2 (C-11), 121.9 (C-12), 143.5 (C-13), 41.5 (C-14), 27.5 (C-15), 23.5 (C-16), 45.8 (C-17), 52.35 (C-18), 39.9 (C-19), 39.9 (C-20), 30.4 (C-21), 36.8 (C-22), 28.1 (C-23), 15.2 (C-24), 15.7 (C-25), 16.9 (C-26), 23.2 (C-27), 180.1 (C-28), 17.0 (C-29), 21.1 (C-30); The molecular formula is C$_{30}$H$_{48}$O$_3$.

2.3.2. Lignoceric acid (2)

White solids; mp: 84.0°C (literature mp 84.2°C); ^{1}H NMR(400 MHz, CDCl$_3$) δ_H: 2.35 (2H, t, H-2), 1.64 (2H, m, C-3), 1.26 (40 H, br s, 20 × CH$_2$), 0.88 (3H, t, Me-20); ^{13}C NMR (175 MHz, CDCl$_3$): δ_C 179.7 (C-1), 34.0 (C-2), 31.9 (C-22), 29.6(18 × CH$_2$), 24.7 (C-3), 22.7 (C-23), 14.1 (Me-24); The molecular formula is C$_{24}$H$_{48}$O$_2$.

2.3.3. β-Sitosterol (3)

White powder; mp 134-136 ºC;^{1}HNMR (400 MHz, CDCl$_3$): δ_H 5.32 (m, 1H, H-6), 4.88 (s, 1OH, H-3), 3.50 (m, 1H, H-3), 1.00 (s, 3H, H-**29**), 0.92 (d, 3H, H-**19**), 0.84 (t, 3H, H-**24**), 0.83 (d, 3H, H-**26**), 0.81 (d, 3H, H-**27**), 0.67 (s, 3H, Me-**28**), 1.11–2.25 (remaining –CH$_2$-) ppm; ^{13}C-NMR (175 MHz, CDCl$_3$): δ 140.8 (C-5), 121.7 (C-6), 71.3 (C-3), 56.8 (C-14), 56.1 (C-17), 50.2 (C-9), 45.8 (C-22), 42.3 (C-4, 13), 42.2 (C-18), 39.8 (C-12), 37.4 (C-1), 36.6 (C-10), 34.0 (C-20), 31.0 (C-2), 32.0 (C-8), 29.7 (C-25), 28.2 (C-16), 26.1 (C-21), 24.4 (C-15), 23.2 (C-23)21.0 (C-11), 20.1 (C-26), 19.8 (C-27), 19.4 (C-28), 19.0 (C-19), 12.3 (C-24), 12.1 (C-29); The molecular formula is C$_{29}$H$_{50}$O.

2.3.4. Heneicosanoic acid (4)

White crystalline solid; mp 47–48ºC (literature mp 48–50ºC); ^{1}H NMR (400 MHz, CDCl$_3$): δ0.86 (t, Me-21), 1.23 (br, 34, 17×CH$_2$), 1.62 (m, 1×CH$_2$CH$_2$COOH) and 2.34 (t, CH$_2$COOH);^{13}C-NMR (175 MHz, CDCl$_3$): δ173.1 (C-1), 33.7 (C-2), 31.8 (C-19), 29.6-29.0 (15 x CH$_2$), 24.6 (C-3), 22.6 (C-20), 14.1(Me-21) ppm; The molecularformula is C$_{21}$H$_{42}$O$_2$.

2.3.5. 5-hydroxy-7-methoxy-2-methylchromone (5)

Mp 106 -108 ºC; ^{1}HNMR (400 MHz, CDCl$_3$): δ_H 12.67 (1H, s, C-5 OH), 6.32 (1H, d, H-8), 6.31 (1H, d, H-6), 6.00 (1H, s, H-3), 3.82 (3H, s, C-7 OMe), 2.32 (3H, s, Me-2);^{13}C-NMR (175 MHz, CDCl$_3$): δ 182.5 (C-4), 166.8 (C-7), 165.4 (C-5), 162.2 (C-8a), 158.2 (C-2), 108.6 (C-3), 105.1 (C-4a), 97.9 (C-6), 92.5 (C-8), 55.5 (OMe-7), 20.8 (Me-2); The molecular formula is C$_{11}$H$_{10}$O$_4$.

2.3.6. 5-hydroxy-7-methoxy-2, 8-dimethylchromone (6)

^{1}HNMR (400 MHz, CDCl$_3$): δ_H 12.72 (1H, s, 2-OH), 6.34 (1H, s, H-6), 6.00 (1H, s, H-3), 3.85 (3H, s, 7-OMe), 2.35 (3H, s, H-11), 2.12 (3H, s, 8-Me); ^{13}C-NMR (175 MHz, CDCl$_3$): $\delta\delta$183.3 (C-4), 166.8 (C-2), 163.2 (C-7), 160.2 (C-5), 155.0 (C-9), 108.3 (C-3), 104.6 (C-10), 103.4 (C-8), 94.8 (C-6), 56.1 (OMe-7), 20.6 (C-11), 7.6 (Me-8); The molecular formula is C$_{12}$H$_{12}$O$_4$.

2.3.7. Vanillin (7)

^{1}H NMR (400 MHz, CDCl$_3$): δ_H 9.81 (1H, s, H-1), 7.44 (1H, dd, H-7), 7.42 (1H, d, H-3), 7.03 (1H, d, H-6), 3.98 (3H, s, 4-OCH$_3$); ^{13}C-NMR (175 MHz, CDCl$_3$): δc 191.0 (C-1), 151.7 (C-5), 147.6 (C-4), 129.9 (C-2), 127.6 (C-7), 114.4 (C-6), 108.8 (C-3), 56.2 (C-8); The molecular formula is C$_8$H$_8$O$_3$.

2.4. Anticonvulsant activity

2.4.1. Picrotoxin-induced convulsion test

Fourteen groups of six animals each, received;the crude extract (5, 10, 15 and 20 mg/kg, i.p.), the pure compounds **1-6** [20 mg/kg; intraperitoneally (i.p.)], or clonazepam (1 mg/kg, i.p.). After an hour, intraperitoneal (i.p) injection of 7.5 mg/kg picrotoxin (PIC) was injected into the mice to induce tonic-clonic seizures. 15 min later, the protective effects of the different treatments were recorded. Mice that did not convulse within the 15 min of observation were, qualified as protected [22].

2.4.2. Pentylenetetrazole-induced convulsion test

Fourteen groups of six animals received treatment as discussed above. However, in this case, the positive control group was treated with 0.1 mg/kg clonazepam (i.p). Intraperitoneal injection of 70 mg/kg pentylenetetrazole (PTZ) was injected into the mice, to induce tonic-clonic seizures. The protective effects of the different treatments given 1 h before PTZ injection were recorded. Mice that did not convulse within the 10 min of observation were qualified as protected [23].

2.4.3. Bicuculine-induced convulsions test

Fourteen groups of six animals each, received;the crude extract (5, 10, 15 and 20 mg/kg, i.p.), the pure compounds **1-6** (20 mg/kg; i.p.), or diazepam (3 mg/kg, i.p.). After one hour, the mice were injected with an intraperitoneal injection of 4 mg/kg bicuculine (BICU) to induce tonic-clonic seizures. Thirty minutes (30 min) later the protective effects of the different treatments were recorded. Mice that did not convulse within the 30min of observation were qualified as protected [22].

2.5. Statistical analysis

The data were expressed as mean ± S.E.M. Graph Pad Prism Software version 4.03 was used to analyze the data. Statistical analysis of data was executed using One-way ANOVA, followed by Dennett's post-hoc test.The Fisher exact test (two tail) was used to compare the percentage of protected mice in each case.A level of $p < 0.05$ was viewed as statistically significant [24].

3. Results and discussion

The dried flower buds of *Syzygium aromaticum* were pulverized and extracted with methanol. Repeated column chromatography of the methanol crude extract afforded seven known compounds. These compounds were identified as; oleanolic acid (1), lignoceric acid (2), β-sitosterol (3), heneicosanoic acid (4), 5-hydroxy-7-methoxy-2-methylchromone (5),5-hydroxy-7-methoxy-2,8-dimethylchromone (6) and vanillin, by comparison of their spectroscopic data with literature values.

3.1. Structural identification of compounds

- Compound 1 was isolated as white powder. It had a positive Liebermann-Burchard test, indicated by the formation of a red-violet colored ring and had a melting point of 305.6-306.6°C in accordance with published literature (306-308°C). From its 1HNMR spectrum (SI Fig 1, SI = Supplementary Information), an olefinic proton of C-12 occurred at δ 5.30. Several tertiary methyl groups were observed at δ 1.24 (s, H-23), 1.01 (s, H-24), 0.97 (s, H-25), 1.04 (s, H-26), 1.29 (s, H-27), 0.97 (s. H-29) and 1.01 (s. H-30). This suggested an olea-12-ene skeleton. The signal at δ 3.44 signified a methine proton which is compatible with at least one hydroxy group attached on the olea-12-ene skeleton. The 13C NMR spectrum (SI Fig 2) displayed 30 signals. Signals of interest included the carbonyl C-atom signal at δ 180.1, and one double bond at δ 121.9 (C-12) and δ 143.5 (C-13) as well as a signal related to an oxygenated C-atom at δ 78.0. Other signals from C-18 to C-22 occurred at δ 52.35 (C-18), 39.9 (C-19), 39.9 (C-20), 30.4 (C-21), 36.8 (C-22) suggesting the presence of oleanolic acid. Its 13CNMR and 1HNMR data are identical with data published in literature [25]. Thus compound 1 is therefore oleanolic acid.

- Compound 2 was isolated as white solids. Its melting point was 84 ºC. The 1H NMR (SI Fig 3), revealed a characteristic long peak at δ 1.26, the typical 40 H of the long chain which was evident as a multiplet that integrated for one proton. A two proton triplet centered at δ 2.35 was ascribed to the methylene protons attached on C-2.On the other hand the triplet of three proton intensity at δ 0.88 could be assigned to the terminal methyl group at C-24. While the multiplet at δ 1.64 represents the methylene protons attached to C-3. The 13C NMR spectrum (SI Fig 4), exhibited signals for 24 carbon atoms. Diagnostic peaks for a long chain fatty acid appeared at δ 179.7, which was assigned to C-1 the carbonyl C-atom of the acidic group and at δ 14.1 for the terminal C-atom C-24. Signals for C-4 to C-21 appeared as a single long peak multiplet at δ 29.6. The C-atoms alpha and beta to the terminal C-atom appeared at δ 22.7 (C-23) and δ 31.9 (C-22) respectively.While the carbon atoms alpha and beta to the carbonyl group, were observed at δ 34.0 ppm and δ 24.7 respectively. Therefore the 13C NMR showed 24 C-atom signals which included 22 methylenes, 1 methyl and 1 quaternary carbon atom. The above spectra characteristics are very similar to those observed for lignoceric acid previously isolated in literature [26].

- Compound 3 was isolated as a white powder. This compound showed a positive Liebermann-Burchard test, indicating its sterol nature. The 1HNMR spectrum (SI Fig 5) showed signals at δ 0.67 and δ 1.00 (3H each) which were attributed to the two tertiary methyl groups at C- 28 and C-29 respectively. Also, the triplet of three proton intensity at δ 0.84 was attributed to the primary methyl group at C- 24. Two doublets centered at δ 0.83 and δ 0.81 could be assigned to two methyl groups at C-26 and C -27 respectively. While the doublet, at δ 0.92 was assigned to the methyl group at C-19. Also, the typical olefinic proton of the steroidal skeleton was revealed as a multiplet at δ 5.32 and there were no protons corresponding to the double bond between C-20/C-21. The 13C NMR spectrum (SI Fig 6) has shown interesting signals 140.8 and 121.7 ppm, which were attributed to C5 and C6 double bonds respectively. The value at 19.4 ppm corresponds to the angular carbon atom (C-28). This 13C-NMR spectrum showed twenty nine carbon signals, which include six methyls, eleven methylenes, nine methines and three quaternary carbons with a hydroxyl group. The above spectral characteristics are similar to those observed for β–sitosterol in literature [27].

- Compound 4 was isolated as white crystalline solids. Its melting point was 47- 48 ºC. Just as in SA2 the 1H NMR spectrum (SI Fig 7) revealed a long peak of multiplet at δ 1.23, the typical 34 H of the long chain was evident as a multiplet that integrated for one proton. A triplet centered at δ 2.34 could be attributed to two methylene protons attached to C-2.The triplet of three proton intensity at δ 0.86 was assigned to the terminal methyl group at C-21. While the multiplet at δ 1.62 represents the methylene protons attached to C-3. The 13C NMR spectrum (SI Fig 8), showed a recognizable signal at δ 173.1, which can be assigned to C-21 the carbonyl C-atom of the acidic group.The terminal C-atom C-21 appeared at δ 14.1. Signals for C-4 to C-18 appeared as a single long peak multiplet at δ 29.6. The C-atoms alpha and beta to the terminal C-atom appeared at δ 22.6 (C-20) and δ 31.8 (C-19) respectively.While the carbon atoms alpha and beta to the carbonyl group, were observed at δ 33.7 ppm and δ 24.6 respectively. Therefore the 13C NMR showed 21 C-atom signals which included 19 methylenes, 1 methyl and 1 quaternary carbon atom. The above spectra characteristics are similar to those observed for heneicosanoic acid previously isolated from literature [28].

- Compound 5 was obtained as a yellow powder. Its 1H NMR spectrum (SI Fig 9) revealed a distinct singlet at δ 2.32 was assigned to the methyl group protons at C-2.The characteristic H-3 signal of the chromone nucleus appears at δ 6.00 and the aromatic region of the spectrum displayed the presence of two aromatic protons at δ 6.31 and δ 6.32 assigned to H-6 and H-8 respectively. The singlet at δ 3.82 was assigned to the methoxy group attached to the chromone ring at C-7. The low field singlet at δ 12.72 was attributed to the hydroxyl group at C-5. These features were further supported by the 13C NMR spectrum (SI Fig 10) data which revealed the expected 11 carbon signals. The methyl carbon atom resonated at δ 20.8 while the methoxy carbon atom

resonated at δ 55.5 and the carbonyl carbon atom resonated at δ 182.5. This spectroscopic data was in line with that reported in literature [29], for 5-hydroxy-7-methoxy-2-methylchromone also known as eugenin.

- Compound 6 was also obtained as a yellow powder. The 1H and 13C NMR spectra data (SI Fig 11 and SI Fig 12 respectively) revealed the presence of three methyls (one oxygenated), two methine and seven non protonated carbon atoms, which were quite similar to those reported for 5-hydroxy-7-methoxy-2-methylchromone. Discreet analysis and comparison of the NMR data disclosed that compound 6 and 5-hydroxy-7-methoxy-2-methylchromone (5), shared the same benzopyran-4-one ring but with different substituents. In compound 6, the aromatic proton at C-8 was replaced with a methyl group, which now resonated at δ 2.12 ppm. Compound 6 was determined to be 5-hydroxy-7-methoxy-2,8-dimethylchromone as also known as isoeugenitin [29].

- Compound 7 was obtained as a white solid. It had a melting point of 81- 83 oC. The 1H NMR spectrum (SI Fig 13) exhibited 8 proton resonates. The prominent peak at δ 9.81 was demonstrative of an aromatic aldehyde proton while the singlet which occurred at δ 3.98 was ascribed to the methoxy group attached on the aromatic ring at C-4. An ABX-trisubstituted benzene ring system consisting of H-6 (δ 7.03), H-7 (δ 7.44) and H-3 (δ 7.42) was also revealed on the 1H NMR spectrum.From the 13C NMR spectrum (SI Fig 14) a carbonyl carbon atom was observed at δ 191.0, while the methoxy carbon atom resonated at δ 56.2. Several signals corresponding to aromatic carbon atoms were observed at δ 129.9, 108.8, 147.6, 151.7, 114.4 and δ 127.6. This was attributed to C-2, C-3, C-4, C-5, C-7 and C-7 respectively. These features were in close agreement with data previously published in literature [30]. Compound 7 was therefore identified as vanillin.

3.2. Anticonvulsant activity of the methanol crude extract

The methanol crude extract of the dried flower buds of *Syzygium aromaticum* was evaluated for its anticonvulsant effects, in mice model seizures induced by picrotoxin (PIC), pentylenetetrazole (PTZ) and bicuculline (BICU), respectively.

3.2.1. Picrotoxin Test

The extract offered 66.66 % (p < 0.01) and 100 % (p < 0.001) protection against PIC-induced convulsions at the respective doses of 15 and 20 mg/kg, The anticonvulsant drug clonazepam produced 100% protection against PIC-induced seizures (Fig 2).

3.2.2. Petylenetetrazole Test

Clonazepam offered total protection against PTZ-induced convulsion (p <0.001). In a similar manner the crude extract at a dose of 20 mg/kg completely protected mice against PTZ-induced Convulsions (p < 0.001). The doses 10 and 15 mg/kg offered 50.00% and 66.66% (p < 0.01) protection, respectively (Fig 2).

3.2.3. Bicuculine Test

The crude extract significantly antagonized Bicuculine-induced convulsions in mice. 50.00% and 100% of mice were protected at the doses of 15 and 20 mg/kg respectively. The crude extract and the drug Diazepam (3 mg/kg, p < 0.001) both totally protected the mice against BICU-induced convulsions (Fig 2).

This crude extract (20 mg/Kg) showed very significant anticonvulsant activity, as it offered 100% protection against epileptic seizures, chemically induced in mice, with ED_{50} values of 2.35 mg/Kg for PIC test, 1.92 mg/Kg for PTZ test and 2.33 mg/Kg for BICU test. This indicated that the extract had very interesting anticonvulsant compounds. Given the positive results from the crude extract, the isolated compounds were then screened using the same experimental models of epileptic convulsions.
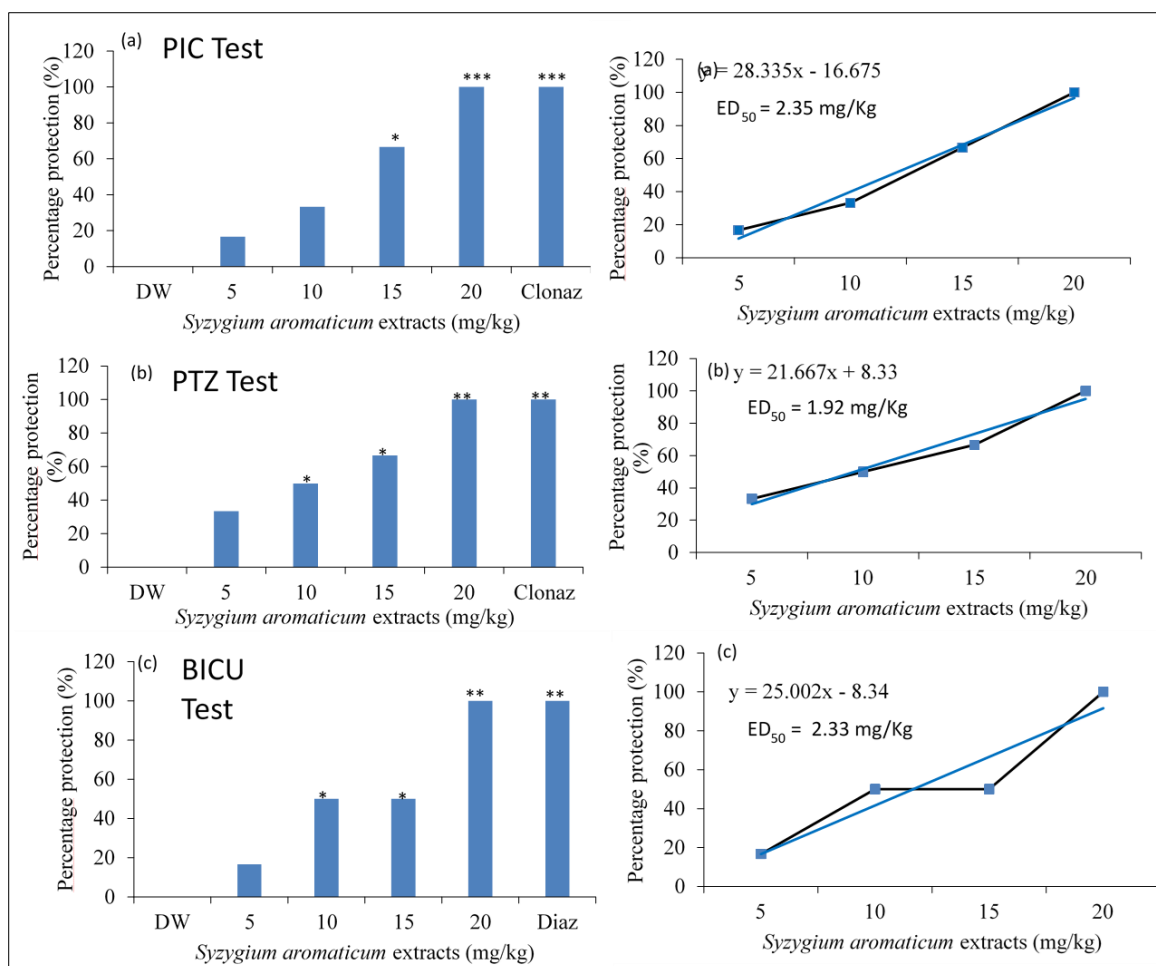
N = 6 per dose *p<0.05, ***p<0.001 (Fisher exact test: two tail). DW = distilled water, Clonaz = clonazepam,Diaz = diazepamLegend: Percentage protection of mice and dose-response values (ED50) from picrotoxin test (PIC Test); Percentage protection of mice and dose-response values (ED50) from pentylenetetrazol test (PTZ Test); Percentage protection of mice and dose-response values (ED50) from bicuculine test (BICU Test)

Figure 2 Effects of the crude extract of *Syzygium aromaticum* against seizures induced by picrotoxin, pentylenetetrazol or bicuculine
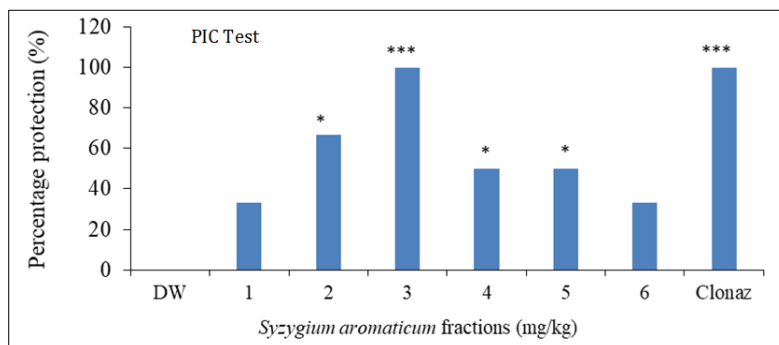
3.3. Anticonvulsant Activity of the Pure Compounds 1–6

3.3.1. Picrotoxin-induced convulsion

Table 1 Effects of compounds 1- 6 on the latency to the seizure induced by picrotoxin (PIC)

Treatment	Dose (mg/kg)	Latency to the seizure(min) [mean±SEM]	PercentageProtection (%)
Control (DW)	-	3.91±1.29	0
1	20	3.87±0.49	33.33
2	20	12.63±0.38**	66.66
3	20	No Convulsion	100.00
4	20	6.32±0.72*	50.00
5	20	12.42±1.95**	50.00
6	20	13.17±1.01**	33.33
Clonaz	1	No Convulsion	100.00

Values represent the mean ± S.E.M for 6 animals per group. *p < 0.05, ***p < 0.001compared to control group (ANOVA followed by Dunnett's post-hoc test) ANOVA: Analysis of Variance; SEM: Standard Error of the Mean

As shown in Table 1 and Fig 3, compounds 1- 6 (20 mg/kg) offered 33.33 to 100.00% protection against convulsions, induced by PIC (7.5 mg/kg, i.p). The reference drug clonazepam (1 mg/kg) also offered 100% protection against convulsions, given none of the mice displayedd tonic-clonic seizures induced by PIC. Statistical analysis showed that compounds 1- 6 (20 mg/kg i.p) increased the latency to the tonic-clonic components of PIC-induced seizures in the unprotected mice. Therefore at 20 mg/kg, compounds 1- 6 reduced the rate of seizures significantly, while the control group had a seizure rate of 100% given it offered no protection (Table 1).

N = 6 per dose, *p<0.05, ***p<0.001 (Fisher exact test: two tail). DW = distilled water, Clonaz = clonazepam
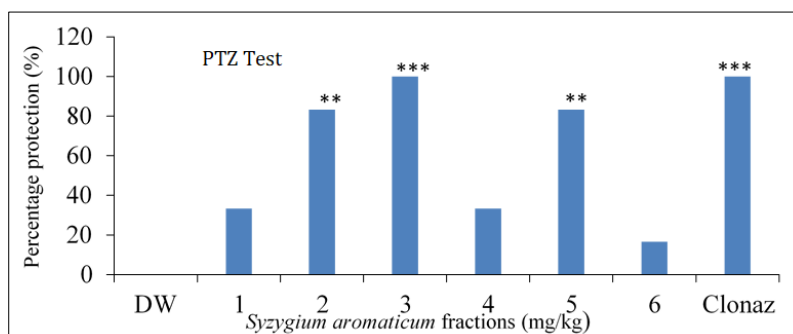
Figure 3 Effects of compounds 1 –6 on the percentage protection of mice against seizuresinduced by picrotoxin

3.3.2. Pentylenetetrazole-induced convulsion

Table 2 Effects of compounds 1- 6 on the latency to the seizure induced by PTZ

Treatment	Dose (mg/kg)	Latency to the seizure(min) [mean±SEM]	PercentageProtection (%)
Control (DW)	-	3.67±0.84	0
1	20	4.96±0.93	33.33
2	20	9.53±0.00**	83.33
3	20	No Convulsion	100.00
4	20	7.87±0.86*	33.33
5	20	8.77±0.35*	83.33
6	20	6.48±1.38*	16.66
Clonaz	1	No Convulsion	100.00

Values represent the mean ± S.E.M for 6 animals per group. *p < 0.05, ***p < 0.001compared to control group (ANOVA followed by Dunnett's post-hoc test) ANOVA: Analysis of Variance; SEM: Standard Error of the Mean

N = 6 per dose, *p<0.05,*p<0.01, ***p<0.001 (Fisher exact test: two tail). DW = distilled water, Clonaz = clonazepam

Figure 4 Effects of compounds 1- 6 on the percentage protection of mice against seizures induced by PTZ

The effects of the intraperitoneal (i.p) injection of compounds 1- 6 (20 mg/kg) on convulsions induced by PTZ (70 mg/kg, i.p) in mice are shown in Table 2 and Fig 4. Compounds 1- 6 (20 mg/kg) offered 16.66% to 100.00% protection

against PTZ-induced convulsions in mice. The reference drug clonazepam (0.1 mg/kg) also offered 100% protection against convulsions, as none of the mice displayed tonic-clonic seizures induced by PTZ. Statistical analysis showed that compounds 1- 6 (20 mg/kg i.p) increased the latency to the tonic-clonic components of PTZ-induced seizures in the unprotected mice (Table 2). Hence at 20 mg/kg, compounds 1- 6 significantly reduced the rate of seizures. And the control group had a seizure rate of 100% given it offered no protection.

3.3.3. Bicuculine-induced Convulsion

The effects of intraperitoneal (i.p) injection of compounds 1- 6 (20 mg/kg) on convulsions induced by BICU (4 mg/kg, i.p) in mice are shown in Table 3 and Fig 5. Compounds 1- 6 (20 mg/kg) offered 33.33 to 100.00% protection against convulsions induced by BICU in mice. The reference drug, diazepam (3 mg/kg) also offered 100% protection against convulsions, given none of the mice displayed tonic-clonic seizures induced by BICU. Statistical analysis showed that compounds 1- 6 (20 mg/kg i.p) increased the latency to the tonic-clonic components of BICU-induced seizures in the unprotected mice (Table 3). Thus at 20 mg/kg, compounds 1- 6 significantly reduced the rate of seizures. And the control group had a seizure rate of 100% given it offered no protection.

Table 3 Effects of compounds 1- 6 on the latency to the seizure induced by BICU

Treatment	Dose (mg/kg)	Latency to the seizure(min) [mean±SEM]	PercentageProtection (%)
Control (DW)	-	11.92±1.37	0
1	20	11.21±0.68	50.00
2	20	25.64±1.48***	66.66
3	20	No Convulsion	100.00
4	20	24.27±0.61***	50.00
5	20	22.06±1.26***	33.33
6	20	21.23±2.05***	33.33
Clonaz	1	No Convulsion	100.00

Values represent the mean ± S.E.M for 6 animals per group. *p < 0.05, ***p < 0.001compared to control group (ANOVA followed by Dunnett's post-hoc test) ANOVA: Analysis of Variance; SEM: Standard Error of the Mean
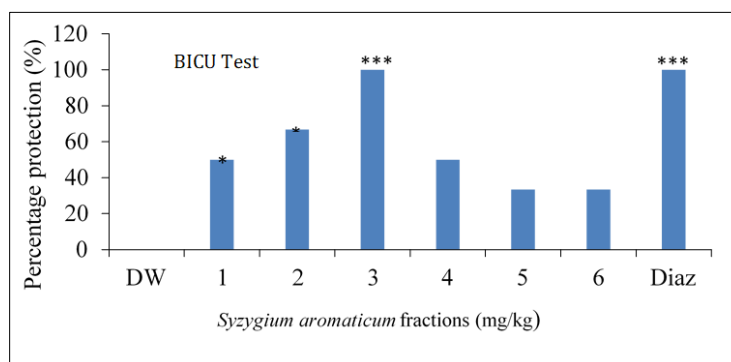
N = 6 per dose, *p<0.05,*p<0.01, ***p<0.001 (Fisher exact test: two tail). DW = distilled water,Diaz = diazepam

Figure 5 Effects of compounds 1 – 6 on the percentage protection of mice against seizures induced by bicuculine

3.4. Anticonvulsant activity of compound 3

Compound 3was subjected to more assessment at varying concentrations of 5, 10, 15 and 20 mg/kg, due to its ability to completely prevent, or delay the onset of tonic-clonic seizures (convulsions) induced by picrotoxin (PIC), pentylenetetrazole (PTZ) and Bicuculline (BICU), all chemoconvulsant agents (7.5, 70 and 4 mg/kg respectively) in mice.

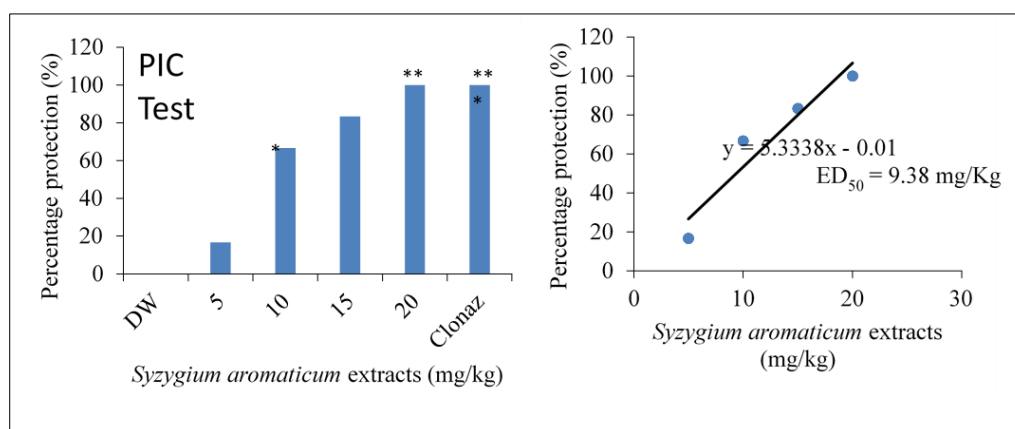### 3.4.1. Effect of compound 3 on picrotoxin-induced convulsion

The effects of compound 3 (5, 10, 15 and 20 mg/kg) on convulsions induced by PIC (7.5 mg/kg, i.p) in mice are shown in Table 4 and Fig 6. This compound (5, 10, 15 and 20 mg/kg) offered 16.66 to 100.00% protection against convulsions

induced by PIC in mice. The reference drug clonazepam (1 mg/kg) also offered 100% protection against convulsions, as none of the mice displayed tonic-clonic seizures induced by PIC (Fig 6). Statistical analysis showed that compound 3 (5, 10, 15 and 20 mg/kg, i.p) greatly increased the latency to the tonic-clonic components of PIC-induced seizures in the unprotected mice as the administered dose increased. This was obvious given that at 20 mg/kg, compound 3 offered 100% protection against seizures. And the control group had a seizure rate of 100% given it offered no protection (Table 4). The ED_{50} value was 09.38 mg/Kg.

Table 4 Effects of compound 3 on the latency to the seizure induced by PIC

Treatment	Dose (mg/kg)	Latency to the seizure(min) [mean±SEM]	PercentageProtection (%)
Control (DW)	-	3.91±1.29	0
3	5	11.93±0.94***	16.66
3	10	13.94±0.60***	66.66
3	15	14.25±0.00***	83.33
3	20	No Convulsion	100.00
Clonaz	1	No Convulsion	100.00

Values represent the mean ± S.E.M for 6 animals per group. *p < 0.05, **p < 0.01, ***p < 0.001compared to control group (ANOVA) followed by Dunnett's post-hoc test

N = 6 per dose, *p<0.05, ***p<0.001 (Fisher exact test: two tail). DW = distilled water, Clonaz = clonazepam

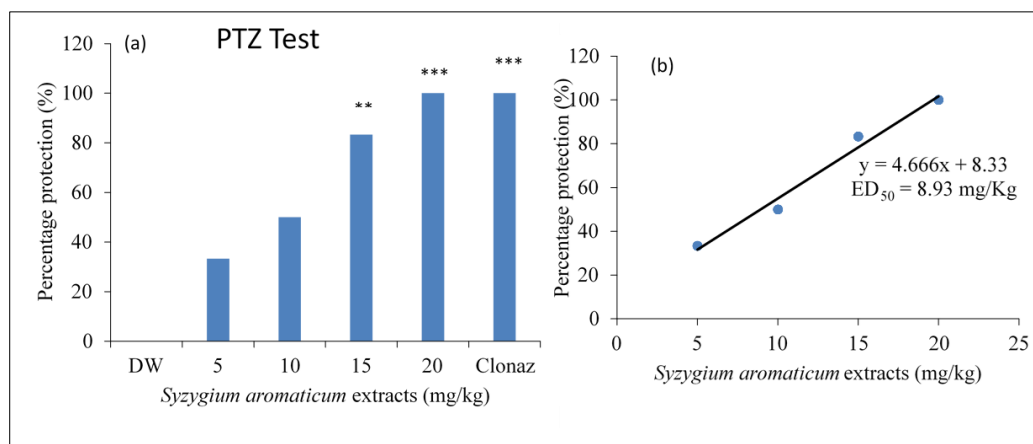**Figure 6** Effects of compound 3 on the percentage protection of mice against seizures induced by picrotoxin

3.4.2. Effect of compound 3 on pentylenetetrazole-induced convulsion

Table 5 Effects of compound 3 on the latency to the seizure induced by PTZ

Treatment	Dose (mg/kg)	Latency to the seizure(min) [mean±SEM]	PercentageProtection (%)
Control (DW)	-	3.47±0.89	0
3	5	4.98±0.75	33.33
3	10	8.65±0.51**	50.00
3	15	8.98±0.54**	83.33
3	20	No Convulsion	100.00
Clonaz	0.1	No Convulsion	100.00

Values represent the mean ± S.E.M for 6 animals per group. **p < 0.01, compared to Values represent the mean ± S.E.M for 6 animals per group. **p < 0.01, compared to control group (ANOVA followed by Dunnett's post-hoc test)

The effects of intraperitoneal (i.p) injection of compound 3 (5-20 mg/kg), on convulsions induced by PTZ (70 mg/kg, i.p) in mice are shown in Table 5 and **Fig 7**. Compound 3 (5-20 mg/kg) offered 33.33 to 100.00% protection against convulsions induced by PTZ in mice. The reference drug clonazepam (0.1 mg/kg) also offered 100% protection against convulsions, as none of the mice displayed tonic-clonic seizures induced by PTZ. Statistical analysis showed that as the concentration of the administered dose increased, compound 3 significantly increased the latency to the tonic-clonic components of PTZ-induced seizures in the unprotected mice (Table 5). This was confirmed at the dose of 20 mg/kg, at which, compound 3 greatly reduced the rate of seizures by 100% from the control, while the control group had a seizure rate of 100% given it offered no protection (Table 5). The ED_{50} value was 8.93 mg/Kg.

N = 6 per dose, *p<0.05, ***p<0.001 (Fisher exact test: two tail). DW = distilled water, Clonaz = clonazepam Legend a = Percentage protection b = Dose response values

Figure 7 Effects of compound 3 on the percentage protection of mice against seizures induced by PTZ

3.4.3. Effect of compound 3 on bicuculine-induced convulsion

The effects of intraperitoneal (i.p) injection of compound 3 (5-20 mg/kg) on convulsions induced by BICU (4 mg/kg, i.p) in mice are shown in Table 6 and **Fig 8**. Compound 3 (5-20 mg/kg) offered 50.00 to 100.00% protection against convulsions induced by BICU in mice. The reference drug diazepam (3 mg/kg) also offered 100% protection against convulsions, as none of the mice displayed tonic-clonic seizures induced by BICU. Statistical analysis showed that compound 3 (5-20 mg/kg) increased the latency to the tonic-clonic components of PTZ-induced seizures in the unprotected mice as the administered dose increased (Table 6). This was very true given that, at 20 mg/kg. This compound significantly reduced the rate of seizures by 100%, while the control group had a seizure rate of 100% given it offered no protection (Table 6). The ED_{50} value was 5.00 mg/Kg.

Table 6 Effects of compound 3 on the latency to the seizure induced by BICU

Treatment	Dose (mg/kg)	Latency to the seizure(min) [mean±SEM]	PercentageProtection (%)
Control (DW)	-	11.92±1.37	0
3	5	23.05±1.00***	50.00
3	10	26.29±1.16***	66.66
3	15	28.15±0.00***	83.33
3	20	No Convulsion	100.00
Diaz	3	No Convulsion	100.00

Values represent the mean ± S.E.M for 6 animals per group. **p < 0.01, compared to Values represent the mean ± S.E.M for 6 animals per group. **p < 0.01, compared to control group (ANOVA followed by Dunnett's post-hoc test)

The results of the anticonvulsant assay showed that compounds 1- 6 all protected the mice against the development of seizures and they all delayed instances of seizure induced by PIC, PTZ or BICU at the tested dose of 20 mg/kg. These six compounds (1- 6)have significantly inhibited the effects of PIC, PTZ and BICU with a high latency suggesting that they might provide 100% protection like the reference drugs clonazepam (1 and 0.1 mg/kg for PTZ and PIC respectively) and diazepam (3 mg/kg for BICU) if a higher dose of about 25 mg/kg was administered. Interestingly, at 20 mg/kg,

compound 3 provided 100% protection against the development of seizure episodes and delayed instances of seizure. This was closely followed by compounds 2 and 5 (each offering 83.33%), while the lowest protection came from compound 1, 4 and 6 (16.66 – 50 00 %).
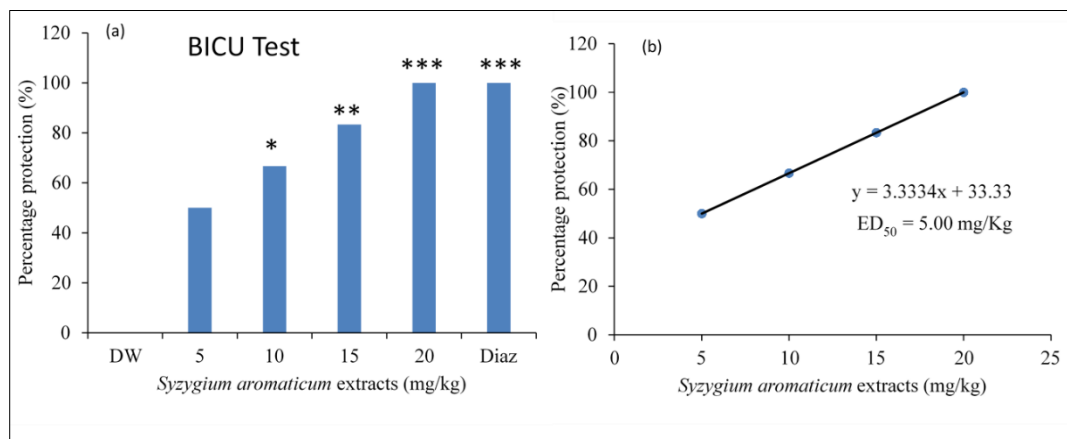
N = 6 per dose, *p<0.05,***p<0.01, ***p<0.001, (Fisher exact test: two tail). DW = distilled water,Clonaz = clonazepam

Legend: Percentage protection; Dose response values

Figure 8 Effects of compound 3 on the percentage protection of mice against seizures induced by BICU

Picrotoxin (PIC), Pentylenetetrazole (PTZ) and Bicuculline (BICU) are chemoconvulsants used regularly to induce seizures in experimental animals [31, 32]. Seizures result from an imbalance between inhibition and excitation signals, often due to a failure of inhibitory neurotransmission [33].Substances that antagonize the postsynaptic GABAA receptor cause excessive neuronal excitation over neuronal inhibition which results in convulsions and death of laboratory animals [33]. PIC is a non-competitive antagonist and provokes seizures by blocking the GABAA receptor chloride ion channel which offers resistance to most anticonvulsant agents [32]. On the other hand, PTZ is a competitive antagonist and induces convulsions by antagonizing GABAA in a competitive manner [32]. While Bicuculline (BICU), acts directly on the postsynaptic GABAA receptor complex, given it is a selective competitive GABAA receptors antagonist and induces hyperactivity behaviour and seizures [31, 34]. This is the first time compounds 2, 4, 5, 6 and 7 are being isolated from this plant, while compounds 1 and 3 have been previously isolated from *Syzygium aromaticum* [35, 36]. Also to the best of our knowledge, this induced seizure by PIC, PTZ or BICU in mice at the tested dose of 20 mg/kg is the first report of the anticonvulsant activity of these compounds 1- 6.

4. Conclusion

From these findings we suggest that the tested compounds (**1**-**6**) could serve as important leads for the development of future antiepileptic drugs (AEDs), given that they offered 16.66 % - 100 % protection in mice against convulsion induced by these 3 chemoconvulsants PIC, PTZ and BICU. This research has therefore added value to the genus *Syzygium* and the Myrtaceae family as a whole, given that this is the first report confirming and validating the antiepileptic potential of these compounds isolated from *Syzygium aromaticum*.

Compliance with ethical standards

Disclosure of conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Statement of ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Also, all procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

Author's contributions

L. N. N. wrote the manuscript text and performed the extraction, isolation and structural elucidation of isolated compounds. G.S.T carried out the biological screening, analysed the data and contributed equally in writing the manuscript. C.R.S. carried out the NMR analysis. J.A.M. designed the research project and supervised the study. All authors read and approved the final manuscript.

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