European Journal of Applied Sciences – Vol. 10, No. 4
Publication Date: August 25, 2022
DOI:10.14738/aivp.104.12640.
Ukwade, C. E., Ebuehi, O. A. T., Adisa, R. A., Odukoya, A., Agaba, O., Adesegun, S. A., Igietseme, J. U., & Omosun, Y. (2022). Antioxidant and Cytotoxic Activities of Fractions of Ethanol Leaf Extract of Byrsocarpus Coccineus Schum. and Thonn. (Connaraceae). European Journal of Applied Sciences, 10(4). 413-428.

Antioxidant and Cytotoxic Activities of Fractions of Ethanol Leaf Extract of Byrsocarpus Coccineus Schum. and Thonn. (Connaraceae)

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ABSTRACT

Medicinal plants have historically been used traditionally for their therapeutic potentials, they are therefore investigated with the hope of developing novel drugs. This study is to investigate the phytochemicals, antioxidant and cytotoxic activities of *Byrsocarpus coccineus* fractions. The ethanol leaf extract of *B. coccineus* was chromatographed on VLC over silica gel (Mesh 70-230) using n-hexane/EtOAc/MeOH eluent system; n-hexane/EtOAc (from100/0 to 0/100), then

ethyl acetate (EtOAc)/MeOH (from 100/0 to 0/100) to yield seven (7) fractions.(F1-F7). The phytochemical screening and antioxidant activities of the different fractions were carried out. All the fractions were subjected to gas chromatography and mass spectrometry (GC-MS) analysis and cytotoxic activities of the different fractions were investigated using brine shrimp lethality assay. The phytochemical result showed that fractions contain tannins, flavonoids, phenols, reducing sugar, alkaloids, steroids, saponin and terpenoid. Antioxidant analysis show that F3 had the highest DPPH (IC_{50}=56.88µg/ml) and NO (IC_{50}=53.02µg/ml) free radical scavenging activities. F7 (IC_{50}=56.25 µg/ml) and F3 (IC_{50} of 56.79 µg/ml), had the highest lipid peroxidation free radical scavenging activity. The brine shrimp toxicity assay result showed that F3 and F4 were cytotoxic to *Artemia salina* with a LD_{50} value of 39.01µg/ml and 53.55 µg/ml respectively. The GC-MS analysis showed the presence in the most active fraction with antioxidant (cis-vaccenic acid, neophytadiene), antitumor and cancer preventive (squalene) and anticancer (9-Octadecenoic acid, 9, 12-Octadecadienoic acid, methyl ester) potentials. This study reveals that *B. Coccineus* contains pharmacologically bioactive compounds with therapeutic potentials.

Keywords: Byrsocarpus coccineus, phytochemicals, antioxidant, brime shrimp lethality assay, GC-MS

INTRODUCTION

Medicinal plants have been used as traditional remedies for ages, they are important source of folk medicines. About 80% of the world population, especially those in the developing countries, rely on medicinal plants for the treatment of various ailments (Vital and Rivera, 2009). Plants are reservoirs for bioactive secondary metabolites and provide a promising line for therapeutics, as these are safer, ecofriendly, low-cost, readily available, biodegradable and less toxic compared with conventional treatment methods (Iqbal et al., 2017).

Byrsocarpus coccineus Schum and Thonn (family Connaraceae) locally known in Western Nigeria as "Amujewewe" (Burkill, 1985), is an ethno-medicinal plant used in traditionally in several parts of West Africa (Dalziel, 1937). Various preparations of the plant; leaves, roots (scraped bark and sap) and whole plant have been used traditionally to treat diverse ailments. Previous studies reported that the leaf extract of *B. coccineus* had various pharmacological activities such as analgesic activities (Akindele and Adeyemi, 2006a), antidiarrhea activities (Akindele and Adeyemi, 2006b), antipyretic activities (Akindele and Adeyemi, 2007a), anti-inflammatory activities (Akindele and Adeyemi, 2007b), anxiolytic/sedative activities (Akindele and Adeyemi, 2010) and antidiabetic activity (Dada et al., 2013). It was also shown that ethanol root extract of *B. coccineus* had anti-inflammatory and *in vitro* antioxidant activities (Dosseh et al., 2015). The aim of this present study is to investigate the phytochemical, antioxidant, cytotoxic activities (against brine shrimp nauplii), and to possibly identify the chemical components of active fractions of *Byrsocarpus coccineus* (B.C) ethanol leaf extract using Gas Chromatography – Mass Spectrophotometry (GC-MS).

MATERIALS AND METHODS

Plant collection and Identification

Byrsocarpus coccineus (BC) is locally known as "Amujewewe" in Western Nigeria (Burkill, 1985), was collected from uncultivated land in Ibadan, Oyo state, Nigeria. The plant sample was

authenticated by Prof J.D. Olowokudejo, at the Department of Botany, University of Lagos, Akoka, Lagos, Nigeria, with the voucher specimens number 7491.

Extraction and separation processes

Powdered BC leaf (1.5 kg) was macerated in 95% ethanol for 48 h in the dark under constant agitation. The extract was filtered using Whatman filter paper (No.1) and the filtrate was evaporated to dryness using a rotary evaporator at reduced pressure to yield the ethanol crude extract (63.8 g).

Fractionation of BC ethanol crude extract

BC ethanol crude extract (60 g) was fractionated using vacuum liquid chromatography (VLC) over silica gel (200–300 mesh) and eluting with different solvent ratios of increasing polarity: 100% N-Hexane (**F1**), 70:30 n-Hexane/Ethylacetate (**F2**), 30:70 n-Hexane/Ethylacetate (**F3**), 100% Ethylacetate (**F4**), 70:30 Ethylacetate/Methanol (**F5**), 30:70 Ethylacetate/Methanol (**F6**) and 100% Methanol (**F7**).. All the fractions were evaporated to dryness using the rotary evaporator.

Antioxidant Activities

DPPH Radical Scavenging Assay

DPPH scavenging effects of the extract and fractions were observed according to previous method of Shimada (Shimada et al.,1992). Briefly 0.1 mM solution of DPPH in methanol was prepared; 1ml of the solution was added to 1 ml of extract in methanol at different concentrations (25-100 µg/ml). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min in the dark cupboard . The absorbance was measured at 517 nm by using a UV-Visible Spectrophotometer. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The percent DPPH scavenging effect was calculated using the following equation

DPPH Scavenging effect (%) = $[(A_0-A_1)/A_0] \times 100$

Where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the standard sample or extract. The IC_{50} value represented the concentration of the compounds that caused 50% inhibition of DPPH radical formation.

Lipid Peroxidation Assay

Lipid peroxidation assay was carried out by the method of Ohkawa et al. (1979). The reaction mixture contained 1 mL of fowl egg yolk emulsified with phosphate buffer (pH 7.4) to obtain a final concentration of 25 g/l, sample (different concentration of 20–100 µg/mL), and 100 µL of 1000 µM $FeCl_2$. The mixture was incubated at 37°C for 1 h before being treated with 0.5 mL of freshly prepared 15% trichloroacetic acid (TCA) and 1.0 mL of 1% thiobarbituric acid (TBA). The reaction tubes were further incubated in boiling water bath for 10 min. Once cooled to room temperature, the tubes were centrifuged at 3500 rpm for 10 min to remove precipitated protein. The absorbance at 532 nm was determined and gallic acid was used as positive control.

The percentage inhibition was calculated from the following equation.

Inhibition (%) = $[(A_b-A_s/A_b) \text{ X } 100$

Where A_b is the absorbance of the blank (DPPH solution without the extract or ascorbic acid), and A_s is the absorbance in the presence of the extract or garlic acid.

Nitric Oxide Assay

Nitric oxide assay was done using the method of Green et al. (1996). Sodium nitroprusside (10 mM) in phosphate buffer saline was mixed with different concentrations (25-100 µg/ml) of each extract, and incubated for 30min, after incubation period, 0.5 mL of Griess reagent (1% sulfanilamide, 2% H_2PO_4 and 0.1% N-(1-naphthyl) ethylene diamine dihydrochloride was added. Absorbance was taken at 550 nm and ascorbic acid was used as standard. IC_{50} which is an inhibitory concentration of each extract required to reduce 50% of nitric oxide formation was determined. The same reaction mixture without the extract, but equivalent amount of methylated spirit was used as control.

$$\text{Nitric oxide (inhibition \%)} = \frac{\text{A (control)} - \text{A (sample)}}{\text{A (control)}} \times 100$$

Reducing power assay

The reducing power was determined according to the method of Oyaizu (1986), with slight modifications. Reaction was carried out in a mixture containing 1 ml of sample (25-100 µg/mL), 2.5 mL of 0.1 M sodium phosphate buffer (pH 6.6) and 2.5 mL of 1%, w/v potassium ferrocyanate $[K_3Fe(CN)_6]$ by incubating at 50°C for 20 min. After addition of 2.5 mL trichloroacetic acid (10%, w/v), the mixture was centrifuged at 5000 rpm for 10 min. The upper layer (5 ml) was mixed with 0.5 ml of fresh $FeCl_3$ (0.1%, w/v), and the absorbance at 700 nm was measured against a blank. Gallic acid was used as the control (Oyaizu, 1986).

Brine Shrimp Lethality Assay

Brine shrimp lethality assay was used to evaluate the cytotoxicity of the extract and different fractions (Krishnaraju et al., 2005). Stock solution was prepared by dissolving 25 mg of plant extract in 5 mL of filtered sea water. Concentrations of 1000 µg/ mL, 100 µg/ mL and 10 µg/ mL were prepared by serial dilution from the stock solution. Ten shrimps nauplii were added to each of the test tubes; the control consisted of seawater to which only 10 nauplii were also added. The surviving (larvae) shrimps were counted after 24 h. Potassium dichromate was used as reference standard. All experimental assays were done in triplicates. The LC_{50} was calculated using Probit analysis.

Phytochemical Screening

The crude extract and the fractions were subjected to phytochemical analysis for the presence of some secondary metabolites such as alkaloids, tannins, glycosides, steroids, terpenoids, flavonoids, saponins and resins, using standard methods of Sofowora (1993) and Evans (2009).

GC-MS Analysis

Gas chromatography/mass spectrometry (GC/MS) analysis of the most potent fraction of BC was carried using Agilent HP 7890/5975C GC–MS system with an HP-5MS capillary column (30 m x 0.25 mm i.d. x 0.25 NM film thickness) (Aligent, Santa Clara, CA, USA). The carrier gas was Helium (purity > 99.999%) and it was used at a flow rate of 1 mL/min. The GC oven was initially 40°C and held for 3 min, it was increased to 290°C at the rate of 3 °C /min and held for 5 min. The injector was maintained at a constant temperature of 250°C and detector at 230°C. Each fraction was reconstituted with methanol and 1µg of each fraction were input into the injector

through a split-splitless injector. The transfer line, MS source with an Agilent HP-5973 mass selective detector in the electron impact mode (Ionization energy:70 eV) and quadruple temperatures set at 150^0C. The analysis was done at a quadruple MS full scan range of 50 to 500 atomic maunits (amu) with a 2.8 min solvent delay time. Identification of components of the most active fraction (was carried out by using the computer to match the peaks with Wiley Ms. Libraries and also comparing mass spectra of the peaks with data from literature (Ahuchaogu et al., 2018).

RESULTS AND DISCUSSION

In this present study antioxidant and cytotoxic activities including preliminary phytochemical screening were carried out on extract and fractions (F1-F7) of BC ethanol leaf extract. The phytochemical analysis of extract and fractions (F1-F7) of BC ethanol leaf extract showed the presence of phenols, flavonoids, steroids, alkaloids, reducing sugar and tannins, only F6 contained saponin, but phlobatanins was absent in all fractions (Table 1). Previous studies on phytochemical constituents of BC ethanol leaf extract revealed the presence of as alkaloids, tannins, saponins, flavonoids, steroid and phenol (Ukwade et al. (2000). Phytochemicals found in medicinal plants contain have physiological effect on the body (Edeoga, 2005); (Olowokudejo et al., 2008), (Okigbo et al., 2008) and possess several biological properties. Phytochemicals are have protective or disease preventive and medicinal properties (Kumar and Goel, 2019). For example terpenoids, alkaloids and phenols possesses antibacterial and antifungal activities, alkaloids, phenols and flavonoids have antioxidant and anticancer activities (Saxena et al., 2013) and saponins, terpenoids, flavonoids, tannins, steroids and alkaloids possess anti-inflammatory properties (Akindele and Adeyemi, 2007); (Manach et al., 1996); (Orhan et al., 2007).

Antioxidants with free radical scavenging activities may have great relevance in the prevention and therapeutics of diseases in which oxidants or free radicals are implicated (Soares et al., 1997). The free radical scavenging potentials of BC ethanol leaf extract fractions (F1-F7), were evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, nitric oxide (NO) scavenging assay and lipid peroxidation assay. This study was carried out to evaluate the antioxidant capacity of the extract and various fractions compared to ascorbic acid which served as control at different concentrations (Table 2).

It can be observed this study that all the fractions exhibited concentration-dependent DPPH radical scavenging activities (Figure 1(A)). F1 and F2 had significantly lower ($p < .05$) scavenging activities, while F4, F5 and F6 had moderate activities, but F3 and F7 displayed significantly higher ($p < .05$) DPPH scavenging activities when compared to the standard (ascorbic acid). F3 had the highest DPPH scavenging activity with an IC_{50} of 53.02 µg/ml compared to ascorbic acid with an IC_{50} of 54.98µg/ml (Table 2; Figure 1 (A)). DPPH is characterized as stable free radical by virtue of the delocalization of the spare electron, where the molecule as a whole do not dimerise, as would be the case with other free radicals. The phenolic compounds present in the BC could be responsible for the observed DPPH radical scavenging activity, since phenols can readily donate hydrogen atom to the radical (Arokiyaraj et al., 2008).

NO scavenging activities were dose dependent in the all fractions (Figure 1 (B)). NO scavenging activity of the F3 (IC_{50} 56.88 µg/ml) was not significantly different from the standard (ascorbic acid) (IC_{50} 61.58 µg/ml) but all the other fractions had significantly lower ($p < .05$) scavenging activities when compared to the standard. The inhibitory potentials of the fractions on NO radical can be attributed to the presence of phenolic compounds which are able to compete with oxygen and its derivatives because they can easily donate electrons to the radicals (Marcocci et al., 1994). They are involved in scavenging the oxygen derived free radicals (Vaya et al., 2003).

All the fractions exhibited concentration-dependent lipid peroxidation activities (Figure 1 (C)). The lipid peroxidation activity results of the fractions (Table 2) showed that F1, F3, F6 and F7 had significantly higher ($p < .05$) free radical scavenging activities but F2 had a significantly lower ($p < .05$) when compared to the standard. F7 and F3 had the highest lipid scavenging activities.

All the fractions showed varying levels of reducing power activities in a dose dependent manner, (Figure 1 (D)) which were lower than the standard (Ascorbic acid). During the process of lipid peroxidation, the oxidized intermediates can be reduced by antioxidant compounds which are electron donors. This can be inferred to be the reducing power property of an antioxidant (Gordon, 1990).

Among the seven different fractions isolated from the crude BC ethanol extract, F3 showed the most potent free radical activity, this is an indication that compounds with high free radical scavenging activity in BC are probably of medium polarity.

Primarily phenolic compounds are of great importance as cellular support material because they form the integral part of cell wall structure by polymeric phenolics (Gupta et al., 2010). Bioactive polyphenols have attracted special attention because they can protect the human body from the oxidative stress which may cause many diseases, including cancer, cardiovascular problems and ageing (Robards et al., 1999). Flavonoids are free radical scavengers, super antioxidants and potent water soluble which prevent oxidative cell damage and have strong anti-cancer activity (Salah et al., 1995). This study shows the phenols and flavonoids are present in these fractions, might be responsible for the free radical scavenging activities observed in the fractions especially F3 (Sagar et al., 2011). Ukwade et al. (2000) had earlier reported that crude ethanol leaf extract of BC has free radical scavenging (antioxidant) properties.

Brine shrimp (*Artemia salina*) lethality assay represents a rapid, cheap and simple bioassay for testing plant extracts bioactivity which correlates reasonably with cytotoxic and anti-tumor properties of the bioactive compounds in medicinal plant (McLaughlin et al., 1993). Brine shrimp assay is useful for the verification of these biogenic compounds (Sam, 1993) particularly the cytotoxic compounds. It is established that substances with LD_{50} values less than 200µg/ml are potential cytotoxic compounds and those with LD_{50} greater 1000 µg/ml are considered inactive (Krishnaraju et al., 2005); (Meyer et al., 1982).

In this present study, the cytotoxic effect of BC ethanol leaf extract and its fractions (F2-F7) were evaluated and result shows that F3 (LD_{50} of 39.01µg/ml) and F4 (53.55µg/ml) were highly

cytotoxic compared to the other fractions with LD_{50} values greater than 1000 µg/ml (Table 3). The cytotoxic activity of F1 was not determined as a result of low yield of the fraction. F3 was the most cytotoxic to brine shrimp followed by F4, F5-F7 were not cytotoxic.

Some secondary metabolites present in F3 and F4, have been reported to have cytotoxic properties for example flavonoids (Urquiaga and Leighton, 2000; Okwu, 2004), steroids (Aziz et al., 2014), tanins (Zarin et al., 2016), terpenoids (Thoppil and Bishayee, 2011). It can be suggested that the cytotoxicity of F3 and F4 could be due to the presence of these secondary metabolites (Table 1). Further purification and isolation of these cytotoxic compounds present would be of great importance.

The results of the GC-MS analysis led to the identification of number of compounds from the GC fractions of BC ethanol leaf extract. These compounds were identified through mass spectrometry attached with GC. The results of the present study were showed in Tables 4-5. The gas chromatogram (Figures 2-3) shows the relative concentrations of various compounds eluted as a function of retention time. The heights of the peak indicate the relative concentrations of the components present in the fractions 3 and 4 of BC ethanol leaf extract. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds present in fractions 3 and 4 of BC ethanol leaf extract. The large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios. These mass spectra (Figures 2-3) are fingerprint of compounds which can be identified from the data library. This report is the first of its kind to analyze the chemical constituents of the fractions of ethanol leaf extract of *B. coccineus* using GC-MS.

The GC-MS analytes show notable compounds with antioxidant, cancer preventive and anticancer properties. F1 contain squalene; antitumor and cancer preventives (Kell et al., 2005); (Smith, 2000). Phytol and phytol acetate are notable antioxidants and cancer preventives (Swamy and Sinniah, 2015); (Wei et al., 2011); (Song and Cho, 2015) are present in F2. 9-Octadecenoic acid, 9-Octadecenoic acid methyl ester, Oleic acid/cis-vaccenic acid; cancer preventives and antioxidants (Simin et al., 2002); (Gideon and Gideon, 2015) are present in F2, F3, F4, F6 and F7 while 9-Octadecadienoic acid methyl ester, 9,12-Octadecadienoic acid, methyl ester possesses anticancer and antioxidant activity (Yu et al., 2005) is present in F2, F4 and F5. Methyl stearate, Hexadecanoic acid and its methyl ester derivative; anticancer (Yu et al., 2005); (Takeara et al., 2008) are present in F2, F3, F5 and F7. Moreover, F3 also possess β-sitosterol which is also an antioxidant and cancer preventives (Shahdaat et al., 2016). Hence, the more cytotoxic effect and antioxidative activity shown by F3 could be as a result of the synergistic activity of these bioactive compounds (Yu et al., 2005); (Takeara et al., 2008). These compounds also act as anti-oxidants, as they are components of cell membrane and are used to replace damage lipids on the membrane as a result of free radicals, which could lead to apoptosis, thereby ameliorating the cell from damage and improving cell proliferation. However, isolation of individual phytochemical constituents may proceed to finding a novel anti-cancer drug.

CONCLUSION

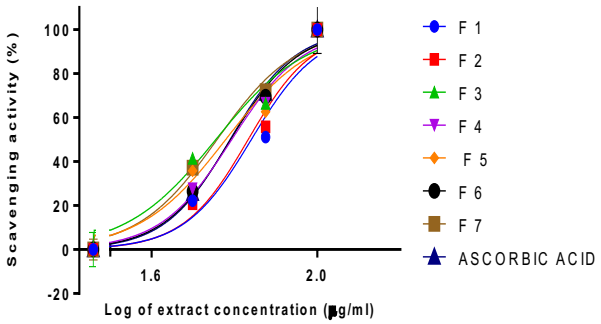
This study shows that fractions (F3 and F4) of ethanol leaf extract of *B. coccineus* are cytotoxic on brine shrimp nauplii, possess antioxidant (cis-vaccenic acid, neophytadiene) potential

antitumor and cancer preventive (squalene) and anticancer (9-Octadecenoic acid, 9, 12-Octadecadienoic acid, methyl ester) compounds. The presence of various bioactive compounds of pharmacological importance in *B. coccineus* can be attributed to its folkloic use as a traditional medicine.

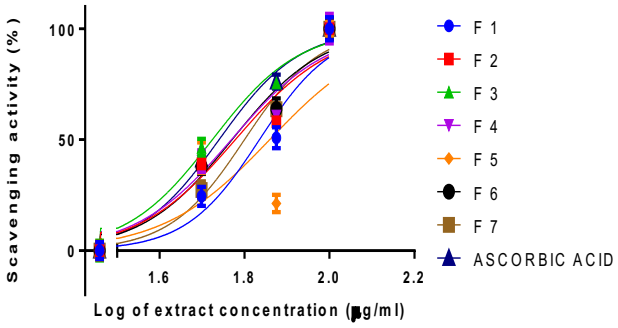
Table 1. Qualitative phytochemical composition of fractions (F1-F2) of BC ethanol leaf extract

Sample	Saponin	Tannins	Flavonoids	Phenols	Reducing Sugar	Alkaloids	Phlobatannin	Steroids	Terpenoids
F1	--	+	+	+	+	+	--	+	+
F2	--	+	+	+	+	+	--	+	+
F3	--	+	+	+	+	+	--	+	+
F4	--	+	+	+	+	+	--	+	+
F5	--	+	+	+	+	+	--	+	+
F6	+	+	+	+	+	+	--	+	+
F7	--	+	+	+	+	+	--	+	+

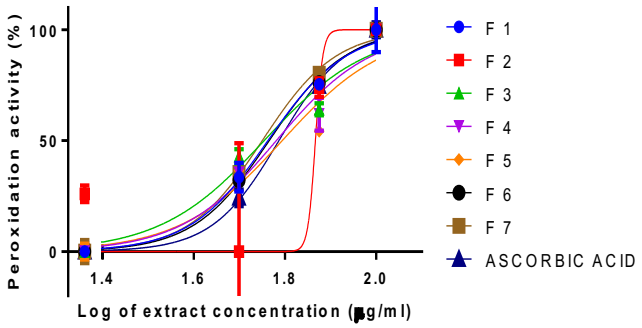
+= Present
-= Absent



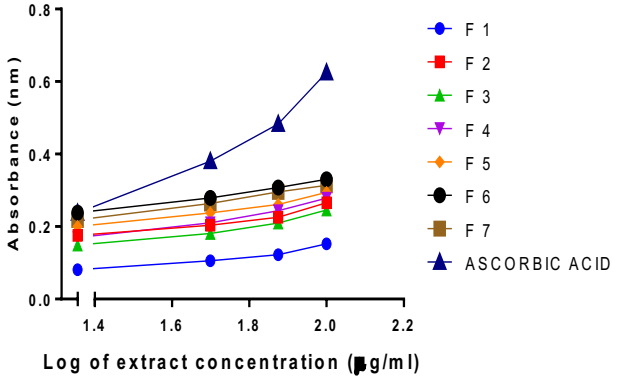
(A)



(B)



(C)



(D)

Fig 1: Antioxidant activity of fractions (F1-F7) of BC ethanol leaf extract: (A)- DPPH scavenging activity (%inhibition), (B)- Nitric oxide scavenging activity (%inhibition), (C)- Lipid peroxidation scavenging activity (%inhibition), (D)- Reducing power activity

Table 2: Antioxidant activity of fractions (F1-F7) of BC ethanol leaf extract

Fraction	DPPH IC_{50} (µg/ml)	Nitric oxide IC_{50} (µg/ml)	Lipid peroxidation IC_{50} (µg/ml)
F 1	69.30 ± 1.05*	68.43 ± 1.06*	57.87 ± 1.03*
F 2	68.09 ± 1.04*	60.04 ± 1.07*	73.55 ± 1.04
F 3	56.88 ± 1.05*	53.02 ± 1.04	56.79 ± 1.04*
F 4	62.16 ± 1.03	58.02± 1.03*	60.39 ± 1.04
F 5	60.10±1.05	60.10±1.05*	62.20±1.05
F 6	61.78 ±1.03	61.78±1.03*	58.25±1.02*
F 7	57.27±1.027*	57.27 ±1.03*	56.25 ±1.02*
Ascorbic acid (control)	61.58 ± 1.02	54.98 ± 1.03	61.18 ± 1.02

Data = mean ± SD; *n* = 3. ∗Statically significant (*p* < .05).

Table 3: Brine shrimp lethality results for BC Fractions (F1-F7)

Fraction	LD_{50}
F 1 (n hexane 100%)	ND
F 2 (n hexane 70% and Ethylacetate 30%)	1303.00 µg/ml
F 3 (n hexane 30% and Ethylacetate 70%)	39.01 µg/ml
F 4 (Ethylacetate 100%)	53.55 µg/ml
F 5 (Ethylacetate 70% and Methanol 30%)	2988.82 µg/ml
F 6 (Ethylacetate 30% and Methanol 70%)	25703.95 µg/ml
F 7 (Methanol 100%)	24484993891.12 µg/ml
Potassium dichromate	49.55 µg/ml

Key: ND - Not Determined

Table 4: Selected bioactive compounds present in the GCMS analysis of F3 (30% n-Hexane + 70% Ethylacetate) fraction of BC ethanol leaf extract.

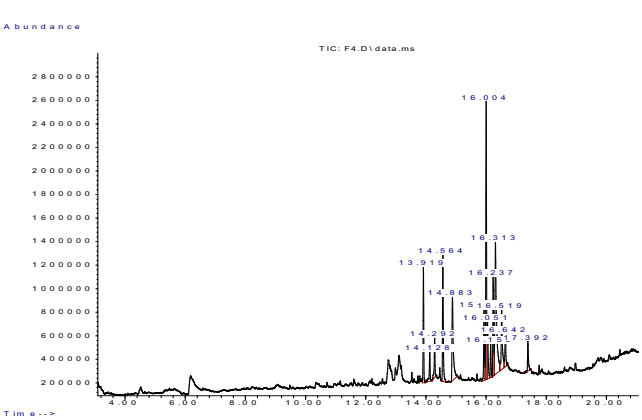
S/No	Retention time	Compound name	Area %	Molecular Weight	Activity
1	12.730	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	17.91	196	No activity
2	13.920	Neophytadiene	3.88	278	Analgesic, Antipyretic, Anti-Inflammatory, Antimicrobial, And Antioxidant Compound (Raman et al., 2012).
3	14.563	Hexadecanoic acid, methyl ester	6.91	270	Antibacterial and antifungal (Chandrasekaran et al., 2011).
4	14.897	n-Hexadecanoic acid	15.76	256	Anti-inflammatory (Aparna et al., 2012), Antioxidant, hypocholesterolemic nematicide, antiandrogenic, hemolytic, 5-Alpha reductase inhibitor (Kumar et al., 2010), potent mosquito larvicide (Rahuman et al., 2000).
5	15.930	9,12/10,13-Octadecadienoic acid, methylester	4.12	294	Hypocholesterolemic, Nematicide Antiarthritic, Hepatoprotective Anti androgenic, 5-Alpha reductase inhibitor, Antihistaminic (Jananie and Priya, 2011).
6	16.006	11/7/9-Octadecenoic acid (Z)-, methyl ester	12.90	296	Anti-inflammatory, antiandrogenic, Cancer preventive, Dermatitigenic, Hypocholesterolemic, 5-alpha reductase inhibitor, anemiagenic, (Gideon and Gideon, 2015).
7	16.049	13/11/9-Octadecenoic acid, methyl ester (E)-	2.89	296	No activity reported
8	16.235	Methyl stearate	4.83	298	GABA aminotransferase inhibitor, Anti-inflammatory, intestinal, Lipid metabolism regulator, Gastrin inhibitor,

					Antihelmintic (Nematodes), Antinociceptive (Adnan et al., 2019).
9	16.316	cis-Vaccenic acid/ Oleic acid	10.76	282	Flavour (Omolosa and Vagi, 2001), cancer preventive (Simin *et al*., 2000) (Gideon and Gideon, 2015), anti-inflammatory (Yunfeng et al., 2007) antimicrobial activity (Wagh et al., 2007) and Antibacterial (Awa et al., 2012).
`10	20.097	Beta/gamma.-Sitosterol	7.73	414	Antioxidant, Antimicrobial, Angiogenic, Antioxidant, Immunomodulatory, Antidiabetic, Anti-inflammatory, anticancer, and antinociceptive activities without major toxicity (Shahdaat et al., 2016).

Table 5: Selected bioactive compounds present in the GCMS analysis of F4 (100% Ethylacetate) fraction of BC ethanol leaf extract

S/No	Retention time	Compound name	Area %	Molecular Weight	Activity
1	13.920	Neophytadiene	6.74	278	Analgesic, antipyretic, anti-inflammatory, antimicrobial, and antioxidant compound (Raman et al., 2012).
2	14.563	Pentadecanoic acid, 14-methyl-, methyl ester. Hexadecanoic acid, methyl ester	9.14	270	No activity reported. Antibacterial and antifungal activity (Chandrasekaran et al., 2011).
3	14.882	n-Hexadecanoic acid	9.71	256	Antioxidant, hypocholesterolemic nematicide, pesticide, antiandrogenic flavor, hemolytic, 5-Alpha reductase inhibitor (Kumar et al., 2010) and potent mosquito larvicide (Rahuman et al., 2000).
4	15.930	9,12-Octadecadienoic acid, methylester. Methyl 10-trans,12-cis-octadecadienoate	5.07	294	Anticancer (Yu et al., 2005).
5	16.001	8/9-Octadecenoic acid (Z)-, methyl ester	18.83	296	Anti-inflammatory, Antiandrogenic, cancer preventive, dermatitigenic hypocholesterolemic, 5-alpha reductase inhibitor and anemiagenic (Gideon and Gideon, 2015).
6	16.049	9/11/13-Octadecenoic acid, methyl ester	4.76	296	Anti-inflammatory, Antiandrogenic, cancer preventive, dermatitigenic

					hypocholesterolemic, 5-alpha reductase inhibitor and anemiagenic (Gideon and Gideon, 2015).
7	16.235	Methyl stearate	6.92	298	GABA aminotransferase inhibitor, Anti-inflammatory, intestinal, Lipid metabolism regulator, Gastrin inhibitor, Antihelmintic (Nematodes), Antinociceptive (Adnan et al., 2019).
8	16.311	Oleic Acid	19.33	280	Cancer preventive (Simin et al., 2002); (Gideon and Gideon, 2015), anti-inflammatory (Yunfeng et al., 2007) and antimicrobial activity (Wagh et al., 2007).
9	16.520	Octadecanoic acid	6.68	284	Antimicrobial activity (Rahuman et al., 2000).
10	16.644	Methyl 9-cis,11-trans-octadecadienoate, Methyl 9,12-heptadecadienoate, Methyl 10-trans,12-cis-octadecadienoate	2.98	294	No activity reported



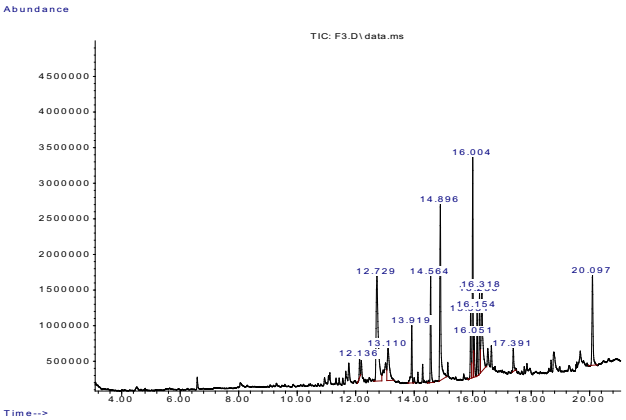


Figure 2: GCMS Chromatogram of F3 (30% n-Hexane and 70% Ethylacetate) fraction of BC ethanol leaf extract.

Figure 3: GCMS Chromatogram of F4 (100% Ethylacetate) fraction of BC ethanol Leaf Extract.

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