



Phytochemical Screening of Fish Poison Tree, *Barringtonia Asiatica* Seed for Potential Biopesticidal Activity and Pharmaceutical Uses

Jomer B. Mangawang^{1*}, Ma Luz F. Cabatan¹, Jonah G. Zante¹, and Chenie Mae T. Bibon¹

¹Department of Agriculture and Aquatic Sciences, Aurora State College of Technology, Bazal Campus, Maria Aurora, Aurora, Philippines

Email for correspondence: jomermangawang@yahoo.com

Submitted March 11, 2020. Accepted June 2, 2020. Published online July 31, 2020.

Abstract

The study was conducted to identify the phytochemical contents in the seeds of fish poison tree, *Barringtonia asiatica* (L.) Kurz. The qualitative and quantitative analyses were ascertained using standard conventional methods and gas chromatography-mass spectroscopy (GC-MS) technique. Phytochemical screening revealed the presence of saponins, alkaloids, cardiac glycosides, flavonoids, terpenoids, coumarin, and tannins in the seeds. In addition, the seed contained 42 compounds which includes Vitamin E, (43.53%), fatty acid esters (LAEE, 40.30%; PAEE, 10.24%; (E)-9-ODAEE, 6.73%), and sterols (chondrillasterol, 14.20%; lanosterol, 7.29%; and stigmasterol, 7.02%). Furthermore, chemical components such saponins, alkaloids, and cardiac glycosides present in the seed were found positive for biopesticidal activity against Golden Apple Snails. The analysis confirmed that fish poison tree seed is a potential source of bioactive substances that may support pharmaceutical uses and biopesticide production.

Key Words: fatty acid esters, fish poison tree, GC-MS, saponins, terpenoids.

Introduction

Barringtonia asiatica (L.) Kurz, commonly known as fish poison tree and locally named as “boton” in the Philippines, is a common strand of tree located along the seashore serving as shade tree along the boulevard and avenues by the sea (Stuart, 2018). This tree favors the wet tropical, moist tropical, and wet subtropical climatic zones and grows about 7–25 meters tall. The stem grows episodically and shows marked segmentation of the axes with prominent leaf scars. Leaf size varies from small- to medium-sized lanceolate leaves in the leptocaul species, 5–20 cm, to huge obovate-lanceolate leaves to 20–40 cm in width.

The leaves are locally used as vegetable food, herb, spice, and medication for backaches, sore joints, rheumatism, hernia, and diarrhea (Barwick, 2004). According to Stuart (2018), in the Philippines, leaves are heated and applied as topical remedy for stomachache. Similarly, Ravikumar et al. (2015) reported that the Nicobari tribe of India utilized leaves for the treatment of fractures, wounds, de-worming, and pain relief. The flowers are large pinkish-white, which give off a sickly-sweet smell to attract bats and moths, which pollinate them in return.

The fruit, otherwise known as box fruit, due to the distinct square-like diagonals jutting out from the cross section of the fruit, measures 9–11 cm in diameter (Figure 1). According to Martin (2005), the fruit, which is extremely water-resistant and buoyant, is dispersed by the ocean current and can survive afloat for up to fifteen years. According to Smith et al. (2005) and Jianse et al. (2004), these fruits are used for food. It is also used to treat swollen spleen after an attack of malaria, treat hernia, cysts, goiter, tumor, boils, and all kinds of lumps or bumps. In addition, it is used to poison fish. The seeds, on the other hand, which often emerge in fruit, can easily grow ashore when soaked in rainwater (Prance, 2012). Generally, all the parts of the fish poison tree contain many natural phytochemicals possessing a wide range of biological effects including antioxidant, antibacterial, antifungal, antitumor, anti-viral, and anti-inflammatory (Omolso, 2002; Burton et al., 2003; Kumar et al., 2007; Brunet et al., 2009; Ravikumar et al., 2015).

Figure 1

Various parts of Barringtonia asiatica known as fish poison tree.



Meanwhile, studies showed different biological activities of the chemical constituents that can be extracted from the seed. Traditionally, the seeds of the fish poison tree are grated and mixed with coconut cream to relieve burns and remedy ringworm (WHO, 1998). According to Ravikumar et al. (2015), throughout the Pacific, the seeds of this tree are also used traditionally to stun or kill fish due to its ichthyotoxic properties. Marston and Hostettmann (1991) explained that the toxin in the seeds can hemolyze red blood cells of cold-blooded animals, hence killing fish. It was reported that the 3-O- $\{[\beta\text{-D-galactopyranosyl-(1}\rightarrow\text{3)-2-}\beta\text{-D-glucopyranosyl-(1}\rightarrow\text{2)]-}\beta\text{-D-glucoronopyranosyloxy}\}$ -21-O- $\{[(2\text{E})\text{-2-methyl-1-oxo-2butenyl]oxy}\}$ -22-O-(2-methyl-1-oxobutoxy)-15,16,28-trihydroxy-(3 β ,15 α ,16 α ,22 α)-olean-12-ene, known as Ranuncoside VIII is the active piscicidal compound contained in the seed extract of fish poison tree. Furthermore, Mojica and Micor (2007), Brunet et al. (2009), and Tanor et al. (2014) reported the presence of saponins and terpenoids (Bakar, et al. 2009) which are known for their active biopesticidal properties. However, there are more to explore when it comes to the phytochemical components of these seeds. Therefore, this study aims to determine the phytochemical constituents in the seed of fish poison tree as potential source of bioactive compounds for biopesticide and pharmaceutical uses.

Materials and Methods

Collection and Authentication of Fish Poison Tree Seeds

The fruits of fish poison tree were gathered alongside the coastline of Dipaculao, Aurora. Matured fruit (either brown or dark green in color) is collected underneath the tree or along the beach areas. It was then brought to ASCOT Science Laboratory at Bazal Campus, Maria Aurora, Aurora for processing.

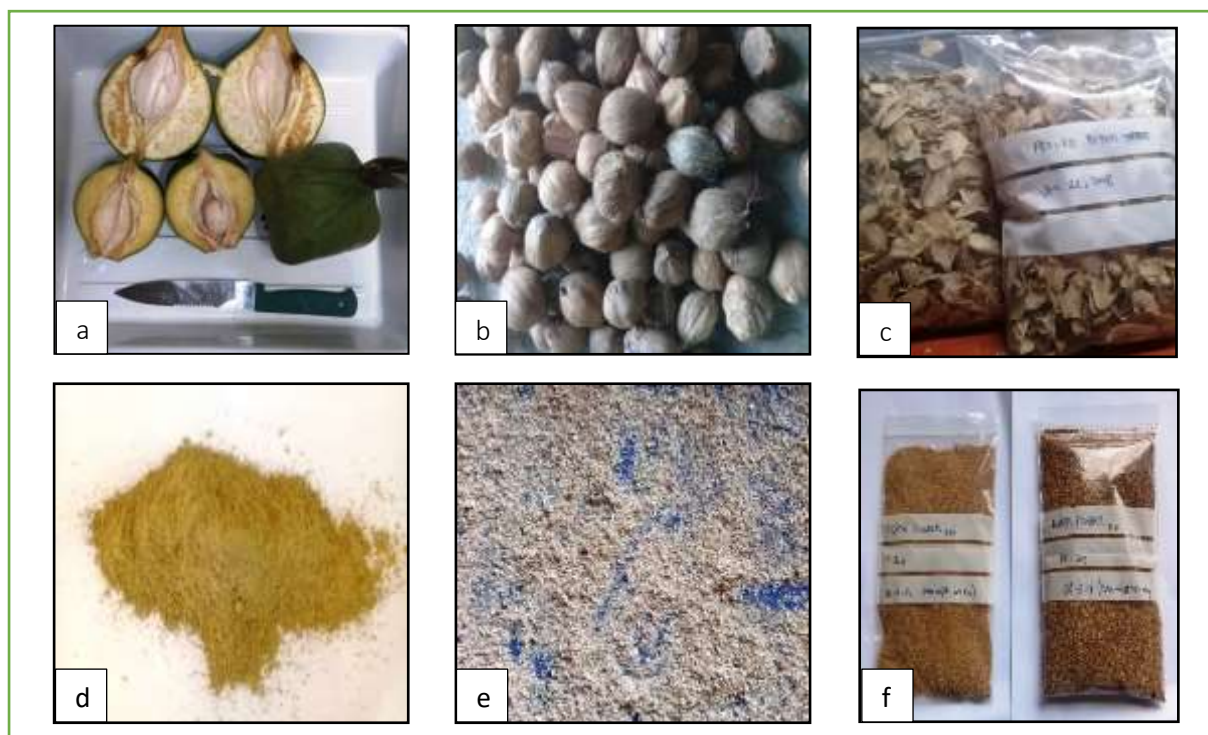
The plant material *Barringtonia asiatica* was inspected and authenticated by Paul Henrico P. Golo Cruz, M.Sc. and Angeles M. de Leon, PhD at the Department of Biology, College of Arts and Sciences, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines.

Preparation of Fish Poison Tree Seed Powder

The collected matured fruits were dehusked and cut open to expose and separate the seeds. The seed coat from the extracted seed was peeled off. The seed was then placed in the shredder machine to cut into pieces, and air dried for 2 days. After air drying, the shredded *Barringtonia asiatica* seeds were placed into a grinder to further pulverize and allow the powder to cool before putting in a container (Figure 2).

Figure 2

Preparation of Barringtonia asiatica Seed Powder



Preparation of the Plant Extract

About 20.0 g of powdered sample was extracted with 100.0 ml of 80% ethanol. The ethanolic extract was concentrated using rotary evaporator and the concentrate is rediluted with 100 ml of distilled water.

Test for Tannins (Ferric Chloride Test)

One gram of powdered sample was boiled with 20 ml distilled water in a water bath for about 5 minutes. The mixture was then filtered while hot. Afterwards, the filtrate was added with 2–3 drops of 0.1% FeCl_3 . The formation of greenish-black precipitate confirms the presence of tannins (Pradeep et al., 2014).

Test for Phlobatannins

About 3 ml of extract was added with 2 ml of 1% HCl and boiled in a water bath. The formation of red precipitate confirms the presence of phlobatannins (Pradeep et al., 2014).

Test for Saponins (Froth Test)

Two grams of powdered sample was boiled with 20 ml distilled water in a water bath. The mixture was then filtered. A 10 ml portion of the filtrate was added with 5 ml of distilled water and was shaken vigorously. The formation of a stable froth denotes the presence of saponins in the sample (Sri Murni et al., 2011).

Test for Flavonoids (Ammonia Test)

One ml of 10% NH_3 solution was added to 3 ml of the extract. The formation of a pale yellow precipitate was taken as a positive result for flavonoids (George & Shanmugam, 2014).

Test for Coumarins (Sodium Hydroxide Test)

One ml chloroform and 1 ml of 10% NaOH solution were added to 3 ml of the extract. The formation of a yellow precipitate was taken as a positive result for coumarins (Vimalkumar, 2014).

Test for Alkaloids (Maeyer's Test)

Two grams of powdered sample was added with 10 ml of diluted HCL and 5 ml of water. The mixture was boiled for 5 minutes and filtered. The filtrate was added with Maeyer's reagent and the formation of flesh precipitate or turbidity denoted the presence of alkaloids (Sri Murni et al., 2011).

Test for Terpenoids (Salowski Test)

About 5 ml of the extract was mixed in 2 ml of chloroform followed by gradual addition of 3 ml concentrated sulfuric acid. The reddish-brown coloration at the interface denoted the presence of terpenoids (Sri Murni et al, 2011).

Test for Cardiac Glycosides (Keller-Killani Test)

To 2 ml of extract, 1 ml of glacial acetic acid and one drop of 5% FeCl_3 were added and mixed. This mixture was then gradually transferred to another test tube containing 2 ml of concentrated sulfuric acid. The appearance of a reddish brown ring at the interface of the two solutions confirmed the presence of cardiac glycosides (Sheel, 2014).

Gas Chromatography –Mass Spectroscopy analysis

A total of 50 μl final metabolite extracts, either the 85% methanol or saponin extract, were completely dried using the ScanVac, operating at 40°C into 150 μl glass insets. The sample was analyzed with and without derivatization. For the derivatization process, immediately after drying, the insets were sealed with airtight magnetic lids into GC-MS vials and derivatized by the addition of 40 μl trimethylsilyl cyanide (TMSCN) (Khakimov et al., 2013). All steps involving sample derivatization and injection were automated using a Dual-

Rail MultiPurpose Sampler (MPS) (Gerstel, Mülheim der Ruhr, Germany). After reagent addition, the sample was transferred into the agitator and incubated at 40°C for 40 minutes at 70 rpm. This procedure ensured precise derivatization time and reproducible sample injection.

Immediately after derivatization, 1 µl of the derivatized sample was injected into a cooled injection system port (CIS4, Gerstel) in splitless mode. The septum purge flow and purge flow to split vent at 2.5 min after injection were set to 25 and 15 ml/min, respectively. Initial temperature of the CIS4 port was 120°C, and heated at 5°C/s to 320 °C (after 30 s of equilibrium time), where it was kept for 10 min. After heating the CIS4 port, it was gradually cooled to 250 °C at 5 °C/s, and this temperature was kept constant during the entire run.

The GC-MS consisted of an Agilent 7890A GC and an Agilent 5975C series MSD (Agilent Technologies, Glostrup, Denmark). GC separation was performed on an Agilent HP-5MS column (30 m x 250 µm x 0.25 µm) by using hydrogen carrier gas at a constant flow rate of 1.2 ml/min. The GC oven temperature program was as follows: initial temperature, 40 °C, heating rate, 12.0 °C.min⁻¹; end temperature, 300 °C; hold time 8.0 min; and post-run time 5 min at 40 °C. Mass spectra were recorded in the m/z range of 50–600 with a scanning frequency of 2.3 scan/s, and the MS detector was switched off during the 8.5 min solvent delay time. The transfer line ion source, and quadrupole temperatures were set to 280°C, 230°C, and 150°C. The mass spectrometer was tuned according to manufacturer recommendations by using perfluorotributylamine. The MPS and GC-MS were controlled using vendor software Maestro (Gerstel). A blank sample containing only the derivatization reagent was run in order to monitor reagent-derived and column-derived non-sample related peaks. An alkane mixture standard sample (all even C10-C40 alkanes at 50 mg/ml in hexane) was used prior to calculate retention indices (Khakimov et al., 2016).

Interpretation of mass spectrum of GC-MS was done using database of National Institute Standard and Technology (NIST). The mass spectrum of unknown component was compared with the spectrum of the known component stored in the NIST library. Major components were identified by with authentic standards and by with recorded from computerized libraries. The compound name, probability, molecular formula, molecular weight, peak area, and biological activity of the test materials were ascertained. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas.

Biopesticidal Activity Testing

Field testing was conducted to test the biopesticidal activity of the powdered seed of fish poison tree against golden apple snail. Twelve plots measuring 3 x 4 m were prepared for four treatments with three replications. One hundred golden apple snails were stocked in each plot with a water level of 2–3 cm for the snails to mobilize the area. After 24 hours of acclimatizing the snails, each plot was sprayed with the different treatments, T1 (control), T2 (50 ml), T3 (100 ml), and T4 (150 ml). Solutions sprayed were taken from a base solution by diluting the different volume of treatments to one liter. After a day, golden apple snails were collected and counted for the mortality rate.

Results and Discussion

Phytochemical Constituents Present in *Barringtonia asiatica* Seed Powder

Out of eight phytochemical components tested, seven were present in the sample which includes saponin, terpenoids, tannins, flavonoids, coumarins, cardiac glycosides, and alkaloids (Table 2). Phlobatannins were absent. According to Buttler (1992), phytochemicals, as naturally occurring chemical compounds found in plants, provide health benefits for humans further than those attributed to macronutrients and micronutrients. Phytochemicals such as alkaloids, saponins, flavonoids, and other phenolic compounds play an important role in the growth and reproduction of most plants. These compounds also act as antifeedants and antipathogens.

Table 2

Result of the Phytochemical Screening of *Barringtonia asiatica* powder

No.	Test of Components	Remarks
1	Saponin	+
2	Terpenoids	+
3	Tannins	+
4	Phlobatannins	-
5	Flavonoids	+
6	Coumarins	+
7	Cardiac glycosides	+
8	Alkaloids	+

Legend: (+) = present; (-) = absent

The result of the phytochemical screening and GC-MS analysis conducted reveals chemical constituents that have pesticidal properties. Picardal (2018) reported the presence of secondary metabolites (flavonoids, tannin, alkaloids, glycosides, etc.) on the plant *Solenostemma argel* which caused snail mortalities. Likewise, Tripathi and Singh (2000), Tiwari and Singh (2004), and Picardal (2018) reported similar biopesticidal activity of the garlic, *A. sativum* against the snails *Indoplanorbis exustus* and *Lymnaea acuminata* when exposed to plant phytochemicals (e.g. eugenol, esters, saponins, etc.). In fact, Metwally (2006), Michail (2010), Mwine and Van Damme (2011), and Meriga et.al, (2012) revealed the toxicity of *A. sativum* towards the numerous rice pests were associated to its molluscicidal phytochemicals, namely; saponins, tannins, phorbol, steroids, flavonoids, terpene, and esters. According to San Martin and Cruz (2013), saponin inhibits certain cholinesterase enzymes that have a lethal effect to the nervous system of the snail and also causes formation of cholesterol in the body of the snail. Large numbers of saponins also affect the snail's membrane permeability by either forming pores in the membrane, altering sodium-potassium and calcium ATPase activity, or insertion of the hydrophobic saponin nucleus into the lipid layer.

Ahmed et al. (2014) also indicated that the molluscicidal activity of *Barringtonia asiatica* seed extract is due to its high alkaloid content, which causes adverse effects in the central nervous system of the snail. Dai et al. (2011) also showed that cardiac glycosides had a significant effect on GAS, resulting to the decrease in the glycogen content of the shell. This resulted to the impairment of the physiological metabolism of the snails and altering the hepatopancreas tissues of *P. canaliculata*, which consequently leads to fatality. Thus, the presence of the different phytochemical constituents in the seeds of fish poison tree such as saponins, alkaloids, cardiac glycosides, are evidence that the seed powder has a high pesticidal property.

Secondary substances in plants are known for a long time for their medicinal and pharmacological properties. These substances have been present in them to thrive in a rather hostile environment. The plant can indeed use its secondary metabolites to be protected against several pest animals and pathogenic microbes. According to Marelli et al. (2016), saponins have been proposed for the treatment of a variety of diseases, including diabetes, obesity, and osteoporosis. Their efficacy against cancer has been attributed to their ability to inhibit cell proliferation, to counteract angiogenesis, and to stimulate apoptosis. Güçlü-Ustündağ and Mazza (2007), Bissinger et al. (2014), and Elekofehinti (2015) also documented the toxicity of saponins to insects (insecticidal activity), parasite worms (anthelmintic activity), mollusks (molluscicidal), and fish (piscidal activity), and their antifungal, antiviral, and antibacterial activity.

Terpenoids and tannins present in the fish poison tree seed help prevent the development of chronic joint swelling (Agnihotri, 2010). The presence of terpenoids in various plants is widely used in herbal medicines (Edeoga et al., 2005). Trease and Evans (2002) reported that tannins have been widely used as an application to sprains, bruises, and superficial wounds. Plants rich in coumarins were used in folk medicine as traditional remedies drugs for the treatment of respiratory diseases (Correa, 1984). Many pharmacological activities have been also ascribed to coumarins such as anticlotting, hypotensive, antimicrobial, anti-inflammatory, and

antitumor activities (Leal et al., 2000). On the other hand, flavonoid compounds have significant biological activities that are key in wound healing such as radical scavenging/anti-oxidative, anti-allergic/anti-inflammatory, antiviral, antibacterial, antifungal, hypoglycemic, and anti-carcinogenic properties/effects (Agrawal, 2011; Stanković, 2011; Kumar & Pandey, 2012).

Cardiac glycosides are considered one of the most useful drugs in therapeutics. Cardiac glycosides are steroids having the ability to exert specific powerful action on the cardiac muscle. It increases the force of heart contraction without a concomitant increase on oxygen consumption. Consequently, the myocardium becomes a more efficient pump and is able to meet the demands of the circulatory system (Franswoth, 1966; Kelly, 1990). Evans (1996) reported that alkaloids are broadly varied in chemical structure and in pharmacological action. According to Ee et al. (2010) and Godlaski (2011), the toxicity of some alkaloids is widely recognized; however, they are a source of many biologically active phytochemicals with great potential for medicinal and agricultural uses. Many alkaloids have attractive pharmacological effects and are used as medications, such as recreational drugs, or in entheogenic rituals.

Percentage Composition of Phytochemical Constituents Using GC-MS Analysis

Further analysis using GC-MS revealed the presence of 42 phytochemical constituents of fish poison tree seed extract, 18 constituents for analysis without sample derivatization, and 25 constituents for analysis with sample derivatization. The active principles with their molecular formula, molecular weight (MW), and composition (%) in the methanol extract are presented in Tables 3 and 4. Most of the constituents were dominated by fatty acids, methyl esters, and sterols. Identified dominating compounds contributed from 5.62% up to 45.89% composition, while the rest had less than 3% composition. For the threshold similarity index, the following were used: 80-100 = certain; 70-79=uncertain/questionable; below 70=unknown.

GC-MS Analysis without Sample Derivation

Bioactive constituents elucidated without sample derivatization include Vitamin E contributing the highest percentage (43.53%), followed by chondrillasterol (14.20%), linoleic acid, methyl ester (8.80%), lanosterol (7.57%), stigmasterol (7.02%), 7,22-ergostadienone (6.19%), and 23-(phenylsulfanyl)lanosta-8,24-dien-3-ol which contributed 5.62% (Table 3). Other phytochemical constituents identified were: lupenone; 4,22-stigmastadiene-3-one; palmitic acid, methyl ester; squalene; (5E)-8-(2,5,5,8a-tetramethyl-1,4,4a,5,6,7,8,8a-octahydro-1-naphthalenyl)-6-methyl-5-octen-2-ol; globulol; lupeol; farnesyl bromide; linoleic acid, methyl ester; alpha-tocopherol-beta-D-mannoside; 3-n-butylthiophene-1,1-dioxide; and 1,3,12-nonadecatriene. Palmitic acid, methyl ester was identified in both analyses. Among the identified chemical components, vitamin E, stigmasterol, squalene, and lupeol are known to have the antioxidant property.

The chromatogram of fish poison tree seed extract analysis without sample derivatization is shown in Figure 3. This chromatogram shows a total of 22 peaks. The largest peak is at a retention time of 39.896 min corresponding to Vitamin E (Peak 5) with percent area of 43.53. Additional two peaks of Vitamin E (Peak 21 and 22) were elucidated at a retention time of 47.482 min with percent area of 2.19 and at a retention time of 48.392 min with percent area of 2.33, making a total percentage composition of 45.89%. It was followed by a retention time of 45.271 min corresponding to chondrillasterol (Peak 14) with percent area of 14.73 and a retention time of 43.794 corresponding to stigmasterol (Peak 11) with percent area of 7.28. The other main compounds from the analysis conducted include lanosterols (Peak 17 and 20) RT=46.224 min, %A=3.01 and RT=40.275 min, %Area=4.56, respectively; 7,22-ergostadienone (Peak 16) RT=45.976 min, %Area=6.42%; and 23-(Phenylsulfanyl)lanosta-8,24-dien-3-ol (Peak 18) RT=46.517, %Area=5.83.

Table 3

Percentage Composition of Barringtonia asiatica Seed Phytochemical Constituents Using GCMS- QP2010 Without Sample Derivatization

Peak No.	Retention Time, min.	Similarity Index, %	Components	Molecular Formula	Molecular Weight	Composition, %
1	28.545	82	Palmitic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270	1.25
2	31.678	95	Linoleic acid, methyl ester	C ₁₉ H ₃₄ O ₂	294	0.31
3	32.884	83	1,3,12-nonadecatriene	C ₁₉ H ₃₄	262	0.06
4	36.932	83	Squalene	C ₃₀ H ₅₀	410	0.44
5	39.896	97	Vitamin E	C ₂₉ H ₅₀ O ₂	430	43.53
6	40.827	63	3-n-Butylthiophene-1,1-dioxide	C ₈ H ₁₂ O ₂	172	0.08
7	41.467	72	alpha-Tocopherol-beta-D-mannoside	C ₃₅ H ₆₀ O ₇	592	0.85
8	42.710	75	(5E)-8-(2,5,5,8a-Tetramethyl-1,4,4a,5,6,7,8,8a-octahydro-1-naphthalenyl)-6-methyl-5-octen-2-ol	C ₂₃ H ₄₀ O	332	0.89
9	43.336	95	Squalene	C ₃₀ H ₅₀	410	0.71
10	43.510	71	Globulol	C ₁₅ H ₂₆ O	222	0.73
11	43.794	95	Stigmasterol	C ₂₉ H ₄₈ O	412	7.02
12	44.788	74	Farnesyl bromide	C ₁₅ H ₂₅ Br	284	0.56
13	45.085	77	Lupenone	C ₃₀ H ₄₈ O	424	3.03
14	45.271	90	Chondrillasterol	C ₂₉ H ₄₈ O	412	14.20
15	45.719	79	Lupeol	C ₃₀ H ₅₀ O	426	0.71
16	45.976	83	7,22-Ergostadienone	C ₂₈ H ₄₄ O	396	6.19
17	46.224	88	Lanosterol	C ₃₀ H ₅₀ O	426	2.73
18	46.517	77	23-(Phenylsulfanyl)lanosta-8,24-dien-3-ol	C ₃₆ H ₅₄ OS	534	5.62
19	47.031	85	4,22-Stigmastadiene-3-one	C ₂₉ H ₄₆ O	410	1.80
20	47.275	88	Lanosterol	C ₃₀ H ₅₀ O	426	4.56
21	47.282	90	Vitamin E	C ₂₉ H ₅₀ O ₂	430	1.15
22	48.392	91	Vitamin E	C ₂₉ H ₅₀ O ₂	430	2.92

Figure 3

Chromatogram of Fish Poison Tree Seed Extract Analysis without Sample Derivatization

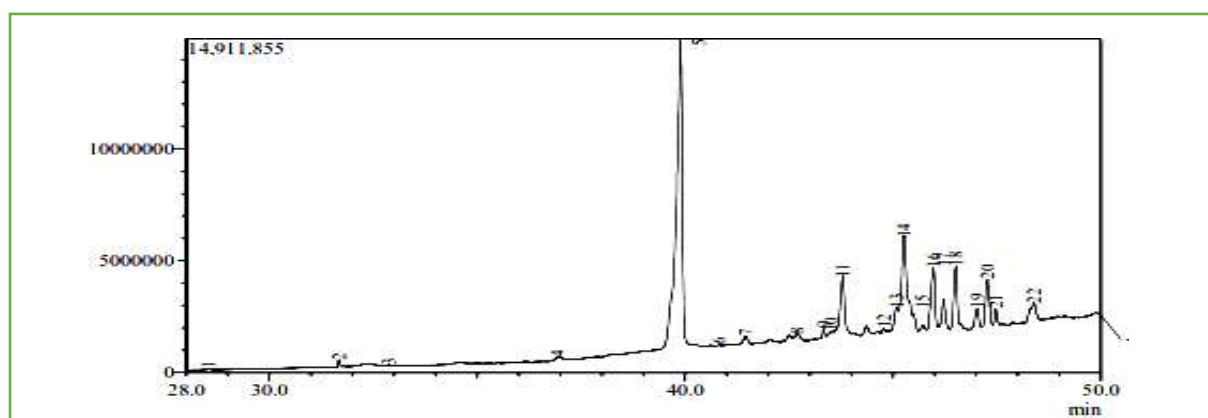


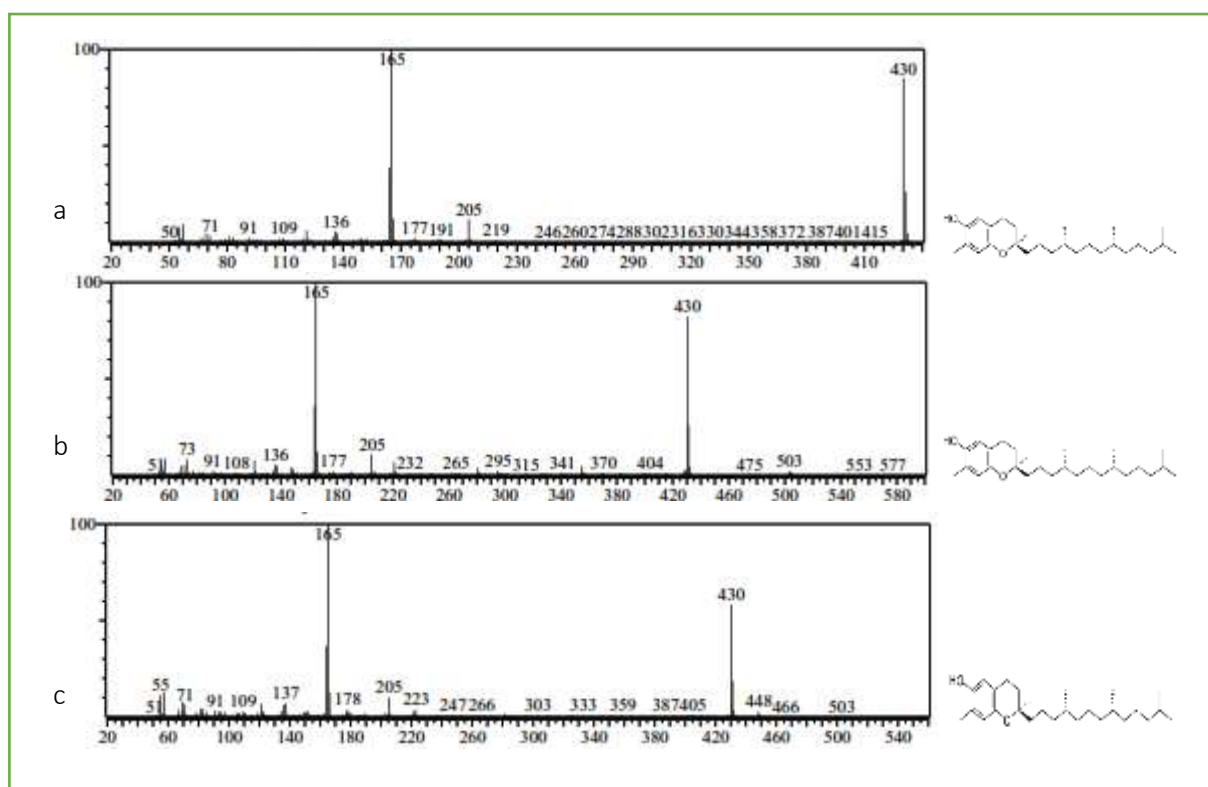
Figure 4 presents the three mass spectra of Vitamin E identified with different retention times (39.896, 47.485, and 48.392 min, respectively) and percent areas (43.53%, 1.15%, and 2.92% respectively). Vitamin E comprised the highest composition of the sample. From the three spectra, peaks occur at 430 m/z which correspond to molecular ion 430. The three spectra show a relative high abundance. Comparing the different spectra to the compound is analogous to the spectra of Vitamin E with a molecular formula of $C_{29}H_{50}O_2$.

The similarity indices for the three peaks of Vitamin E were equal or above 90. The detection of two peaks (Peaks 21 and 22) with smaller percent areas similar to Vitamin E needs further investigation for the confirmation of the compounds. The analysis was screening only due to limitations in the reference standard so confirmatory analysis was not done.

Vitamin E is the major lipid-soluble antioxidant in the cell antioxidant defense system and is exclusively obtained from the diet. The major biological role of vitamin E is to protect polyunsaturated fatty acids (PUFAs) and other components of cell membranes and low-density lipoprotein (LDL) from oxidation by free radicals. According to Kagan (1998), although vitamin E is primarily located in cell and organelle membranes where it can exert its maximum protective effect, its concentration may only be one molecule for every 2000 phospholipid molecules. This suggests that after its reaction with free radicals, it is rapidly regenerated, possibly by other antioxidants.

Figure 4

Mass Spectra and Structures of Vitamin E Elucidated in Fish Poison Tree Seed Extract



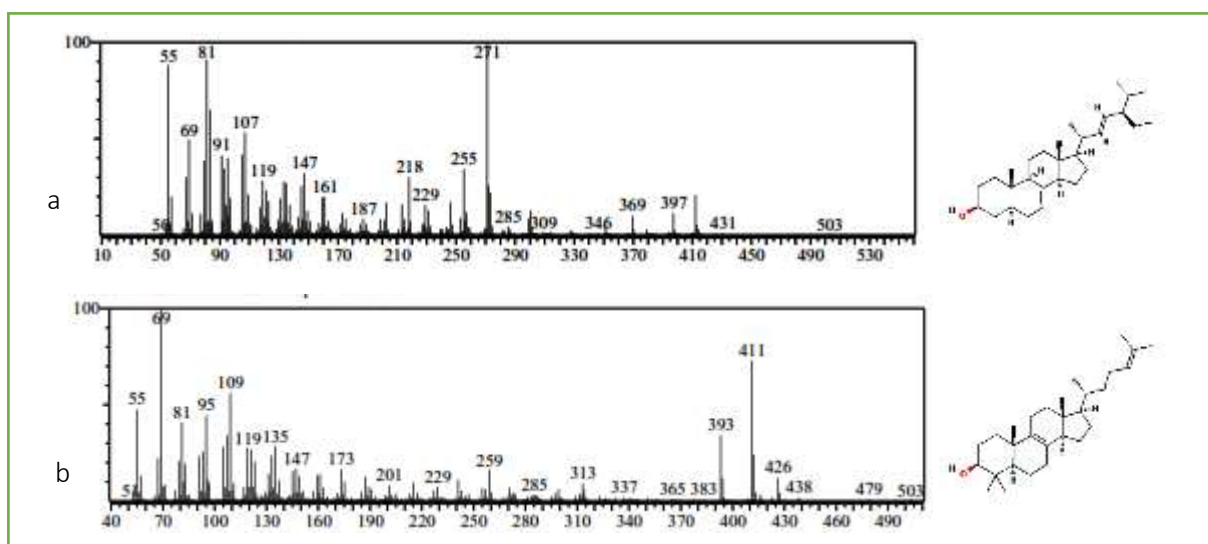
Vitamin E compounds are also known for different biological activities such as antiageing, analgesic, antidiabetic, anti-inflammatory, antioxidant, antidermatitic, antileukemic, antitumor, anticancer, hepatoprotective, hypocholesterolemic, antiulcerogenic, vasodilator, antispasmodic, antibronchitic, and anticoronary (Dr. Duke's Phytochemical and Ethnobotanical Databases).

The mass spectra of chondrillasterol and lanosterol elucidated in the fish poison tree seed extract are shown in Figure 5. From these spectra, a peak occurs at m/z 412 (Figure 5a) which corresponds to molecular ion 412, showing 20% relative abundance. Comparing to the NIST known spectra library, the compound is analogous to the spectrum of chondrillasterol with a molecular formula of $C_{29}H_{50}O_2$, while in Figure 5b spectrum, a peak occurs at m/z 426 which corresponds to molecular ion 426, showing 100% relative abundance. The compound was ascertained as lanosterol with a molecular formula $C_{30}H_{50}O$ using the NIST database.

In terms of its biological activity, Mozirandi et al. (2019) reported that chondrillasterol isolated from *Veronica adonis* (Asteraceae) has antibacterial properties against *S. aureus*, *K. pneumonia*, and *P. aeruginosa*. This active phytochemical could be a useful template for the development of new antimicrobial agents with both antibacterial and antibiofilm activity. According to Chen et.al (2006), this organic compound is also potential for cytotoxicity activities. This bioactive compound was also isolated from *Gambeya boiviniana* Pierre (Rasoanaivo, 2014) and *Lagenaria leucantha* var. *gourda* (Itoh, 1981) crude extract as building blocks for new antibiotics which provide novel opportunities in developing new effective antibiotics.

Figure 5

Mass Spectra and Structures of a) Chondrillasterol and b) Lanosterol Elucidated in Fish Poison Tree Seed Extract



Moreover, Balashova et. al (2018) reported that lanosterol present in Lanomax can stabilize rapid progressive cataracts, making it possible for nonsurgical dissolution of human cataracts.

Figure 6 shows the mass spectra of stigmasterol and squalene elucidated in the fish poison tree seed extract. From the spectra in Figure 6a, a peak occurs at m/z 412 which corresponds to molecular ion 412 showing 40% relative abundance. Using the NIST spectra database, the compound is analogous to the spectrum of stigmasterol with a molecular formula of $C_{29}H_{48}O$. On the other hand, Figure 6b shows a peak that occur at m/z 410 corresponding to molecular ion 410, showing a very low relative abundance. The compound was ascertained squalene with a molecular formula of $C_{30}H_{50}$ through the NIST spectra database

Stigmasterol and squalene exhibit cancer-preventive and anti-inflammatory activity (Fethi et al., 2012). Stigmasterol is used as a precursor in the manufacture of semi-synthetic progesterone, a valuable human hormone that plays an important physiological role in the regulatory and tissue rebuilding mechanisms related to estrogen effects, as well as acting as an intermediate in the biosynthesis of androgens, estrogens, and corticoids. Panda et. al (2009) stated that stigmasterol has a high potential for thyroid inhibitory,

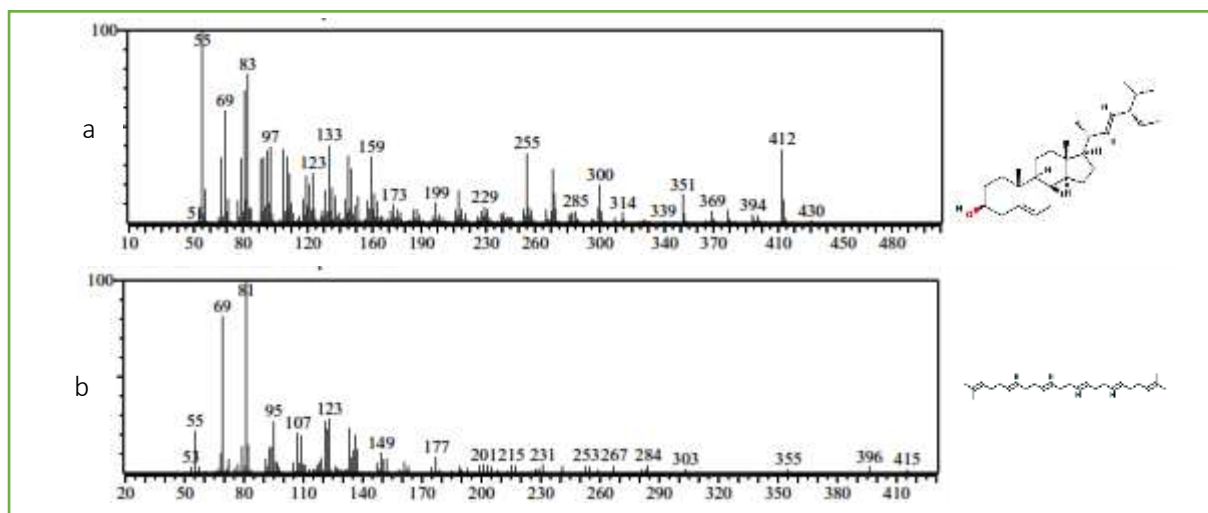
antiperoxidative, and hypoglycemic effects. Stigmasterol has been reported earlier as a strong antioxidant having antibacterial activity against multidrug resistant mycobacteria (Hamdam et.al, 2011; Navarro-Garcia et. al., 2011).

In addition, squalene was reported with the following potential properties like antibacterial (Newmark, 1997), antioxidant, antitumor, and cancer preventive properties (Kelly, 1999; Smith, 2000), immune-stimulant (Salman et al., 2006) and also as a lipoxygenase inhibitor (Wei & Shibamoto, 2007) by several studies. In addition to this, squalene is also known for antiageing, analgesic, antidiabetic, anti-inflammatory, antioxidant, antidermatitic, antileukemic, antitumor, anticancer, hepatoprotective, hypocholesterolemic, antiulcerogenic, vasodilator, antispasmodic, antibronchitic, and anticoronary properties.

Furthermore, according to cancer researchers Reddy and Couvreur (2009), squalene possesses an anti-carcinogenic agent with the observed correlation between a high amount of squalene in shark fatty tissues and the absence of cancer in this species. According to Smith and Theresa (2000), squalene can act as a free radical scavenger and seems to enhance the anti-carcinogenic effect of co-ingested drug treatments and shows synergism against cancer with oleic acid, another constituent found in the *Chrysophyllum albidum* seed oil.

Figure 6

Mass Spectra and Structures of A) Stigmasterol and B) Squalene Elucidated In Fish Poison Tree Seed Extract.



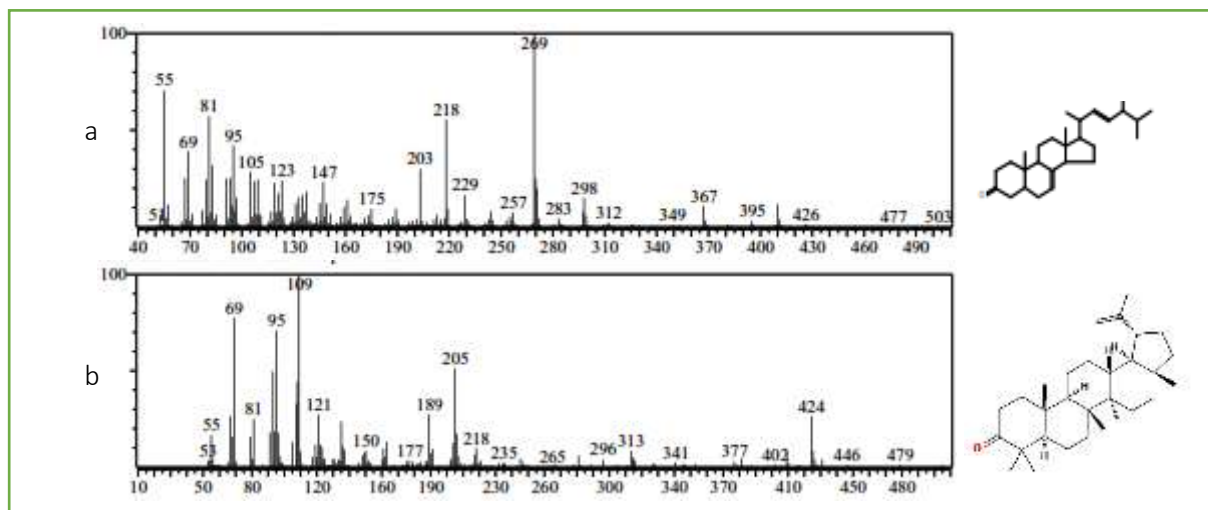
The mass spectra for 7,22-ergoestadione and lupenone are shown in Figure 7. Figure 7a shows a peak that occurs in m/z 396 which corresponds to molecular ion 396. Using the NIST spectra database, the compound was ascertained to 7,22-ergoestadione with a molecular formula of $C_{28}H_{44}O$. Figure 7b on the other hand, shows a peak that occurs at m/z 424 which corresponds to molecular ion 424, showing 30% relative abundance. Compared to the NIST spectra database, the compound is analogous to the spectrum of lupenone with a molecular formula of $C_{30}H_{48}O$.

Mutai et. al. (2004) reported that lupenone which is obtained from *A. mellifera*, has been shown to exhibit significant cytotoxicity against non-small-cell lung carcinoma-N6 (NSCLC-N6) cell line. Na et al. (2009) added that lupenone together with lupeol, inhibit protein tyrosine phosphatase 1B (PTP 1B) which appears to be an attractive target of new drugs development for type 2 diabetes and obesity. Gupta et. al (2011) selected lupenone and β -sitosterol as index components in *Rhizoma Musae*, because some literature has shown that the lupenone and β -sitosterol have anti-diabetic activity. Lupenone, lupeol, and taraxerol inhibited the gene

expression and production of MUC5AC mucin induced by TNF- α from NCI-H292 cells, respectively. The results indicated that lupenone, lupeol, and taraxerol derived from *Adenophora triphylla* var. *japonica* regulates the production and gene expression of mucin, by directly acting on airway epithelial cells. In addition, the results partly explain the mechanism of *Adenophora triphylla* var. *japonica* as a traditional remedy for diverse inflammatory pulmonary diseases (Yoon et.al, 2015).

Figure 7

Mass Spectra and Structures of a) 7,22-ergoestadione, and b) Lupenone Elucidated in Fish Poison Tree Seed Extract

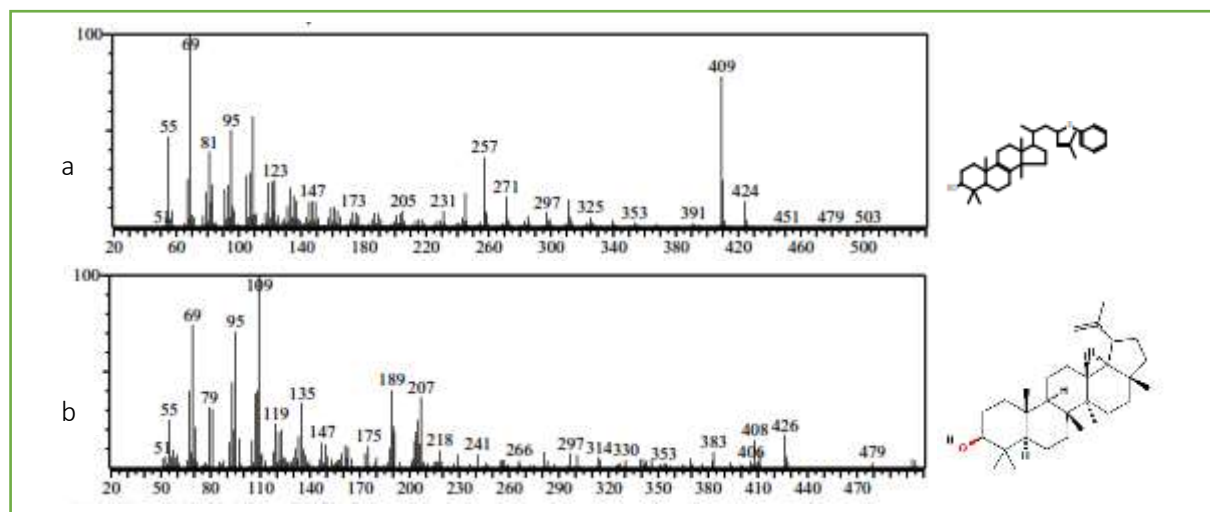


The mass spectra for 23-(phenylsulfanyl) lanosta-8,24-dien-3-ol and lupeol are shown in Figure 8. Figure 8a shows a peak that occurs at m/z 534 which corresponds to molecular ion 534. Compared to the NIST spectra database, the compound is analogous to the spectrum of 23-(phenylsufanyl) lanosta-8,24-dien-3-ol with a molecular formula of $C_{36}H_{54}OS$. On the other hand, Figure 8b shows a peak that occurs in m/z 426 which corresponds to molecular ion 426, showing 20% relative abundance. Using the NIST spectra database, the compound was ascertained as lupeol with a molecular formula of $C_{30}H_{50}O$.

Aside from this, fish poison tree seed extract contains lupeol which exhibits biological activities and can be used as antiprotozoal, anti-inflammatory, antitumor and chemopreventive agents (Gallo et al., 2008). In addition, it has hypoglycemic and thyroid inhibiting properties, precursor of progesterone, antimicrobial, anticancer, anti-arthritis, anti-asthma, and anti-inflammatory. Lupeol has also potentials for anti-inflammatory activity (Geetha & Varalakshmi, 2001) and anticancer (Saleem, 2009).

Figure 8

Mass Spectra and Structures of a) 23-(phenylsulfanyl) lanosta-8,24-dien-3-ol and b) Lupeol Elucidated in Fish Poison Tree Seed Extract



GC-MS Analysis with Sample Derivatization

Table 4 presents the dominating compounds elucidated with sample derivatization which include linoleic acid, ethyl ester contributing the highest composition (40.30%), palmitic acid, ethyl ester (10.24%), linoleic acid, methyl ester (8.80), (E)-9-octadecenoic acid, ethyl ester (6.73%), and 17-methyloctadecanoic acid, methyl ester (6.05%). The remaining compounds contributed less than 3% which includes mostly fatty acid esters. These include O-trifluoroacetyl-(+)-alpha-tocopherol; (9Z)-9,17-octadecadienal; heptanoic acid, docosyl ester; heptanoic acid, docosyl ester; oleanitrile; cholesterol isocaproate; gamma-sitosterol; palmitic acid, methyl ester; 11,14-eicosadienoic acid, methyl ester; stigmaterol O-trifluoroacetate; 3,6,9-triethyl-3,6,9-trimethyltetracyclo[6,1,0(2,4), 0(5,7)] nonane; oleic acid, methyl ester, eicosanoic acid, ethyl ester, stearic acid, methyl ester; 4,8,12,16-tetramethylheptadecan-4-olide; phthalic acid, bis(2-ethylhexyl)ester; 2-hexyldecanol; olean-12-en-3-one; 11-octadecenoic acid, methyl ester; Z-7-tetradecenal; and 9-hexadecenoic acid, ethyl ester. Most of the identified phytochemical constituents were methyl and ethyl esters.

Table 4

Percentage Composition of *Barringtonia asiatica* Seed Phytochemical Constituents Using GCMS- QP2010 with Sample Derivatization

Peak No.	Retention Time, min	Similarity Index, %	Components	Molecular Formula	Molecular Weight	Composition, %
1	27.059	91	(9Z)-9,17-Octadecadienal	C ₁₈ H ₃₂ O	264	2.87
2	27.190	88	Z-7-Tetradecenal	C ₁₄ H ₂₆ O	210	0.28
3	27.722	91	2-Hexyldecanol	C ₁₆ H ₃₄ O	242	0.38
4	28.455	96	Palmitic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270	1.25
5	29.356	88	9-Hexadecenoic acid, ethyl ester	C ₁₈ H ₃₄ O ₂	282	0.20
6	29.765	96	Palmitic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	284	10.24
7	31.646	96	Linoleic acid, methyl ester	C ₁₉ H ₃₄ O ₂	294	8.80
8	31.762	86	Oleic acid, methyl ester	C ₁₉ H ₃₆ O ₂	296	0.91
9	31.861	89	11-Octadecenoic acid, methyl ester	C ₁₉ H ₃₆ O ₂	296	0.30
10	32.230	95	Stearic acid, methyl ester	C ₁₉ H ₃₈ O ₂	298	0.69
11	32.873	95	Linoleic acid ethyl ester	C ₂₀ H ₃₆ O ₂	308	40.30
12	32.968	89	(E)-9-Octadecenoic acid ethyl ester	C ₂₀ H ₃₈ O ₂	310	5.45
13	33.074	93	(E)-9-Octadecenoic acid ethyl ester	C ₂₀ H ₃₈ O ₂	310	1.28
14	33.435	95	17-methyloctadecanoic acid methyl ester	C ₂₀ H ₄₀ O ₂	312	6.05
15	36.195	76	4,8,12,16-Tetramethylheptadecan-4-olide	C ₂₁ H ₄₀ O ₂	324	0.49
16	36.824	88	Eicosanoic acid, ethyl ester	C ₂₂ H ₄₄ O ₂	340	0.79
17	38.402	63	Oleanitrile	C ₁₈ H ₃₃ N	263	1.55
18	39.273	89	Pthalic acid, bis(2-ethylhexyl)ester	C ₂₄ H ₃₈ O ₄	390	0.44
19	40.818	78	Heptanoic acid, docosyl ester	C ₂₉ H ₅₈ O ₂	438	1.61
20	42.905	70	11,14-Eicosadienoic acid, methyl ester	C ₂₁ H ₃₈ O ₂	322	1.01
21	44.295	63	3,6,9-triethyl-3,6,9-trimethyltetracyclo[6,1,0(2,4),0(5,7)] nonane	C ₁₈ H ₃₆	246	0.92
22	44.528	72	O-trifluoroacetyl-(+)-alpha-Tocopherol	C ₃₁ H ₄₉ F ₃ O ₃	526	2.96
23	47.343	63	Stigmasterol O-trifluoroacetate	C ₃₁ H ₄₇ F ₃ O ₂	508	0.94
24	47.468	57	Cholesterol isocaproate	C ₃₃ H ₅₆ O ₂	484	1.51
25	48.187	55	Olean-12-en-3-one	C ₃₈ H ₄₈ O	424	0.38
26	49.796	70	gamma-Sitosterol	C ₂₉ H ₅₀ O	414	1.28

The chromatogram of fish poison tree seed extract analysis with sample derivatization is shown in Figure 9. This chromatogram shows a total of 26 peaks. The largest peak is at a retention time of 32.873 min corresponding to linoleic acid, ethyl ester (Peak 11) with percent area of 43.39. It was followed by a retention time of 29.765 min corresponding to palmitic acid, ethyl ester (Peak 6) with percent area of 11.03 and a retention time of 31.646, corresponding to linoleic acid, methyl ester (Peak 7) with percent area of 9.47. The other main compounds elucidated from the analysis conducted include 17-methyloctadecanoic acid, methyl ester (Peak 14) RT=33.435 min, %A=6.52; (E)-9-octadecenoic acid methyl ester (Peak 12 and 13) RT=32.968 min, %Area=5.45, and RT=33.074 min, %Area=1.80 respectively; O-trifluoroacetyl-(+)- α -tocopherol (Peak 22) RT=44.528 min, %Area=3.18%; and (9Z)-9,17-octadecadienal (Peak 1) RT=27.059, %Area=3.09. The remaining 18 phytochemical constituents have less than 3.00% area composition.

Figure 9

Chromatogram of Fish Poison Tree Seed Extract Analysis with Sample Derivatization

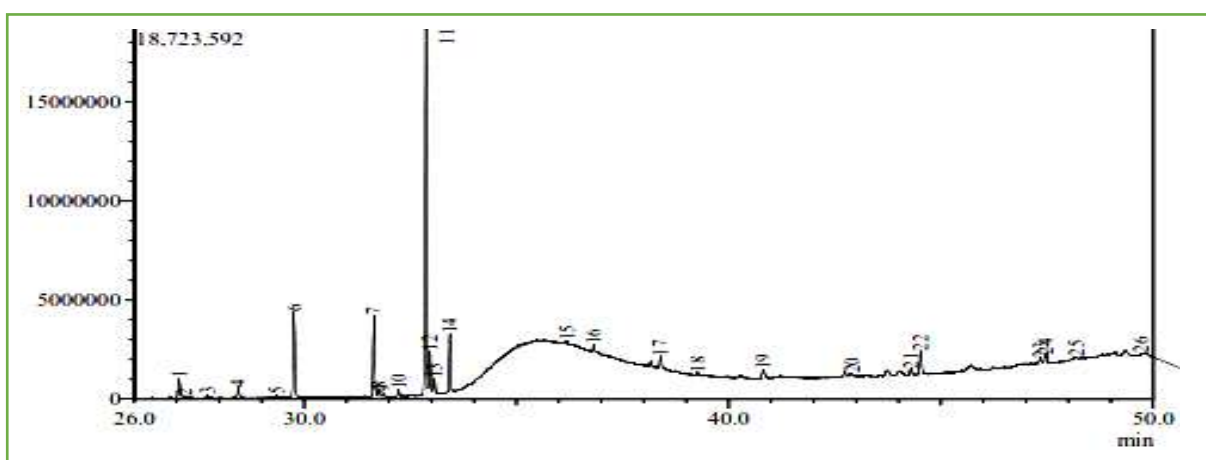


Figure 10 shows the mass spectra and structures of linoleic acid, ethyl ester, palmitic acid, ethyl ester, and 9-hexadecenoic acid, ethyl ester in the fish poison tree seed extract. In Figure 10a, the spectrum occurs in m/z 308 which corresponds to molecular ion 308. Using the NIST spectra database, the compound is similar to the spectrum of linoleic acid, ethyl ester with a molecular formula of $C_{20}H_{36}O_2$. The spectrum in Figure 10b shows a peak that occurs in m/z 284 which corresponds to molecular ion 284. Comparing to the NIST spectra database, the compound is analogous to the spectrum of palmitic acid, ethyl ester with a molecular formula $C_{18}H_{36}O_2$, while the spectrum in Figure 10c shows a peak that occurs in m/z 282 which corresponds to molecular ion 282. The compound was ascertained as 9-hexadecenoic acid, ethyl ester with a molecular formula $C_{18}H_{34}O_2$ in comparison to the NIST spectra database.

Fatty acid ester such as linoleic acid, ethyl ester, palmitic acid, ethyl ester and 9-hexadecenoic acid, ethyl ester, has antifungal, antitumor, and antibacterial properties. linoleic acid, ethyl ester is known for many biological properties such as hypocholesterolemic, nematocide, antiarthritic, hepatoprotective, antiandrogenic, hypocholesterolemic, 5- α reductase inhibitor, antihistaminic, anticoronary, insectifuge, antieczemic, and antiacne. palmitic acid, ethyl ester was reported with the following activities: antibacterial (Kujumgiev et al., 1993), hypercholesterolemic, lubricant (Ivanova et al., 2002), cosmetic and perfumery (Kroes et al., 1992). On the other hand, (E)-9-octadecenoic acid ethyl ester which is also a fatty acid ester was reported to have potentials in perfume production (Harborne & Baxter, 1983; Ross, 2003).

Figure 10

Mass Spectra and Structures of a) Linoleic Acid, Ethyl Ester, b) Palmitic Acid, Ethyl Ester, and c) 9 Hexadecenoic Acid, Ethyl Ester Elucidated in Fish Poison Tree Seed Extract

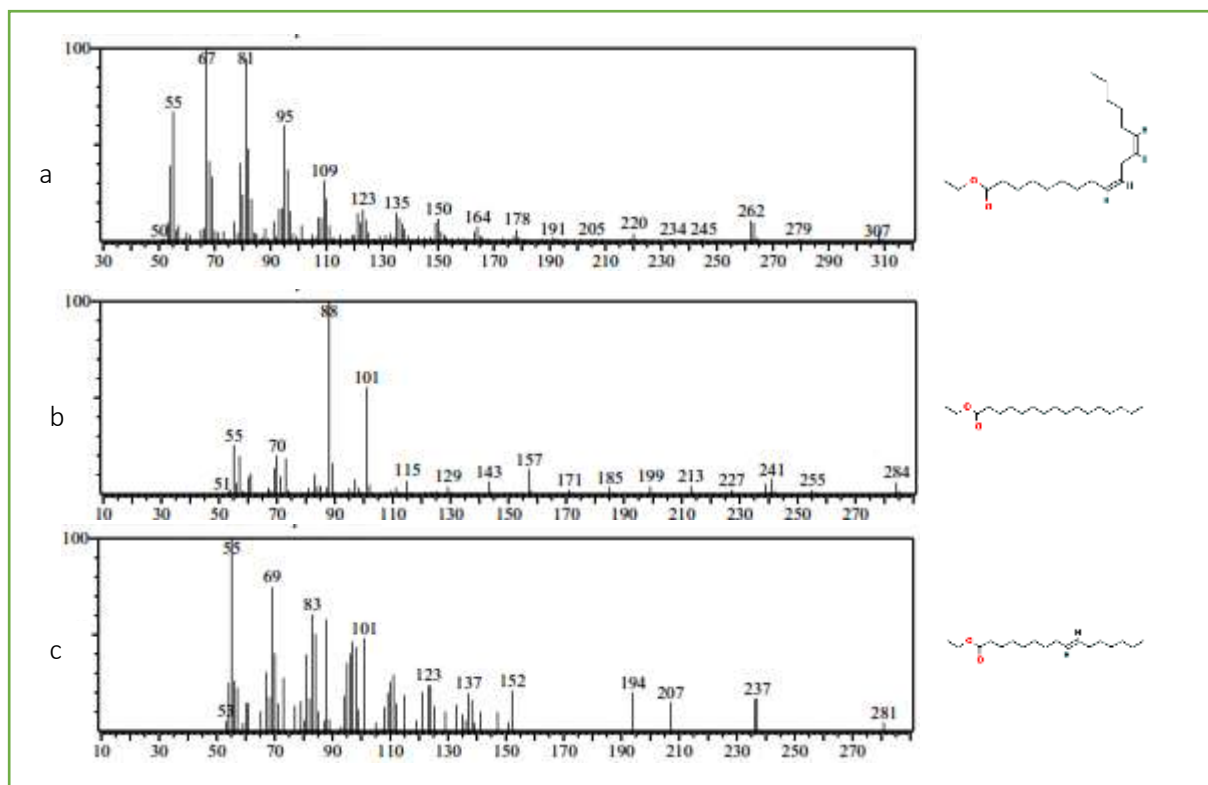
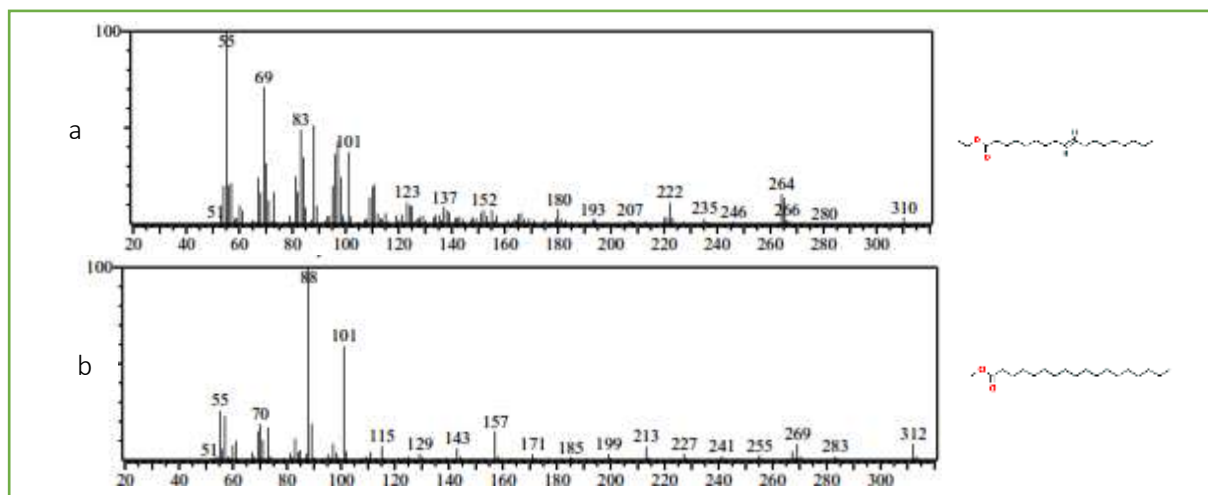


Figure 11 shows the mass spectra and structures of (E)-9-octadecenoic acid ethyl ester and 17-methyloctadecanoic acid methyl ester in the fish poison tree seed extract. In Figure 11a, the spectrum occurs in m/z 310 which corresponds to molecular ion 310. Using the NIST spectra database, the compound is similar to the spectrum of (E)-9-octadecenoic acid ethyl ester with a molecular formula of $C_{20}H_{38}O_2$. Spectrum in Figure 11b shows a peak that occurs in m/z 312 which corresponds to molecular ion 312. When compared to the NIST spectra database, the compound is analogous to the spectrum of 17-methyloctadecanoic acid methyl ester with a molecular formula $C_{20}H_{40}O_2$.

(E)-9-octadecenoic acid ethyl ester is also known as oleic acid, ethyl ester. This compound was reported to have antimicrobial and nematocidal properties ([Chandrasekharan et al., 2008](#)). On the other hand, 17-methyloctadecanoic acid methyl ester belongs to fatty acid methyl ester group. Fatty acid methyl esters are known to possess antifungal properties (Agoramoorthy et al., 2007). Lima et al. (2011) have reported that fatty acid methyl esters of *Annona cornifolia* seeds such as oleic acid methyl ester, linoleic acid methyl ester, and palmitic acid methyl ester have inhibited the growth of 12 strains of a clinical pathogenic fungus *Paracoccidioides brasiliensis*.

Figure 11

Mass Spectra and Structures of A) (E)-9-Octadecenoic Acid Ethyl Ester and B) 17-Methyloctadecanoic Acid Methyl Ester Elucidated in Fish Poison Tree Seed Extract



Biopesticidal Activity Testing against Golden Apple Snail

Table 5 shows the percent mortality rate of golden apple snails using fish poison tree seed extract applied as spray. Treatment 2 (49.33), Treatment 3 (65.55), and Treatment 4 (67.33) are not significantly different from each other. All treatments are significantly different to control. Treatment 3 and Treatment 4 can cause more than sixty percent mortality rate on golden apple snails. It is highly significant at 5% and 1% levels.

The presence of the different secondary metabolites in the fish poison tree seed extract may greatly affect the mortality of golden apple snails. Picardal (2018) reported the presence of secondary metabolites (flavonoids, tannin, alkaloids, glycosides etc.) on the plant *Solenostemma argel* which caused snail mortalities. According to San Martin and Cruz (2013), saponin inhibits certain cholinesterase enzymes that have a lethal effect to the nervous system of the snail and also causes formation of cholesterol in the body of the snail. Ahmed et al. (2014) also indicated the high alkaloid content of *Barringtonia asiatica* seed causes adverse effects in the central nervous system of the snail. Dai et al. (2011) also showed that cardiac glycosides had a significant effect on GAS, resulting to the decrease in the glycogen content of the shell. This resulted to the impairment of the physiological metabolism of the snails and altering the hepatopancreas tissues of *P. canaliculata* which consequently leads to fatality.

Thus, the presence of the different phytochemical constituents in the seeds of fish poison tree such as saponins, alkaloids, cardiac glycosides, are evidence that the seed powder has a high pesticidal property.

Table 5

Percent Mortality of Golden Apple Snails Using Fish Poison Tree Seed Extract Applied as Spray

Treatment	Replication			Treatment Total	Mean
	1	2	3		
I – (control)	0	0	0	0	0.00 ^b
II – 50g	37	44	67	148	49.33 ^a
III – 100g	72	62	63	197	65.55 ^a
IV –150g	68	60	74	202	67.33 ^a

Note: Treatments with the same letter notation are of the same effect

Conclusion

Firstly, phytochemical screening and GC-MS analysis of *Barringtonia asiatica* seed powder pave ways to research and development of the biopesticidal and pharmaceutical uses of fish poison tree. Furthermore, the presence of the phytochemical constituents such as saponins, alkaloids, and cardiac glycosides, confirmed the potential of fish poison tree as a source of bioactive substances for organic pesticides supporting organic agricultural sustainability. Lastly, a high percent composition of vitamin E, linoleic acid, chondrillasterol, and palmitic acid, ethyl ester identified through GC-MS confirmed that the fish poison tree seed to be a potential source for bioactive substances which may support several pharmaceutical uses and therapeutic value.

Acknowledgements

This project was funded by the Department of Agriculture-Bureau of Agrarian Research. Authors thank Jefcor Analytical Service Laboratory at Dasmariñas, Cavite for the GC-MS analysis of boton seed, and their colleagues in Aurora State College of Technology for the support in this endeavor.

References

- Agarwal, M. & Kamal R. (2007). Studies on flavonoid production using in-vitro cultures of *Momordica charantia*. *Indian Journal of Biotechnology* 6(2), 277–279.
- Agnihotri, S., Wakode, S., & Agnihotri, A. (2010). An overview on anti-inflammatory properties and chemo-profiles of plants used in traditional medicine. *Indian Journal of Natural Products and Resources*. 1(2),150–167.
- Agoramoorthy, G., Chandrasekaran, M., Venkatesalu, V. & Hsu, M.J. (2007). Antibacterial and antifungal activities of fatty acid methyl esters of the blind-your-eye mangrove from India. *Braz J Microbiol*. 38, 739–742.
- Ahmed, E. A., Babiker, O. F., & Abdalla, R. M. (2014). Molluscicidal activity of aqueous leaf extract of *Solenostemma argel* (Del Hayne) on *Biomphalaria pfeifferi* Snails. *Journal of Basic and Applied Science Research* 4(1),179–184.

- Bakar, M. F. A., Mohamed, M., Rahmat, A., & Fry, J. (2009). Phytochemicals and antioxidant activity of different parts of bambangan (*Maningerapajang*) and tarap (*Artocarpusodoratissimus*). *Food Chem* 113:479–483.
- Balashova, L. M., Namiot, V. A., Kolesnichenko, I. I., & Udaltsov, S. N. (2018). Lanomax as a drug in cataract treatment: A case study. *Biophysics* 63(4), 655–651.
- Barwick, M. (2004). *Tropical and Subtropical Trees: A Worldwide Encyclopedia Guide*. Thances and Hudson.
- Bibi, G., & Shanmugam, S. (2014). Phytochemical screening and antimicrobial activity of fruit mextract of *Sapindus mukorossi*. *International Journal of Current Microbiology and Applied Sciences* 3(10), 604–611.
- Bissinger, R., Modicano, P., Alzoubi, K., Honisch, S., Faggio, C., Abed, M., & Lang, F. (2014). Effect of saponin on erythrocytes. *International Journal of Hematology* 100(1), 51–59.
- Brunet, J. M. C., Cetkovic, G. S., Djilas, S. M., Tumbas, V. T., & Savatovic, T.S. S. (2009). Radical scavenging and antimicrobial of horsetail (*Equisetum arvense* L.) extracts. *Int. J. Food Sci. Technology* 44, 269–278.
- Burton, R. A., Wood, S. G., Owen, N. L. (2003). Elucidation of a new oleanane glycoside from *Barringtonia asiatica*. *Arkivoc* 13, 137–146.
- Butler, L. G. (1992). Anti-nutritional effects of condensed and hydrolysable tannins. *Cancer* 3, 768–80.
- Chandrasekharan, M., Kannathasan, K., & Venkatesalu, V. (2008). Antimicrobial activity of fatty acid methyl esters of some members of chenopodiaceae. *Z Naturforsch C*. 63, 331–336.
- Chen, J. J., Duh, C. Y., & Chen, I. S. (2005). Cytotoxic chromenes from *Myriactis humilis*. *Planta Med.*, 71, 370–371.
- Correa, P. M. (1984). Dicionário das plantas úteis do Brasil e das exóticas cultivadas. *Rio de Janeiro*, 2, 108–129.
- Dai, L., Wang, W., Dong, X., Hu, R., & Nan, X. (2011). Molluscicidal activity of cardiac glycosides from *Nerium indicum* against *Pomacea canaliculata* and its implications for the mechanisms of toxicity. *Environmental Toxicology and Pharmacology*. 32(2), 226–32.
- Edeoga, H, Okwu, D., & Mbaebie, B. (2005). Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology* 4(7):685–688.
- Ee, G. C. L., Lim, C. M., Rahmani, M., Shaari, K., & Bong, C. F. J. (2010). Pellitorine, a potential anti-cancer lead compound against HL60 and MCT-7 cell lines and microbial transformation of piperine from *Piper nigrum*. *Molecules* 15(4), 2398–2404.
- Elekofehinti, O. (2015). Saponins: Anti-diabetic principles from medicinal plants—A review. *Pathophysiology* 22, 95–103. <https://doi.org/10.1016/j.pathophys.2015.02.001>
- Evans, W. C. (1996). Pharmacopoeial and related drugs of biological origin. Alkaloids. In E. Trease & W. C. Evans (Eds.), *Trease and Evans' Pharmacognosy* (14th ed.). G. W. B. Saunders.
- Farnsworth, N. F. (1966). Biological and phytochemical screening of plants. *Journal of Pharmaceutical Sciences*. 55, 225–276.

- Fethi, B. J., Mohamed, A. M., Samia, A. M., Karima, B. S., Ahmed, N. H., & Zine, M. (2012). Chemical composition, *in vitro* antifungal and antioxidant activities of essential oil from *Cotula coronopifolia* L. growing in Tunisia. *Africal Journal of Microbiology Research* 6 (20), 4388–4395.
- Gallo, M. B. C., & Sarachine, M. J. (2009). Biological activities of lupeol. *Int. J. Biomed. Pharmaceut. Sci.* 3, 46–66.
- Geetha, T., & Varalakshmi, P. (2001). Anti-inflammatory activity of lupeol and lupeol linoleate in rats. *J. Ethnopharmacol.* 76:77–80.
- Godlaski, T. M. (2011). “Gods of drugs: the God within.” *Substance use and misuse.* 46(10): 1217–1222.
- Güçlü-Ustündağ, O., & Mazza, G. (2007). Saponins: properties, applications and processing. *Critical Reviews in Food Science and Nutrition.* 47(3):231–258.
- Gupta, R., Sharma, A. K., Dobhal, M. P., Sharma, M. C., & Gupta, R. S. (2011). Antidiabetic and antioxidant potential of β -sitosterol in streptozotocin-induced experimental hyperglycemia. *Journal of Diabetes* 3, 29–37.
- Hamdan, D., El-Readi, M. Z., & Tahrani A. (2011). Secondary metabolites of ponderosa lemon (*Citrus pyriformis*) and their antioxidant, anti-inflammatory, and cytotoxic activities. *Zeitschrift für Naturforschung C.* 66(7–8), 385–393.
- Itoh, T., Kikuchi, Y., Tamura, T., & Matsomoto, T. (1981). Co-occurrence of chondrillasterol and spinasterol in two Cucurbitaceae seeds as shown by ^{13}C NMR. *Phytochemistry.* 20,761–764.
- Jianshe, H., Xiaoyan, L., Mingjun, Z., & Si, Z. (2004). A survey of the chemical constituents and pharmacological activities of mangrove medicinal plant (*Barringtonia*). *Natural Product Research and Development* 16(2), 167–171.
- Kagan, V. E. (1998). Recycling and redox cycling of phenolic antioxidants. *Annals of the New York Academy of Sciences* 854, 425–434.
- Kelly R. A. (1990). Cardiac glycosides and congestive heart failure. *American Journal of Cardiology.* 65, 10–16
- Kelly, G. S. (1999). Squalene and its potential clinical uses. *Alternative Medicine Review* 4(1), 29–36.
- Khan, M. R., & Omoloso, A. D. (2002). Antibacterial, antifungal activities of *Barringtonia asiatica*. *Fitoterapia* 73(3), 255–260.
- Khakimov, B., Motawia, M. S., Bak, S., & Engelsens, S. B. (2013). The use of trimethylsilyl cyanide derivatization for robust and broad-spectrum high-throughput gas chromatography-mass spectrometry based metabolomics. *Anal. Bioanal. Chem.* 405, 9193–9205.
- Khakimov, B., Tseng, L. H., Godejohann, M., Bak, S., & Engelsens, S. B. (2016). Screening for triterpenoid saponins in plants using hyphenated analytical platforms. *Molecules* 21, 1614–1632.
- Kujumgiev, A., Bankova, V., Ignatova, A. & Popov, S. (1993). Antibacterial activity of propolis, some of its components and their analogs. *Pharmazie* 48, 785–786.
- Kumar, B., Vijayakumar, M., Govindarajan, R., & Pushpangadan, P. (2007). Ethnopharmacological approaches to wound healing-exploring medicinal plants of India. *Journal of Ethnopharmacology* 114(2), 103–113.

- Kumar, S. & Pandey, A. K. (2012). Antioxidant, lipo-protective, and antibacterial activities of phytoconstituents present in *Solanum xanthocarpum* root. *International Review of Biophysical Chemistry*. 3(3), 42–47.
- Kroes, B. H., Van Der Berg, A. J. J., Quarles Van Ufford, H. C., Van Dijk, H., & Labadie, R. P. (1992). Anti-inflammatory activity of gallic acid. *Plants Med*. 58, 499–504.
- Leal, L. K. A. M., Ferreira, A. A. G., Bezerra, G. A., Matos, F. J. A., & Viana, G. S. B. (2000). Anticonceptive, anti-inflammatory, and bronchodilator activities of Brazilian medicinal plants containing coumarin: a comparative study. *Journal of Ethnopharmacology*. 70(2), 151–159.
- Lima, L. A. R., Johann, S., Cisalpino, P. S., Pimenta, L. P. S., Boaventura, M. A. D. (2011). In vitro antifungal activity of fatty acid methyl esters of the seeds of *Annona cornifolia* A.St.-Hil. (Annonaceae) against pathogenic fungus *Paracoccidioides brasiliensis*. *Revista da Sociedade Brasileira de Medicina Tropical* 44(6):777-780. <https://doi.org/10.1590/S0037-86822011000600024>
- Marrelli, M., Conforti, F., Araniti, F., & Statti, G. A. (2016). Effects of saponins on lipid metabolism: A review of potential health benefits in the treatment of obesity. *Molecules* 21(10), 1404-1423. doi: 10.3390/molecules21101404
- Marston, A. & Hostettmann, K. (1991). Plant saponins: Chemistry and molluscicidal action. In J. B. Harborne & P. M. Dey (Eds.), *Ecological Chemistry and Biochemistry of Plant Terpenoids. Proceedings of the Phytochemical Society of Europe* Vol. 31, pp. 264–286. Clarendon Oxford.
- Martin, T. & Gutow, L. (2005). The ecology of rafting in the marine environment. The floating substrata. *Oceanography and Marine Biology: An annual review* 42, 181–264.
- Metwally, N. S. (2006). Potency of *Allium sativum* L. and *Allium cepa* oils against *Schistosoma mansoni* infection in mice. *The Egyptian Journal of Hospital Medicine*. 23, 319–332.
- Meyer, B. N., Ferrigni, N. R., Putnam, J. E., Jacobsen, L. B., & Nichols, D. J. (1982). Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Medica* 45(5), 31–34.
- Mikail, H. G. (2010). Phytochemical screening, elemental analysis and acute toxicity of aqueous extract of *Allium sativum* bulbs in experimental rabbits. *Journal of Medicinal Plant Research*. 4(4), 322–326.
- Mojica, E. R., Micor, J. R. L. (2007). Bioactivity study of *Barringtonia asiatica* (Linnaeus) Kurz seed aqueous extract in *Artemia salina*. *International Journal of Botany* 3(3), 325–328.
- Mozirandi, W., Tagwireyi, D., & Mukanganyama, S. (2019). Evaluation of antimicrobial activity of chondrillasterol isolated from *Vernonia adoensis* (Asteraceae). *BMC Complementary Medicine and Therapies*.
- Mutai, C., Abatis, D., Vagias, C., Moreau, D., Roussakis, C., & Roussis, V. (2004). Cytotoxic lupane-type triterpenoids from *Acacia mellifera*. *Phytochemistry* 65, 1159–64.
- Mwine, J., Ssekyewa, C., Kalanzi, K., & Van Damme, P. (2013). Evaluation of selected pesticidal plant extracts against major cabbage insect pests in the field. *Journal of Medicinal Plants Research* 7(22), 1580–1586.
- Na, M., Kim, B. Y., Osada, H., & Ahn, J. S. (2009). Inhibition of protein tyrosine phosphatase 1B by lupeol and lupenone isolated from *Sorbus commixta*. *J Enzyme Inhib Med Chem*. 24, 1056–1059.

- Navarro-García, V. M., Luna-Herrera, J., Rojas-Bribiesca, M. G., Álvarez-Fitz, P., & Ríos, M. Y. (2011). Antibacterial activity of *Aristolochia brevipes* against multidrug-resistant *Mycobacterium tuberculosis*. *Molecules* 16(9), 7357–7364.
- Newmark, H. L. (1997). Squalene, olive oil, and cancer risk: A review and hypothesis. *Cancer Epidemiology Biomarkers and Prevention* 6, 1101–1103.
- Panda, S., Jafri, M., Kar, A., & Meheta, B. K. (2009). Thyroid inhibitory, antiperoxidative and hypoglycemic effects of stigmasterol isolated from *Butea monosperma*. *Fitoterapia* 80, 123–126.
- Picardal, M. T., Panlaan, K. T., Castaño, P. M. L., Peña, L. G., Abella, K.T., & Picardal, J.P. (2018). Molluscicidal activity of the aqueous extract of garlic (*Allium sativum* L.) bulb against golden apple snail (*Pomacea canaliculata* L.). *International Journal of Bioscience* 13(2), 75–87. <http://dx.doi.org/10.12692/ijb/13.2.75-87>
- Pradeep, A., Dinesh, M., Govindaraj, A., Vinothkumar, D., & Ramesh, N. G. (2014). Phytochemical analysis of some important medicinal plants. *International Journal of Biological and Pharmaceutical Research*. 5(1), 48–50.
- Prance, G. (2012). A Revision of *Barringtonia* (Lecythidaceae). *Allertonia* 12, 1–161.
- Prohp, T. P. & Onoagbe, I. O. (2012). Determination of phytochemical composition of the stem bark of *Triplochiton scleroxylon* K. Schum (Sterculiaceae). *International Journal of Applied Biology and Pharmaceutical Technology* 3(2), 68–76.
- Rasoanaivo, L. H., Wadouachi, A., Andriamampianina, T. T., Andriamalala, G. S., Jeannot, E. B., Raharisololalao, A. (2014). Triterpenes and steroids from the stem bark of *Gambeya boiviniana* Pierre. *J Pharmacogn Phytochem* 3, 68–72.
- Ravikumar, T., Nagesh-ram, Dam-Roy, S., Krishnan, P., Grinson-George, Sankaran, M., & Sachithanandam, V. (2015). Traditional usages of ichthyotoxic plant *Barringtonia asiatica* (L.) Kurz. *Journal of Marine and Island Cultures* 4(2), 76–80.
- Reddy, L. H. & Couvreur, P. (2009). Squalene, A natural triterpene for use in disease management and therapy. *Adv Drug Deliv Rev*. 61 (15), 1412–26.
- Ross, I. A. (2003). *Medicinal plants of the world: Chemical constituents, traditional, and modern medicinal uses* (Vol. 1). Humana Press.
- Saleem, M. (2009). Lupeol, a novel anti-inflammatory and anti-cancer dietary triterpene. *Cancer Lett.* 285, 109–115.
- Salman, Z., Mohdazizi, C. Y., Niknorulaini, N. A., & Mohd Omar, A. K. (2006). Gas chromatography/time-of flight mass spectrometry for identification of compounds from *Parkia speciosa* seeds extracted by supercritical carbon dioxide. In *Proceedings of the First International Conference on Natural Resources Engineering & Technology* (pp. 112–120), Putrajaya, Malaysia.
- Sheel, R., Nisha, K., & Kumar, J. (2014). Preliminary phytochemical screening of methanolic extract of *Clerodendron infortunatum*. *IOSR Journal of Applied Chemistry* 7(1), 10-13.
- Smith, T. J. (2000). Squalene: potential chemopreventive agent. *Expert Opinion on Investigational Drugs* 9(8), 1841–1848.

- Smith, R. L., Cohen, S. M., Doull, J., Feron, V. J., & Goodman, J. I. (2005). A procedure for the safety evaluation of natural flavor complexes used as ingredients in food: Essential oils. *Food Chemistry and Toxicology* 43, 345–363.
- Sri Murni, A., Mimi Sakinah, A. M., Retno Andayani, B. M., & Awalludin, R. (2011). Determination of saponin compound from *Anredera cordifolia* (ten) steenis plant (Binahong) to potential treatment for several diseases. *Journal of Agricultural Science* 3 (4), 224-232 doi:10.5539/jas.v3n4p224
- Stuart, Jr., G. U. (2018). Philippine Medicinal Plants: Boton. Stuart Xchange. <http://www.stuartxchange.org/Botong.html>
- Tan, R. (2001). Sea poison Tree. Mangrove and wetland wildlife at Sungei Buloh Nature Park. Singapore. https://web.archive.org/web/20111202005137/http://www.naturia.per.sg/buloh/plants/sea_poison.htm
- Tanor, M. T., Abdul, L. A., Bambang, T. R., & Pelealu J. (2014). Isolation and identification of triterpenoid saponin from *Barringtonia asiatica*. *The Journal of Tropical Life Science*. 4(2), 119-122 doi:10.11594/jtls.4.2.%x
- Tiwari, F., & Singh, D. F. (2004). Attraction to amino acids by *Lymnaea acuminata*, the snail host of Fasciola species. *Brazilian Journal of Medical and Biological Research* 37(4), 587–590.
- Trease, G. E., & Evans, W. C. (2002). *Pharmacognosy* (15th ed.). Saunders Publishers.
- Tripathi, S. M., & Singh, D. K. (2000). Molluscicidal activity of *Punica granatum* bark and *Canna indica* root. *Brazilian Journal of Medical and Biological Research* 33(11), 1351–135.
- Vimalkumar, C. S., Hosagaudar, V. B., Suja, S. R., Vilash, V., Krishnakumar, N. M., & Latha, P. G. (2014). Comparative preliminary phytochemical Analysis of ethanolic extracts of leaves of *Olea dioica* Roxb infected with the rust fungus *Zaghouania oleae* (E.J. Butler) Cummins and non-infected plants. *Journal of Pharmacognosy and Phytochemistry* 3(4), 69–72.
- World Health Organization. Regional Office for the Western Pacific. (2009). *Medicinal plants in Papua New Guinea*. <https://apps.who.int/iris/handle/10665/206954>
- World Health Organization. Regional Office for the Western Pacific. (1998). *Medicinal plants in the South Pacific*. <https://apps.who.int/iris/handle/10665/207584>
- Yoon, Y., Lee, H., Lee, S., Hong, J., & Lee, C. (2015). Effects of lupenone, lupeol, and taraxerol derived from *Adenophora triphylla* on the gene expression and production of airway MUC5AC mucin. *Tuberculosis and Respiratory Diseases* 78(3), 210–217. doi: 10.4046/trd.2015.78.3.210