Phytochemical Investigation of the Stem Bark of *Securidaca longipedunculata* Fresen (Polygalaceae) (I)

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Abstract

Securidaca longipedunculata is a medicinal plant with a long history of use in Nigeria and in many parts of Africa. Different classes of phytochemicals were isolated and identified from the root of this plant, however, the phytochemistry of the stem bark is not widely investigated; thus, this study was aimed at identifying the phytochemical constituents of the stem bark. Liquid Chromatography-Mass Spectrometry (LCMS/MS) analysis of a fraction (fraction DDK-6) obtained from the column chromatography of the ethylacetate extract of *S. longipedunculata* stem bark was conducted on a Waters Synapt G2 quadrupole time of flight mass spectrometer using positive mode of ionization. The results suggested that 5,7-dihydroxy-6-methyl-3-(2',4'-dihydroxybenzyl)-chroman-4-one was the major component of fraction DDK-6. Other compounds identified in this fraction include geranyl hydroquinone, 5,7-dihydroxy-2-(4-hydroxyphenyl)-6,8-dimethyl-3,4-dihydro-2H-1-benzopyran-4-one,4-hydroxy-3,4-dihydro-1H-isoquinoline-2- carboxidamide, 3-(1,3-benzodi-oxol-5-yl-4-morpholinyl)-3-(2-hydroxy-4,6-dimethoxyphenyl)-1-propanone, 1,3,7-trihydroxy-2-(3-methyl-2-butenyl)-8-(3-hydroxy-3-methylbutyl)xanthone, 5,7-dihydroxy-6-methyl-3-(4-hydroxybenzyl)-chroman-4-one,[(7R,8R)-7-[(Z)-2-methylbut-2-+enoyl]oxy-5,6,7,8-tetrahydro-3H-pyrrolizin-1-yl]methyl(2R)-2,3-dihydroxy-2-[(1S)-1-hydroxyethyl]-3-methyl butanoate, (Z)-Octadec-9-enamide, [(2R,3S,4S,5R,6S)-3,4,5-trihydroxy-6-[2-(hydroxymethyl)phenoxy]oxan-2-yl]methylbenzoate and (1S,4R)-7-Methoxycalamenen-3-one. The present study has tentatively identified the presence of these compounds in the stem bark of *S. longipedunculata*, and studies are currently on-going to isolate them in their pure forms.

Keywords: Chromatography, Medicinal plant, Mass spectrometer, Phytochemistry

Introduction

Securidaca longipedunculata is a small tree with a pale grey, smooth bark and hairless alternate leaves which are variable in size and shape. Its flowers are small, pink or purple in colour, sweet scented and are usually produced in early summer (Van Wyk *et al*., 2009), while its fruits are heavily veined, smooth, oblong and purplish-green when young. It is commonly known as violet tree, fibre tree or Rhodesian violet tree, while its local names include *Uwar magunguna* or *Sanya* in Hausa language, *Ipeta* in Yoruba language and *Umfufu* in Swahili language (Coates-Palgrave, 2005). The root of *S.*

longipedunculata is traditionally used to manage fever, malaria, gonorrhoea, headaches, rheumatism, diabetes, sexual impotence, toothache, fungal infections, epilepsy, cancer, convulsions, constipation, pneumonia, backache, blood purification, sexually transmitted infections, skin infections among others (Chhabra *et al*., 1991; Moshi *et al*., 2007; Viol, 2009; Ogunmefun and Gbile, 2012; Maroyi, 2013; Mustapha, 2013), while the stem bark is traditionally used to treat epilepsy, stomach ache, skin diseases, dysentery, malaria, typhoid, inflammation, chest complaints, abortion, constipation, snake bites and infertility problems (Das, 2009; Bruschi *et al*., 2011; Oladunmoye and Kehinde, 2011; Kadiri *et al*., 2013). Some of the biological activities of *S. longipedunculata* include antibacterial and antifungal (Adebayo and Osman 2012; Karou *et al*., 2012; Musa *et al*., 2013; Ndamitso *et al*., 2013), antioxidant (Karou *et al*., 2012), antiplasmodial activities (Bah *et al*., 2007; Haruna *et al*., 2013), antiinflammatory (Muanda *et al*., 2010), insecticidal, molluscicidal and pesticidal activities (Boeke *et al*., 2004; Olofintoye, 2010; Afful *et al*., 2012; Eziah *et al*., 2013). Phytochemically, different classes of compounds have been reported from the root of *S. longipedunculata*, examples of those compounds include quercetin, gallic acid, chlorogenic acid, cinnamic acid, apigenin, quercetin glucosyl, caffeic acid, epicatechic acid, rutin, 1,6,8-trihydroxy-2,3,4,5-tetramethoxyxanthone, 1,6,8-Trihydroxy-2, 3, 4, 7-tetramethoxyxanthone, 1,6-dihydroxy-2,3,4,5,8-pentame-thoxyxanthone,4,6,8-trihydroxy,1,2,3,5-tetramethoxyxanthone, muchimangins A-D (Muanda *et al*., 2010; Dibwe *et al*., 2012) presenegenin, securinine, β-sitosterol, quercetin-3-O-D-xyloside, benzyl-2-hydroxy-6-methoxybenzoate, 1, 7-dihydroxy-4- methoxyxanthone, methyl salicylate among others (Debella *et al*., 2000; Lognay *et al*., 2000; Jayasakara *et al*., 2002; Van Wyk *et al*., 2005; Meli *et al*., 2007).

It is evident that many compounds have been isolated, characterized and identified from the root of this plant species, however, there is need to further explore the phytochemistry of the stem bark and leaves, therefore, this study was aimed at identifying the phytochemical constituents of a fraction (fraction DDK-6) obtained from the column chromatography of the ethylacetate extract of *S. longipedunculata* using Liquid Chromatography-Mass Spectrometry (LCMS/MS).

Materials and methods

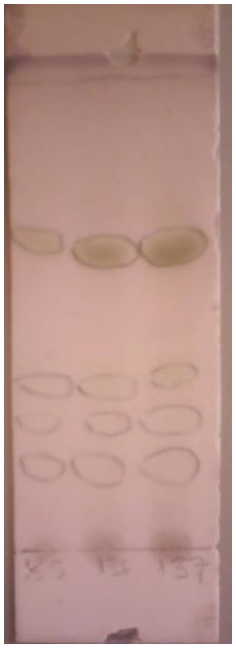
Collection, identification and preparation of plant material

The plant was collected in April, 2018 from the rocky areas of Jugwa Village, Gwaram Local Government Area of Jigawa State, Nigeria, it was then taken to the Herbarium of Ethnobotany and Multidisciplinary Research Division of Bioresources Development Centre, Kano for identification and authentication, and its voucher number was BDCKN/EB/1898. The stem bark was air dried and then ground into fine powder using mortar and pestle, 1 kg of the powdered sample was then macerated with ethylacetate for 48 hours, and the mixture was shaken occasionally. The filtrate obtained was evaporated to dryness at 40 ℃ using rotary evaporator and water bath.

Column chromatography of ethylacetate extract

The ethyl acetate extract (4 g) was chromatographed on 100 g of silica gel using gradient elution, eluates of 20 ml were collected and monitored with hexane: ethylacatate (9:1) as the solvent system for thin layer chromatography (TLC), then the TLC plates were visualized with 10 % H_2SO_4 in methanol spray reagent. Fraction 135-137

revealed the same thin layer chromatography (TLC) profiles in Hexane: Ethylacetate (6.5:3.5) as shown on plate I, and the three fractions were combined together and labelled as fraction DDK-6. However, the quantity of this fraction was too small for further purification, therefore, liquid chromatography-mass spectrometry analysis was carried to identify the compounds present.



Hexane: Ethylacetate (6.5:3.5)

Plate I: TLC Profile of Fraction DDK-6

Liquid Chromatography-Mass Spectrometry (LCMS/MS) Analysis of Fraction DDK-6

The LCMS/MS analysis was performed at the Central Analytical Facilities, Mass Spectrometry Unit of Stellenbosch Bosch University, South Africa. The analysis was conducted on a Waters Synapt G2 quadrupole time-of-flight mass spectrometer (Milford, MA, USA). The instrument was connected to a Waters Acquity ultra-performance liquid chromatograph (UPLC) and Acquity photo diode array (PDA) detector. Ionisation was achieved with an electrospray source using a cone voltage of 15 V and capillary voltage of 2.5 kV, and positive mode of ionisation was then utilized. Nitrogen was used as the desolvation gas at 650 L/hour and the desolvation temperature was set to 275 °C.

Results

LCMS/MS Analysis of Fraction DDK-6

The LCMS/MS analysis of fraction DDK-6 detected 12 peaks as shown in figure 1; the most prominent peaks have M/Z of 192.14,

317.14, 444.18, 301.14, 282.28 and 247.17 at retention time of 6.43, 7.56, 8.13, 9.50, 11.11 and 13.68 minutes respectively. The tentative identification of the compounds was achieved by comparing the obtained molecular ions and fragmentation patterns with different chemical databases and other published literature. The databases employed were Chemical Entities of Biological Interest (CHEBI), Drug Bank Database (DrugBank), Universal Natural Products Database (UNPD), KNApSAcK Family Database, Human Metabolome Database (HMDB), Food Database (FoodDB), Yeast Metabolome Database (YMDB), Northern African Natural Products Database (NANPDB), PubChem, PlantCyc, Lipid Metabolites and Pathways Strategy (Lipid MAPS) and *Escherichia coli* Metabolome Database (ECMDB).

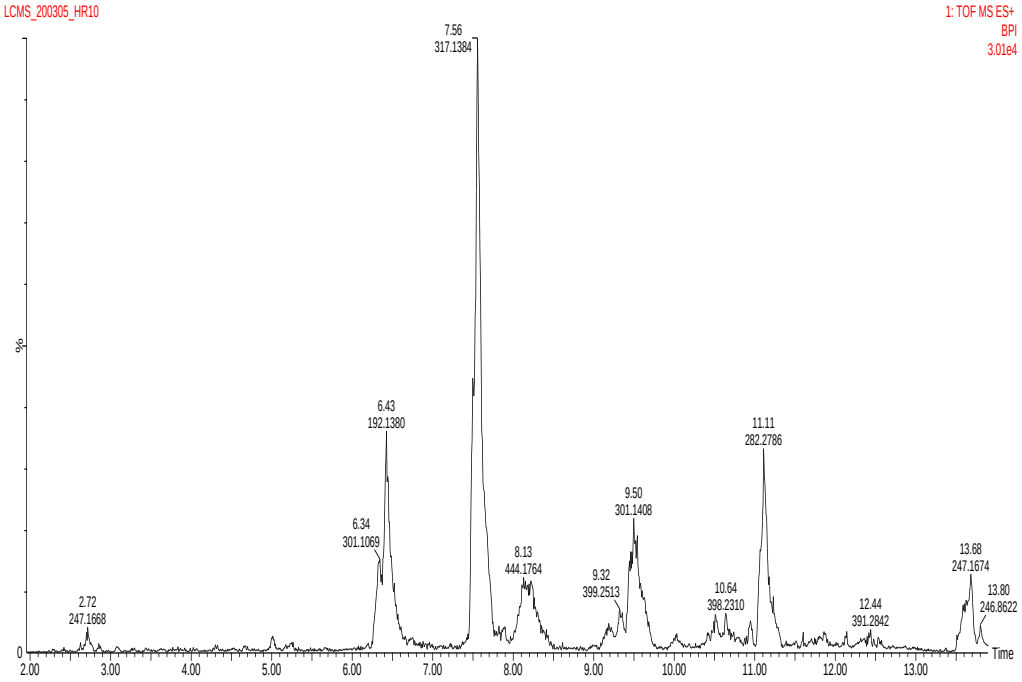
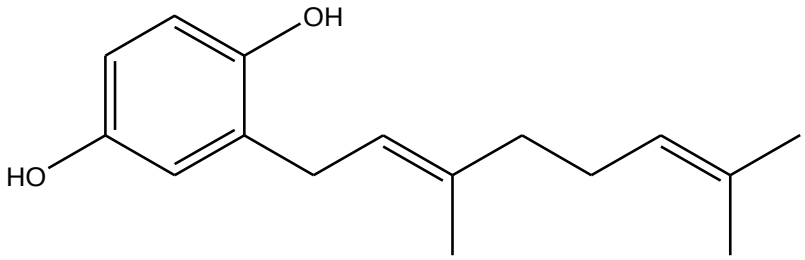


Figure 1: Total Ion Chromatogram of Fraction DDK-6

Table 1: Summary of the Total Ion Chromatogram of Fraction DDK-6

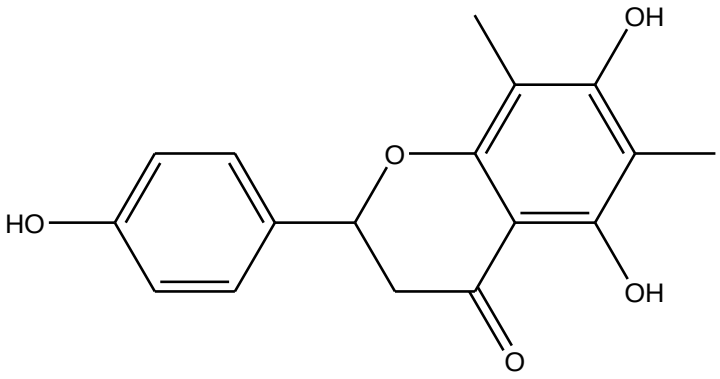
S/N	M/Z $[M+H]^+$	Retention Time (Minutes)	Proposed Compound	Ontology	Molecular Formula	Exact Mass ($gmol^-$)
1	247.17	2.72	Geranyl hydroquinone	Prenylated hydroquinones	$C_{16}H_{22}0_2$	246
2	301.11	6.34	5,7-dihydroxy-2-(4-hydroxyphenyl)-6,8-dimethyl-3,4-	Flavonones	$C_{17}H_{16}O_5$	300

			dihydro-2H-1-benzopyran-4-one			
3	192.14	6.43	4-hydroxydebrisoquine	Tetrahydro isoquinolines	$C_{10}H_{13}N_3O$	191
4	317.14	7.56	5,7-dihydroxy-6-methyl-3-(2',4'-dihydroxybenzyl)-chroman-4-one	Homoiso flavonoids	$C_{17}H_{16}0_6$	316
5	444.18	8.13	3-(1,3-benzodi-oxol-5-yl-4-morpholinyl)-3-(2-hydroxy-4,6-dimethoxyphenyl)-1-propanone	Methoxy phenols	$C_{24}H_{29}NO_7$	443
6	399.25	9.32	1,3,7-trihydroxy-2-(3-methyl-2-butenyl)-8-(3-hydroxy-3-methylbutyl) xanthone	Xanthones	$C_{23}H_{26}O_6$	398
7	301.14	9.50	5,7-dihydroxy-6-methyl-3-(4-hydroxybenzyl)-chroman-4-one	Homoiso flavonoids	$C_{17}H_{16}0_5$	300
8	398.23	10.64	Echimidine	Alkaloids	$C_{20}H_{31}NO_7$	397
9	282.28	11.11	Oleamide	Fatty amides	$C_{18}H_{35}NO$	281
10	391.28	12.44	Populnin	Glucoside	$C_{20}H_{22}0_8$	390
11	247.17	13.68	(1S,4R)-7-Methoxycalamenen-3-one	Sesquiterpenoids	$C_{16}H_{22}0_2$	246

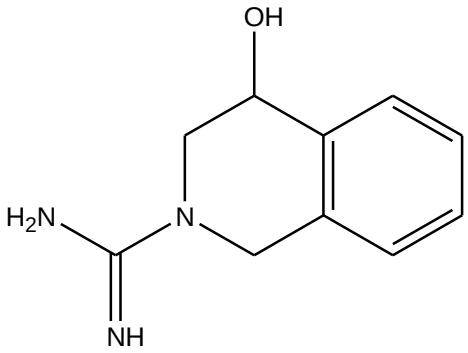


2-[(2E)-3,7-dimethylocta-2,6-dien-1-yl]benzene-1,4-diol

(Geranyl hydroquinone)



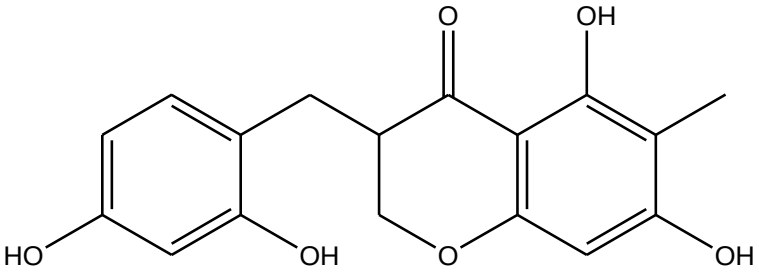
5,7-dihydroxy-2-(4-hydroxyphenyl)-6,8-dimethyl-3,4-dihydro-2H-1-benzopyran-4-one



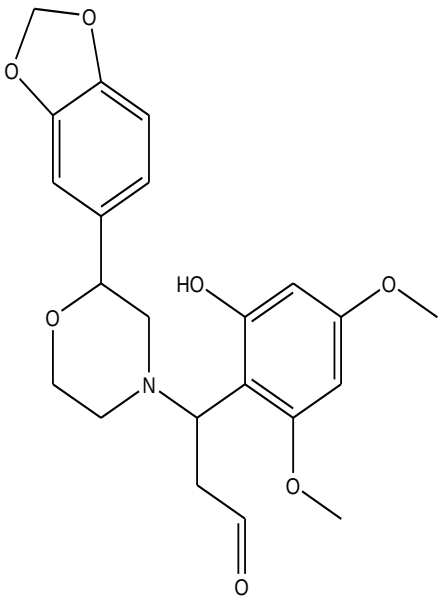
4-hydroxy-3,4-dihydro-1H-isoquinoline-2-carboximidamide

(4-hydroxydebrisoquine)

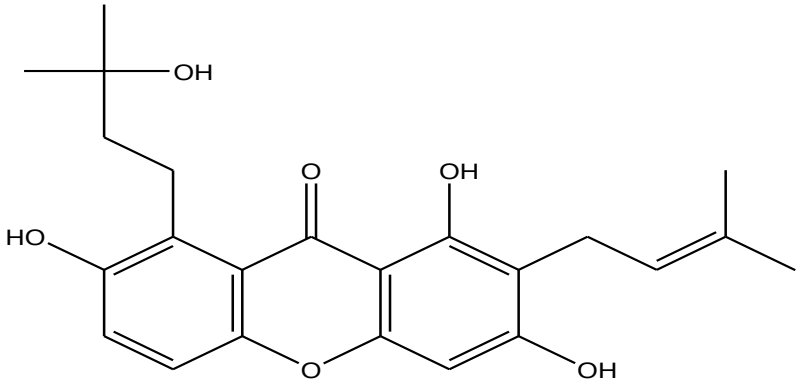
Figure 2: Chemical Structures of the Compounds



5,7-dihydroxy-6-methyl-3-(2',4'-dihydroxybenzyl)-chroman-4-one

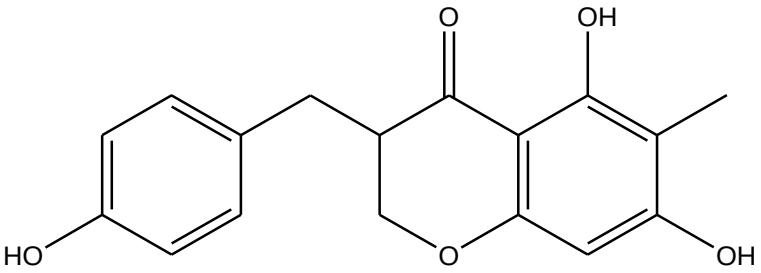


3-(1,3-benzodi-oxol-5-yl-4-morpholinyl)-3-(2-hydroxy-4,6-dimethoxyphenyl)-1-propanone

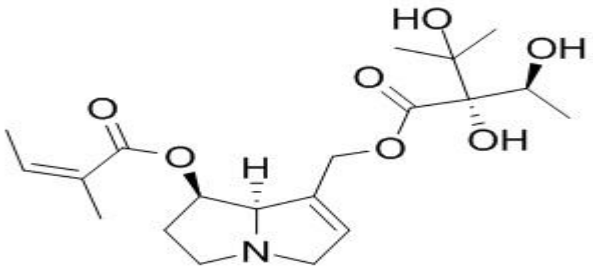


1,3,7-trihydroxy-2-(3-methyl-2-butenyl)-8-(3-hydroxy-3-methylbutyl) xanthone

Figure 2 (Continued): Chemical Structures of the Compounds

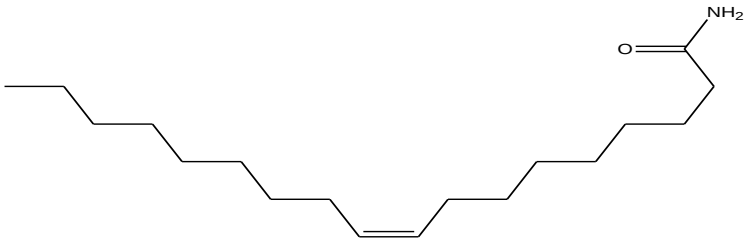


5,7-dihydroxy-6-methyl-3-(4-hydroxybenzyl)-chroman-4-one



[(7R,8R)-7-[(Z)-2-methylbut-2-enoyl]oxy-5,6,7,8-tetrahydro-3H-pyrrolizin-1-yl]methyl (2R)-2,3-dihydroxy-2-[(1S)-1-hydroxyethyl]-3-methylbutanoate

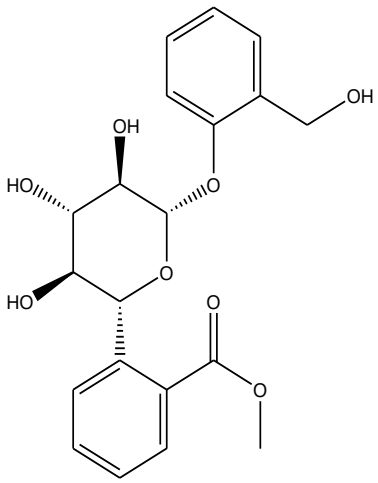
(Echimidine)



(Z)-Octadec-9-enamide

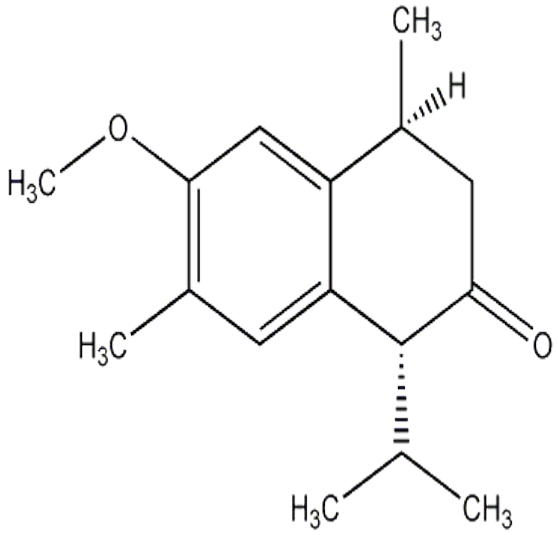
(Oleamide)

Figure 2 (Continued): Chemical Structures of the Compounds



[(2R,3S,4S,5R,6S)-3,4,5-trihydroxy-6-[2-(hydroxymethyl)phenoxy]oxan-2-yl]methyl benzoate

(Populin)



(1S,4R)-7-methoxycalamenen-3-one

Figure 2 (Continued): Chemical Structures of the Compounds

Discussion

The LCMS/MS analysis of fraction DDK-6 showed that 5,7-dihydroxy-6-methyl-3-(2',4'-dihydroxybenzyl)-chroman-4-one was the major constituent with M/Z 317.14; a homoiso-flavonoid with molecular formula of $C_{17}H_{16}O_6$ which corresponds to the molecular weight of 316 $gmol^{-}$. This compound was first reported from *Polygonatum sibiricum*, which is one of the constituents of 'Gan Luo Xin', a traditional Chinese medical formula indicated for the treatment of hepatitis B (Li-Ming *et al*., 2014). Other compounds identified in this fraction include geranyl hydroquinone, 5,7-dihydroxy-2-(4-hydroxyphenyl)-6,8-dimethyl-3,4-dihydro-2H-1-benzopyran-4-one, 4-hydroxy-3,4-dihydro-1H-isoquinoline-2-carboxidamide (also knowns as 4-hydroxydebrisoquine), 3-(1,3-benzodi-oxol-5-yl-4-morpholinyl)-3-(2-hydroxy-4,6-dimethoxyphenyl)-1-propanone, 1,3,7-trihydroxy-2-(3-methyl-2-butenyl)-8-(3-hydroxy-3-methylbutyl) xanthone, 5,7-dihydroxy-6-methyl-3-(4-hydroxybenzyl)-chroman-4-one, [(7R,8R)-7-[(Z)-2-methylbut-2-enoyl]oxy-5,6,7,8-tetrahydro-3H-pyrrolizin-1-yl]methyl(2R)-2,3-dihydroxy-2-[(1S)-1-hydroxyethyl]-3-methylbutanoate (also known as echimidine), (Z)-Octadec-9-enamide (also known as oleamide), [(2R,3S,4S,5R,6S)-3,4,5-trihydroxy-6-[2-(hydroxymethyl)phenoxy]oxan-2-yl]methylbenzoate (also known as populnin) and (1S,4R)-7-methoxycalamenen-3-one, with M/Z 247.17, 301.11, 192.14, 444.18, 399.25, 301.14, 398.23, 282.28, 391.28 and 247.17 respectively. However, the compound with M/Z 246.86 and retention time 13.80 could not be identified.

Echimidine is a hepatotoxic pyrrolizidine alkaloid first reported from *Echium plantagineum* (Cao *et al*., 2013), while punicic acid is a polyunsaturated fatty acid which possesses a wide array of biological properties such as antidiabetic, antiobesity, antiproliferative and anticarcinogenic

activity against various forms of cancer, thus, the antidiabetic, antiproliferative and anticarcinogenic activities of *S. longipedunculata* could be attributed to the presence of punicic acid (Lawal *et al*., 2012; Aruna *et al*., 2016). On the otherhand, geranylhydroquinone is a marine natural products but also isolated in some plants (Manners and Jurd, 1977; Reynaulds and Rodriguez, 1979; Manners, 1983). It has been reported to exhibit antibacterial activity, anti-inflammatory and cytotoxic effects against the leukemia cell lines of Rous sarcoma and mammary cincinoma (Fenical, 1974).

Conclusion

The study has tentatively identified the presence of these compounds in the stem bark of *S. longipedunculata*, studies are currently on-going to isolate these compounds in their pure forms in order to elucidate and characterize their structures using different spectroscopic techniques.

Acknowledgement

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Conflict of Interest

The authors declare no conflict of interest.

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