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LCMS/MS Analysis of the Ethylacetate Extract of *Securidaca longipedunculata* Fresen (Polygalaceae) Stem Bark

Abubakar U.S.[1,*], Danmalam U.H.[2], Ibrahim H.[2], Maiha B.B.[3], Hadiza R.J.[4], Abdullahi M.S.[5], Bashir M.[6]

Abstract

The present study aimed to identify some of the phytoconstituents of a fraction (fraction DDK-4) obtained from the column chromatography of the ethylacetate extract of Securidaca longipedunculata Fresen (Polygalaceae). Liquid Chromatography-Mass Spectrometry (LCMS/MS) analysis of fraction DDK-4 was conducted on a Waters Synapt G2 quadrupole time-of-flight mass spectrometer (Milford, MA, USA) using positive mode of ionization. The results showed that 1,7-dihydroxy-2,8-dimethoxy xanthone, 3-(1,3-benzodi-oxol-5-yl-4-morpholinyl)-3-(2-hydroxy-4,6-dimethoxyphenyl)-1-propanone, 5,7-dihydroxy-6-methyl-3-(2',4'-dihydroxybenzyl)-chroman-4-one, trans-O-methyl-grandmarin, dioctylamine, rhodioloside, punicic acid, echimidine, N-[(2R,3S)-2-[[(3,5-dimethyl-4-isoxazolyl)sulfonyl-methylamino]methyl]-5-[(2R)-1-hydroxypropan-2-yl]-3-methyl-6-oxo-3,4-dihydro -2H-1,5-benzoxazocin-10-yl]-1,3-benzothiazole-2-carboxamide, scilliroside and geranyl hydroquinone were identified as the phytoconstituents present in fraction DDK-4. The presence of these compounds in the stem bark of S. longipedunculata has been reported for the first time. Also, 1,7-dihydroxy-2,8-dimethoxy xanthone could be very useful in the chemotaxonomy of the genus Securidaca and family Polygalaceae. 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.

Keywords: Chemotexonamy, Column chromatography, Family, Genus, LCMS/MS, Phytoconstituents

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INTRODUCTION

Securidaca longipedunculata belongs to the Family Polygalaceae, it is commonly known as violet tree or fibre tree. The plant is also locally known as *Uwar magunguna* or *Sanya* in Hausa language, *Ipeta* in Yoruba language and *Alali* in Arabic. *S. longipedunculata* is a small tree up to 6 metre high with a smooth bark and hairless alternate leaves which are variable in size and shape [1]. The fruits of the violet tree are smooth and purplish green when young and possess a membranous wing of about 4 centimetre long [2]. Traditionally, the root of this plant is used to manage many diseases such as diabetes malaria, headaches, toothache, fungal infections, epilepsy, cancer, sexually transmitted infections, skin infections, gonorrhoea among others [3–7]. The stem bark of the plant is also used to treat snake

bites, epilepsy, stomach ache, skin diseases, dysentery, rheumatism, malaria, typhoid, inflammation, infertility problems etc [8–11].

Preliminary phytochemical screening of different extracts of root, leaf and stem bark of this plant revealed the presence of alkaloids, saponins, terpenoids, flavonoids, cardiac glycosides, carbohydrates, volatile oils, tannins and steroids [12–14]. Some of the pure compounds isolated from the root of the violet tree include quercetin, gallic acid, chlorogenic acid, cinnamic acid, apigenin, quercetin glucosyl, caffeic acid, epicatechic acid, rutin, p-coumaric acid, 6-hydroxy-2-methoxy benzoic acid, 1,6,8-trihydroxy-2,3,4,5-tetramethoxy xanthone, 1,6,8-trihydroxy-2,3,4,7-tetramethoxy xanthone, 1,6-dihydroxy-2,3,4,5,8-pentamethoxy xanthone, 5-O-prenyl-1-hydroxy-2,3,6,7,8-pentamethoxy xanthone, 8-hydroxy-1,4,5,6-tetramethoxy-2,3-methylenedioxy xanthone, 4,6,8-trihydroxy,1,2,3,5 tetramethoxy xanthone and four highly oxygenated xanthones (i.e muchimangins A-D) [15–17]. Presenegenin, securinine, β-Sitosterol, Quercetin-3-O-D-xyloside, benzyl-2-hydroxy-6-methoxybenzoate, 1, 7-dihydroxy-4-methoxyxanthone and methyl salicylate were also isolated from the leaves and root of this plant [18-20]. However, the chemistry of the stem bark of *S. longipedunculata* is not widely investigated, therefore, this study was aimed at identifying some of the chemical constituents of a fraction obtained from the column chromatography of the ethylacetate extract of the violet tree.

MATERIALS AND METHODS

Collection, Identification and Preparation of Plant Material

The plant was collected from Gwaram Town, Jigawa State, Nigeria, it was identified in the field using taxonomic characters and then taken to the Herbarium of Ethnobotany and Multidisciplinary Research Division of Bioresources Development Centre, Kano for authentication, a reference voucher number: BDCKN/EB/1898 was deposited in the Herbarium. The stem bark was air dried and then ground into fine powder using mortar and pestle.

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, 8:2 and 7: 3), and then visualized with a general spray reagent. Fraction 89-92 afforded 4 prominent spots, which were combined together and labelled as fraction DDK-4, however, the quantity of this fraction was too small for further purification, therefore, LCMS/MS analysis was carried to identify the compounds present (Plate 1).

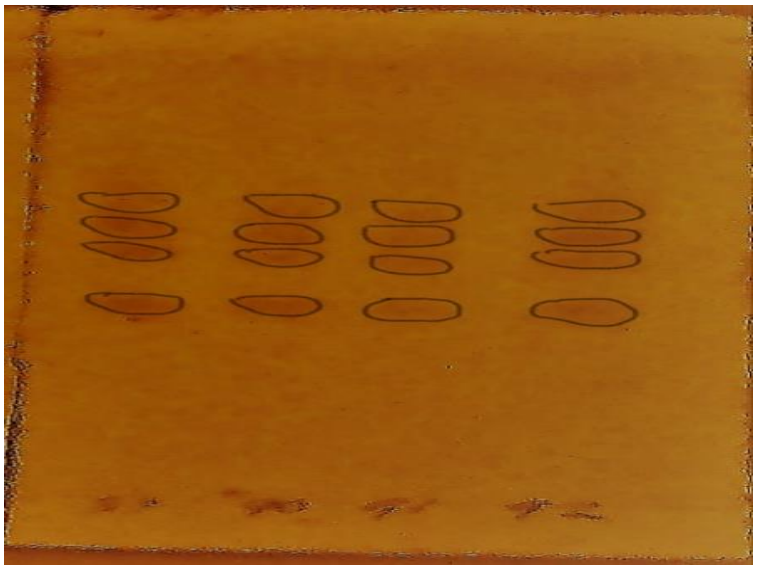


Plate 1. Thin Layer Chromatographic Profile of Fraction DDK-4 (Hexane: Ethylacetate (7:3)).

LCMS/MS Analysis

LCMS/MS analysis (positive ionization mode) of fraction DDK-4 was carried at the Central Analytical Facilities, Mass Spectrometry Unit of Stellenbosch Bosch University, South Africa in order to identify the phytochemical constituents present in the fraction. 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

The LCMS/MS analysis of fraction DDK-4 detected 11 peaks, the most prominent peaks have M/Z 289.11, 279.23, 621.30 and 247.17 at retention time of 6.38, 9.79, 12.38 and 13.58 minutes respectively (Figure 1).

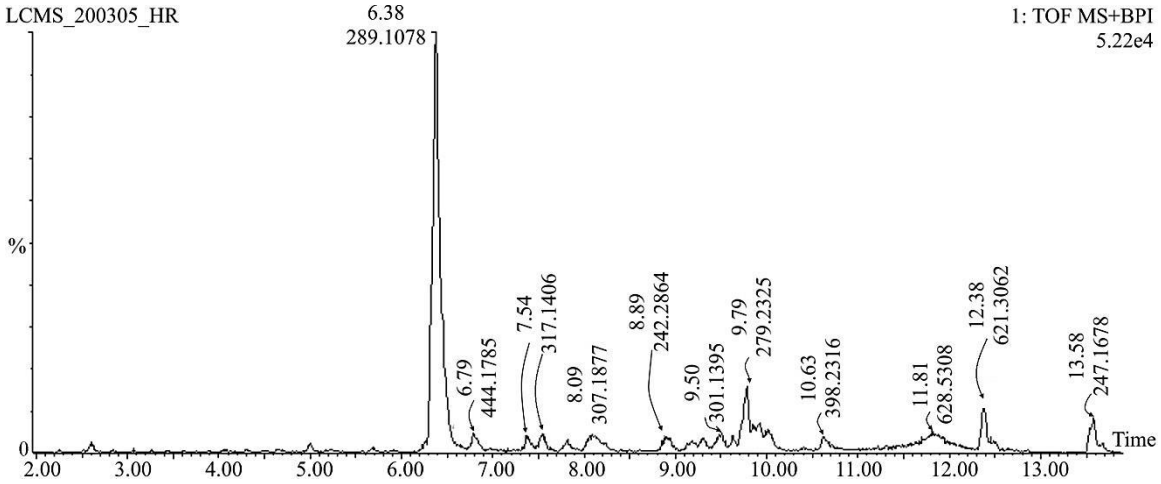
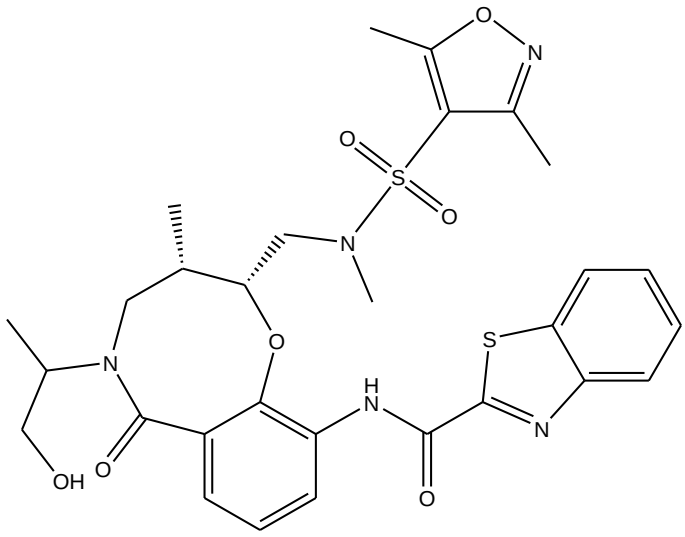
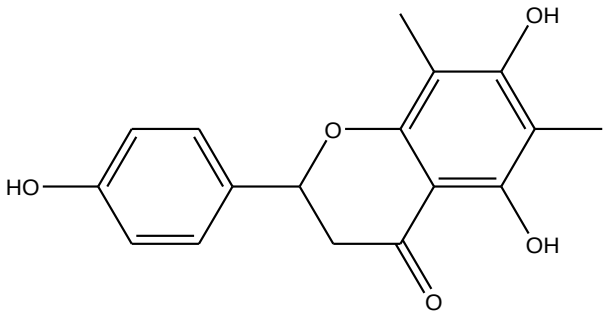


Figure 1. Total ion chromatogram of fraction DDK-4



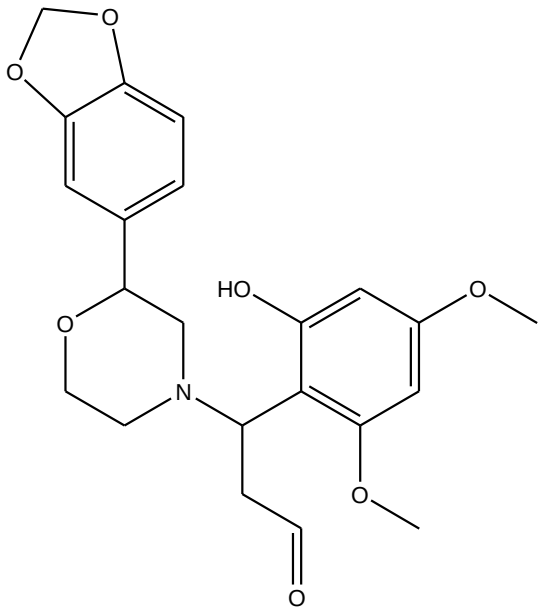
N-[(2R,3S)-2-[[(3,5-dimethyl-4-isoxazolyl)sulfonyl-methylamino]methyl]-5-[(2R)-1-hydroxypropan-2-yl]-3-methyl-6-oxo-3,4-dihydro-2H-1,5 benzoxazocin-10-yl]-1,3-benzothiazole-2-carboxamide

(a)



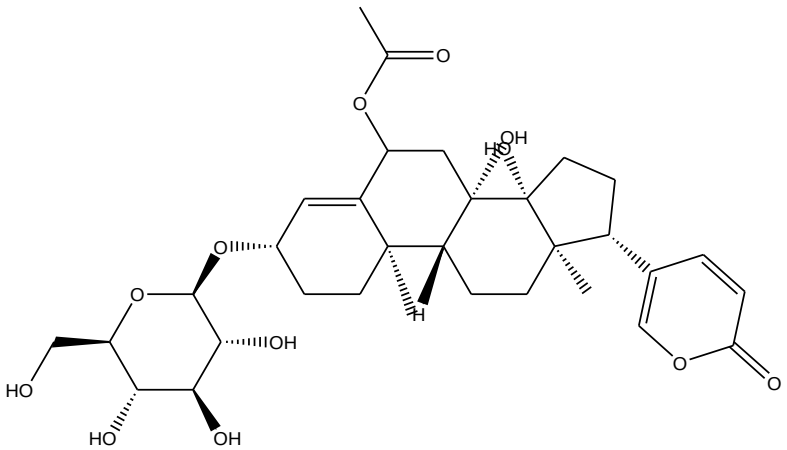
5,7-dihydroxy-2-(4-hydroxyphenyl)-6,8-dimethyl-3,4-dihydro-2H-1-benzopyran-4-one

(b)



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

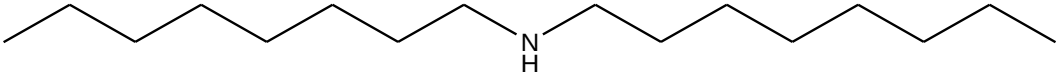
(c)



[(3S,6R,8S,9R,10R,13R,14R,17R)-8,14-dihydroxy-10,13-dimethyl-17-(6-oxopyran-3-yl)-3-[(2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-2,3,6,7,9,11,12,15,16,17-decahydro-1H-cyclopenta[a]phenanthren-6-yl] acetate

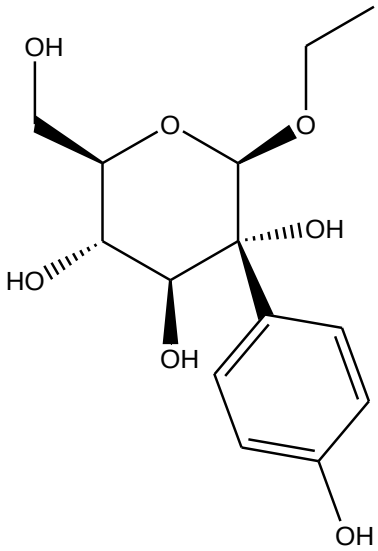
(Scilliroside)

(d)



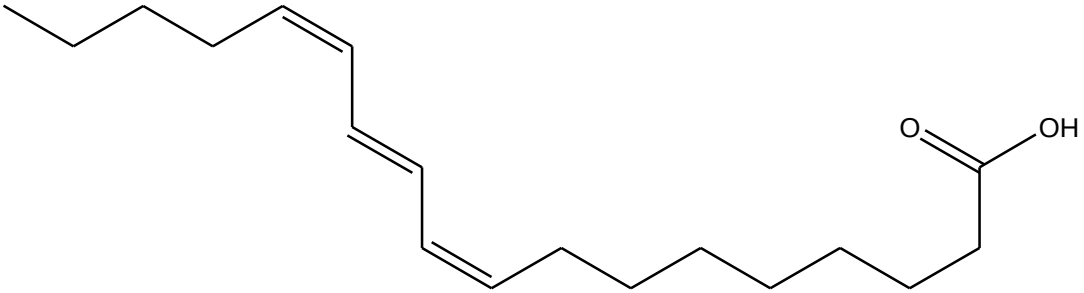
N-octyloctan-1-amine
(Dioctylamine)

(e)



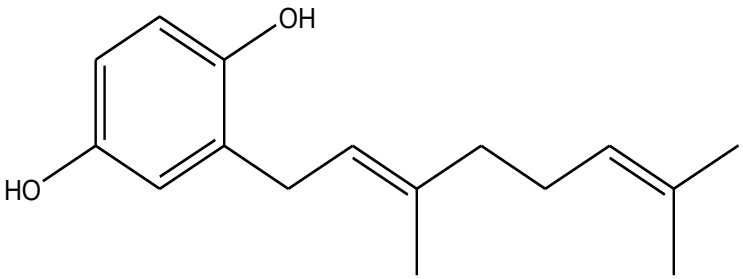
2-(4-Hydroxyphenyl) ethyl beta-D-glucopyranoside
(Rhodioloside)

(f)



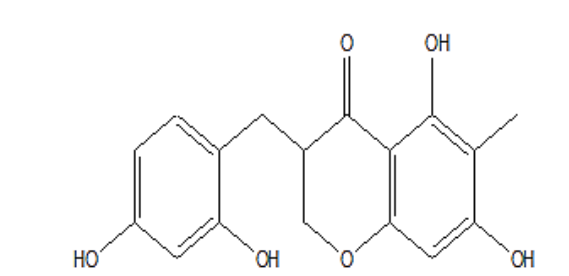
9Z,11E,13Z-octadeca-9,11,13-trienoic acid
(Punicic acid)

(g)



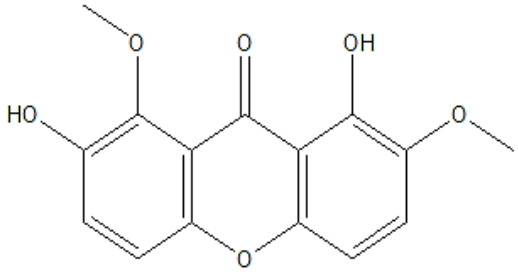
2-[(2E)-3,7-dimethylocta-2,6-dien-1-yl]benzene-1,4-diol
(Geranyl hydroquinone)

(h)



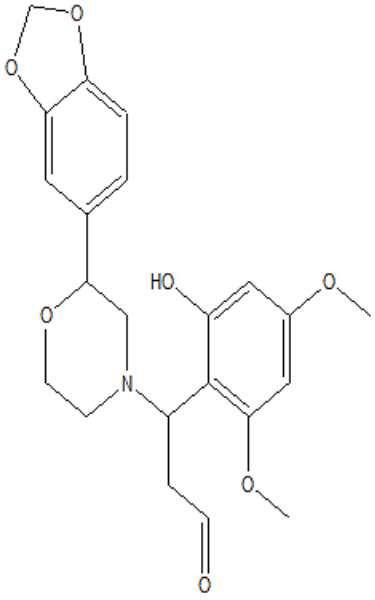
5,7-dihydroxy-6-methyl-3-(2',4'-dihydroxybenzyl)-chroman-4-one

(i)



1,7-dihydroxy-2,8-dimethoxy xanthone

(j)



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

(k)

Figure 2. Chemical structures of the compounds identified (a-k).

DISCUSSION

The LCMS/MS analysis of fraction DDK-4 was carried out using positive mode of ionisation ($M + H^+$), and the tentative identifications of all the compounds were achieved by comparing the obtained molecular ions (precursors) and fragmentation patterns (product ions) with different chemical databases and other published literature. The databases employed were Universal Natural Products Database (UNPD), Chemical Entities of Biological Interest, (CHEBI), Drug Bank Database (DrugBank), Northern African Natural Products Database (NANPDB), PubChem, PlantCyc, KNApSAcK Family Database, Human Metabolome Database (HMDB), Food Database (FoodDB), Lipid Metabolites and Pathways Strategy (Lipid MAPS), *Escherichia coli* Metabolome Database (ECMDB) and Yeast Metabolome Database (YMDB).

The study showed that 11 peaks were detected in the fraction, and this indicates that the four spots observed on the TLC plate above contained 11 compounds as shown in Figure 1. The most prominent peak has M/Z 289.11 which was identified as 1,7-dihydroxy-2,8-dimethoxy xanthone. Other compounds identified in this fraction include 3-(1,3-benzodi-oxol-5-yl-4-morpholinyl)-3-(2-hydroxy-4,6-dimethoxyphenyl)-1-propanone (M/Z 444.18), 5,7-dihydroxy-6-methyl-3-(2',4'-dihydroxybenzyl)-chroman-4-one (M/Z 317.14), trans-O-methyl-grandmarin (M/Z 307.19), dioctylamine (M/Z 242.29), rhodioloside (M/Z 301.14), punicic acid (M/Z 279.23), echimidine (M/Z

398.23), N-[(2R,3S)-2-[[(3,5-dimethyl-4-isoxazolyl)sulfonyl-methylamino]methyl]-5-[(2R)-1-hydroxypropan-2-yl]-3-methyl-6-oxo-3,4-dihydro-2H-1,5 benzoxazocin-10-yl]-1,3-benzothiazole-2-carboxamide (M/Z 628.53) scilliroside (M/Z 621.30) and geranyl hydroquinone (M/Z 247.17) (Figure 2). The molecular weight and molecular formula of these compounds were stated in Table 1.

Table 1. Summary of the total ion chromatogram of fraction DDK-4

S/N	M/Z (M + H^+)	Retention Time (Minutes)	Proposed Compound	Ontology	Molecular Formula	Exact Mass ($gmol^-$)
1	289.11	6.38	1,7-dihydroxy-2,8-dimethoxy xanthone	Xanthones	$C_{15}H_{12}O_6$	288
2	444.18	6.79	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
3	317.14	7.54	5,7-dihydroxy-6-methyl-3-(2',4'-dihydroxybenzyl)-chroman-4-one	Homoiso flavonoids	$C_{17}H_{16}0_6$	316
4	307.19	8.09	Trans-O-methyl-grandmarin	Angular pyrano coumarins	$C_{16}H_{18}0_6$	306
5	242.29	8.89	Dioctylamine	Dialkylamines	$C_{16}H_{35}N$	241
6	301.14	9.50	Rhodioloside	O-glycosyl compound	$C_{14}H_{20}O_7$	300
7	279.23	9.79	Punicic acid	Poly unsaturated fatty acid	$C_{18}H_{30}0_2$	278
8	398.23	10.63	Echimidine	Alkaloids	$C_{20}H_{31}NO_7$	397
9	628.53	11.81	N-[(2R,3S)-2-[[(3,5-dimethyl-4-isoxazolyl)sulfonyl-methylamino]methyl]-5-[(2R)-1-hydroxypropan-2-yl]-3-methyl-6-oxo-3,4-dihydro-2H-1,5-benzoxazocin-10-yl]-1,3-benzothiazole-2-carboxamide	Benzothioazoles	$C_{29}H_{33}N_50_70_2S_2$	627
10	621.30	12.38	Scilliroside	Glycoside	$C_{32}H_{44}0_{12}$	620
11	247.17	13.58	Geranyl hydroquinone	Prenylated hydroquinones	$C_{16}H_{22}0_2$	246

The presence of 1,7-dihydroxy-2,8-dimethoxy xanthone in the root bark of *S. longipedunculata* was previously reported by [17], thus, this compound could be of chemotexonamic significance. Xanthones are considered to be one of the chemical markers of the Family Polygalaceae [21]. Echimidine is a hepatotoxic pyrrolizidine alkaloid first reported from *Echium plantagineum* [22], while punicic acid is a polyunsaturated fatty acid which has been reported to have many biological properties such as antiproliferative and anticarcinogenic activity against various forms of cancer, antiobesity and antidiabetic, therefore, the antidiabetic, antiproliferative and anticarcinogenic activities of *S. longipedunculata* could be attributed to the presence of punicic acid [23, 24].

Scilliroside is a well known rodenticide first reported in the bulb of the Mediterranean squill plant, the presence of this compound in the stem bark of *S. longipedunculata* may be responsible for the rodenticidal activity of *S. longipedunculata* [25, 26]. On the otherhand, geranylhydroquinone is a marine natural product but also isolated in some plants, which has been reported to exhibit antibacterial activity, anti-inflammatory and cytotoxic effects against the leukemia cell lines of Rous sarcoma and mammary cincinoma [27–30].

CONCLUSION

The presence of these compounds in the stem bark of *S. longipedunculata* has been reported for the first time. Also, 1,7-dihydroxy-2,8-dimethoxy xanthone could be very useful in the chemotaxonomy

of the Genus Securidaca. 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.

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