

# GC-MS Analysis of n-hexane Extract of Stem Bark of *Symplocos crataegoides* Buch.-Ham. ex D. Don

Nartunai Govindarajan<sup>1\*</sup>, Uma Maheswara Reddy Cheekala<sup>2</sup>, Shantha Arcot<sup>3</sup>, Susikumar Sundaramoorthy<sup>1</sup>, Ramasamy Duraisamy<sup>4</sup>, Ilavarasan Raju<sup>5</sup>

Nartunai Govindarajan<sup>1\*</sup>,  
Uma Maheswara Reddy  
Cheekala<sup>2</sup>, Shantha Arcot<sup>3</sup>,  
Susikumar  
Sundaramoorthy<sup>1</sup>,  
Ramasamy Duraisamy<sup>4</sup>,  
Ilavarasan Raju<sup>5</sup>

<sup>1</sup>Department of Pharmacognosy, &  
<sup>5</sup>Department of Pharmacology Captain  
Srinivasa Murthy Regional Ayurveda  
Drug Development Institute, CCRAS,  
Govt. of India, Arumbakkam, Chennai –  
600 106, Tamil Nadu, INDIA.

<sup>2</sup>Department of Pharmacology, Sri  
Ramachandra College of Pharmacy,  
Sri Ramachandra University, Porur,  
Chennai - 600116, Tamil Nadu, INDIA.

<sup>3</sup>Department of Pharm. Analysis,  
C.L.Baid Metha College of Pharmacy,  
Thoraipakkam, Chennai – 600 097,  
Tamil Nadu, INDIA.

<sup>4</sup>Department of Chemistry, Govt. Arts  
College, Ariyalur - 621 713, Tamil Nadu,  
INDIA.

## Correspondence

Nartunai Govindarajan, Department  
of Pharmacognosy, Captain Srinivasa  
Murthy Regional Ayurveda Drug  
Development Institute, CCRAS, Govt. of  
India, Arumbakkam, Chennai – 600 106,  
Tamil Nadu, INDIA.

Email: natrunai@yahoo.co.in

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## ABSTRACT

**Context:** The stem barks of the plant *Symplocos crataegoides* Buch.-Ham. ex D. Don (syn. *Symplocos paniculata* (Thunb.) Miq.), Fam. Symplocaceae is extensively used in Indian medicine under the names of *Lodhra*/*Patikalodhra*. Mainly it is used to cure uterine complaints, vaginal and menstrual disorders. **Aim:** To investigate the phytochemicals from the n-hexane extract of the stem bark of *Symplocos crataegoides* Buch.-Ham. ex D. Don., using GC-MS analysis. **Materials and Methods:** Stem bark of *S. crataegoides* was extracted by Soxhlet extraction method using n-hexane. The extract was injected by splitless injection mode into the GC MS 5975 C Agilent equipped with a QP- 5000 (quadrupole) Gas Chromatography - Mass Spectrometer. **Results:** Identification of 57 compounds from n-hexane extract. Those compounds were identified by close matches with standard MS spectra and compared with NIST - 11 and WILEY library data. Dodecane (7.51%) was found as major compound followed by Isopropyl myristate, Dodecane, 1,2,4-trimethyl-benzene, Octacosane, 2-methyl-decane, 2-ethyl-1,2-dimethyl-benzene, 1,2,3,5-tetramethyl-benzene etc., other constituents were found to be in traceable quantities. **Conclusion:** GC-MS analysis of *S. crataegoides* revealed certain interesting facts of presentation of various phytoconstituents in the stem bark. The presence of various phytoconstituents contributes to the medicinal activity of the plant.

**Key words:** *Symplocos Crataegoides*, *Patikalodhra*, GC-MS Analysis, n-hexane extract.

## INTRODUCTION

The stem bark of the plant *Symplocos crataegoides* Buch.-Ham. ex D. Don, Fam. Symplocaceae is extensively used in Indian medicine under the names of *Lodhra*/*Patikalodhra*. *S. crataegoides* is a deciduous shrub or a tree, distributed in the Himalayas from Punjab to Assam, Khasi hills and Burma.<sup>1,2</sup> As per Ayurvedic references the term *Lodhra* denotes the stem bark of *Symplocos racemosa*/*Symplocos crataegoides* and considered as most useful remedy for uterine complaints, vaginal and menstrual disorders. In Sanskrit the name *Lodhra* means that it stops ocular discharges. The drug is useful in digestive disorders, eye diseases and ulcers. Decoction of the bark is used as a gargle in bleeding gums. Its astringent property is utilized for curing loose motions. It is used as aphrodisiac, useful in dropsy, elephantiasis and in fat in urine (Lipiduria). The bark is used in the treatment of ophthalmia, tonic and to prevent abortion.<sup>1,3-7</sup> The important Ayurvedic formulations in which *Lodhra* is used as one of the ingredients are *Lodhrasavam*, *Gandhatailam*, *Dasamularistam*, *Draksadi kasayam*, *Pusyanugacurnam*, etc.<sup>8</sup> The *Nyagrodhadi Kvatha Curna* contains both *Symplocos racemosa* and *Symplocos*

*crataegoides* as ingredients and *Nyagrodhadi curna* contain only *Symplocos racemosa*.<sup>9</sup>

Most of the Books on Indian Materia Medica equate *Symplocos racemosa* as well as *S. crataegoides* as the botanical source of *Lodhra* or *Rodhra*.<sup>2</sup> Two varieties of *Lodhra* are described in the texts viz. *Sabara lodhra* and *Patika lodhra*. *Sabara lodhra* is equated with *Symplocos racemosa* and *Patika lodhra* is equated with *Symplocos crataegoides*.<sup>8</sup> Botanically *S. racemosa* and *S. crataegoides* are different species called by similar vernacular name *Lodhra*. The petroleum ether (60-80°) extract of leaves of *Symplocos crataegoides* shows the presence of Octacos-1-ene, Stigmasterol and Lupeol, whereas ethanolic extract yields Salirepin.<sup>10</sup> The following compounds isolated from the ethanolic extract of the stem bark of *Symplocos crataegoides* (syn: *S. paniculata*) were reported. 4-(8-hydroxyethyl) cyclohexan-1-oic acid; androst-5(6)-ene 17-one 3 $\beta$ -O-( $\beta$ -D-glucopyranoside); 9 $\beta$ , 25-cyclo 3 $\beta$ -O-( $\beta$ -D-glucopyranosyl)-echynocystic acid; 9 $\beta$ ,19-cyclo 24-methylcholan-5,22-diene 3 $\beta$ -O-( $\beta$ -D-glucopyranosyl (1---6) $\alpha$ -D-rhamnopyranoside); 30-Et 2 $\alpha$ , 16 $\alpha$ -dihydroxy 3 $\beta$ -O- ( $\beta$ -D-glucopyranosyl) hopan-24-oic acid; 32,33,34-trimethyl-bacteriohopan-16-ene 3-O- $\beta$ -D-glucopyranoside; and flavones 3',4',5',6'-tetramethoxy

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**Table 1: GC-MS Data of n-hexane extract of stem bark of *S. crataegoides* Buch.-Ham. ex D. Don**

Identified Peak No	$t_R$ (Mins.)	Name of the compound	peak area %	SI factor (%)
1	6.141	Mesitylene	1.18	95
2	6.617	1,3-diethyl -benzene	0.83	91
3	6.683	1-methyl-3-propyl benzene	1.53	93
4	6.899	2-methyl-decane	2.28	90
5	6.951	1-methyl-2-propyl-benzene	0.59	92
6	7.010	3-methyl decane	1.57	92
7	7.144	2-ethyl-1,4-dimethyl benzene	0.74	97
8	7.196	O-cymene	1.00	95
9	7.315	2-ethyl-1,2-dimethyl-benzene	2.01	94
10	7.567	Undecane	7.51	90
11	7.679	4-ethyl-1,4-dimethyl-benzene	0.58	95
12	7.924	2-methyl-trans-decalin	1.20	97
13	7.961	1,2,3,4-tetramethyl-benzene	0.94	95
14	8.021	3,7-dimethyl-decane	0.76	90
15	8.214	1-methyldecahydronaphthalene	0.69	92
16	8.243	Pentyl- cyclohexane	0.53	94
17	8.526	1, 2, 3, 5- tetramethyl-benzene	2.0	94
18	8.555	1,2-bis(1-methylethyl)-benzene	0.94	93
19	8.600	4-methyl-undecane	0.65	91
20	8.689	2-methyl-undecane	1.00	93
21	8.801	3-methyl-undecane	0.91	90
22	8.890	1,2,4-trimethyl-benzene	3.24	92
23	9.187	Azulene	1.67	91
24	9.313	Dodecane	4.60	93
25	9.514	3,6-dimethyl-undecane	1.21	90
26	10.465	2,6-dimethyl -octane	0.99	90
27	10.918	Tridecane	1.65	98
28	11.014	2-methyl-naphthalene	0.32	96
29	12.411	Tetradecane	0.61	98
30	13.800	Pentadecane	0.34	98
31	15.100	Hexadecane	0.44	97
32	16.333	Heptadecane	0.18	98
33	17.507	Octadecane	1.14	98
34	17.767	Isopropyl myristate	7.45	94
35	19.505	Butyl myristate	0.33	98
36	19.676	Eicosane	1.71	98
37	21.504	Hexadecanoic acid butyl ester	1.09	99
38	21.652	Docosane	1.50	95
39	23.071	Octatriacontyl pentafluoropropionate	0.20	90
40	23.338	Octadecanoic acid -2-methylpropyl ester	0.65	90
41	23.465	Tetracosane	1.24	98
42	23.524	2,6,10,14-tetramethyl-hexadecane	0.24	94
43	24.081	Tricosane	0.28	95
44	24.319	Heneicosane	0.49	95
45	24.601	2-(octadecyloxy)-ethanol	0.89	90
46	24.921	1-iodo-hexadecane	0.59	95

Continued...

Table 1: Contd.

Identified Peak No	$t_r$ (Mins.)	Name of the compound	peak area %	SI factor (%)
47	25.144	1-bromo-octadecane	1.49	91
48	25.188	Nonahexacontanoic acid	0.37	93
49	25.426	1-Hexacosene	0.60	91
50	25.723	Hexacosane	0.59	95
51	26.176	1-iodo-octadecane	0.46	95
52	26.221	1-bromodocosane	0.87	91
53	26.741	Octacosane	2.77	99
54	28.642	1-chloro-heptacosane	1.86	93
55	29.816	Hentriacontane	1.72	91
56	31.205	Nonacosane	1.22	91
57	32.862	11-cyclopentyl-heneicosane	0.92	90

7-O- $\beta$ -D-glucopyranosyl (1--3)  $\beta$ -D-glucopyranoside.<sup>11</sup> The present investigation is carried out to determine the possible chemical components of n-hexane extract of stem bark of *S. crataegoides* by GC-MS analysis.

## MATERIALS AND METHODS

### Procurement of Plant Materials

The authentic samples of stem bark of *Symplocos crataegoides* Buch.-Ham. ex D. Don. (Figure 1) was supplied by Dr.G.C.Joshi, Research Officer, Regional Research Institute of Himalayan Flora, CCRAS, Thapala, Ranikhet – 263645, Almora dist., Uttarakhand. The museum specimen (C/222B SB26) is deposited in the department of Pharmacognosy, Captain Srinivasa Murthy Regional Ayurveda Drug Development Institute, Arumbakkam, Chennai, India for future reference.

### Preparation of Extract

The shade dried and coarsely powdered (10/44) stem bark of *S. crataegoides* (4 g) were extracted using n-hexane (AR) by soxhlet extraction method. The extract was filtered through Whatman No.1 filter paper and concentrated in vacuum to constant weight. The yield of the extract is 0.4 %. The n-hexane extract of the plant was used for GC-MS analysis.

### Gas Chromatography – Mass Spectrometer Conditions

Gas chromatography combined with mass spectroscopy is a preferable methodology for routine analysis of compounds. n-hexane extract was injected by split less injection mode into the GC MS 5975 C Agilent equipped with a QP- 5000 (quadrupole) mass spectrometer, fitted with a DB -5 MS ultra inert 30 m x 250  $\mu$ m, film thickness 0.25  $\mu$ m capillary GC column, coated with polydimethyl siloxane. Helium was the carrier gas at a flow rate of 1.5 ml/min. The injector port temperature was 250°C, the detector temperature was 250°C and the oven temperature was maintained at 70°C for 3 mins, 10°C/min up to 300°C for 9 mins. The ionization voltage was 70 eV. It is separated into various constituents with different retention time which are detected by mass spectrophotometer. The chromatogram shows a plot of intensity against retention time was recorded by the software attached to it. The constituents were identified by comparing retention times of the GC peaks with those of reference compounds run under identical conditions and fragmentation patterns in mass spectra were matched with those of the NIST - 11 & WILEY library and published mass spectra.<sup>12</sup>

## RESULTS AND DISCUSSION

GC-MS analysis of the n-hexane extracts of the stem bark of *S. crataegoides* is given in Table 1. Fifty seven compounds were identified by comparison with the authentic spectra obtained from GC-MS library (NIST - 11 and WILEY). Even though several peaks were present in the GC-MS chromatogram (Figure 2), the identification of only 57 constituents was established by comparison with the authentic spectra obtained from GC-MS library with the SI factor. Qualitative GC-MS analysis of n-hexane extract results revealed that Undecane (7.51 %, Figure 3 & 4) was found as major compound. It is a liquid alkane hydrocarbon with the chemical formula  $\text{CH}_3(\text{CH}_2)_9\text{CH}_3$ . It is reported as a mild sex attractant for various types of moths and cockroaches, and an alert signal for a variety of ants.<sup>13</sup> It has 159 isomers. Isopropyl myristate is the ester of isopropanol and myristic acid was found second major compound in the extract of the plant (7.45%, Figure 5 & 6). It is used in cosmetic and topical medicinal preparations where good absorption through the skin is desired. It is also used as a pesticide-free treatment against head lice which works by dissolving the wax that covers the exoskeleton of head lice, killing them by dehydration. Dodecane is present third major percentage present in the extract (4.60%, Figure 7 & 8). It is a liquid alkane hydrocarbon with the chemical formula  $\text{CH}_3(\text{CH}_2)_{10}\text{CH}_3$ , an oily liquid of the paraffin series. It has 355 isomers. Other compounds like 1,2,4-trimethyl-benzene (3.24%), Octacosane (2.77%), 2-methyl-decane (2.28%), 2-ethyl-1,2-dimethyl-benzene (2.01%), 1, 2, 3, 5- tetramethyl-benzene (2.00%), 1-chloro-heptacosane (1.86%), Hentriacontane (1.72%), Eicosane (1.71%), Azulene (1.67%), Tridecane (1.65%), 3-methyl decane (1.57%), 1-methyl-3-propyl benzene (1.53%), Docosane (1.50%), 1-bromo-octadecane (1.49%), Tetracosane (1.24%), Nonacosane (1.22%), 3,6-dimethyl-undecane (1.21%), 2-methyl-trans-decalin (1.20%), Mesitylene (1.18%), Octadecane 91.14%), O-cymene (1.00%), 2-methyl-undecane (1.00%) and 2,6-dimethyl -octane (0.99%). The other compounds such as 1,3-diethyl -Benzene, 1-methyl-2-propyl-benzene, 2-ethyl-1,4-dimethyl benzene, 4-ethyl-1,4-dimethyl-benzene, 1,2,3,4-tetramethyl-benzene, 3,7-dimethyl-decane, 1-methyldecahydronaphthalene, Pentyl- cyclohexane, 1,2-bis(1-methylethyl)-benzene, 4-methyl-undecane, 3-methyl-undecane, 2-methyl-naphthalene, Tetradecane, Pentadecane, Hexadecane, Heptadecane, Butyl myristate, Octatriacontyl pentafluoropropionate, Octadecanoic acid -2-methylpropyl ester, 2,6,10,14-tetramethyl-hexadecane, Tricosane, Heneicosane, 2-(octadecyloxy)-ethanol, 1-iodo-hexadecane, Nonahexacontanoic acid, 1-Hexacosene, Hexacosane, 1-iodo-octadecane, 1-bromodocosane and 11-cyclopentyl-heneicosane were found to

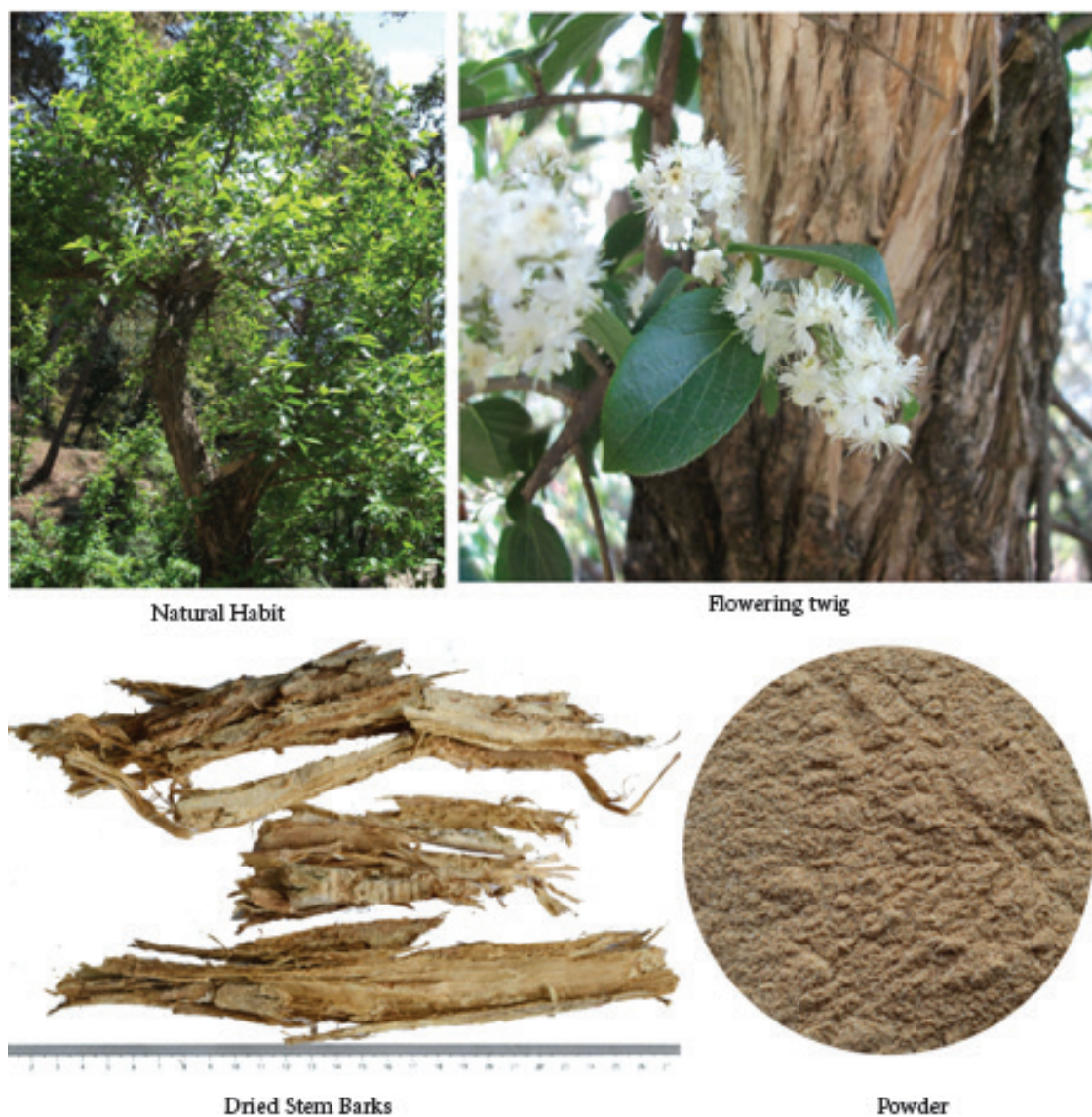


Figure 1: *Symplocos crataegoides* Buch.-Ham. ex D. Don

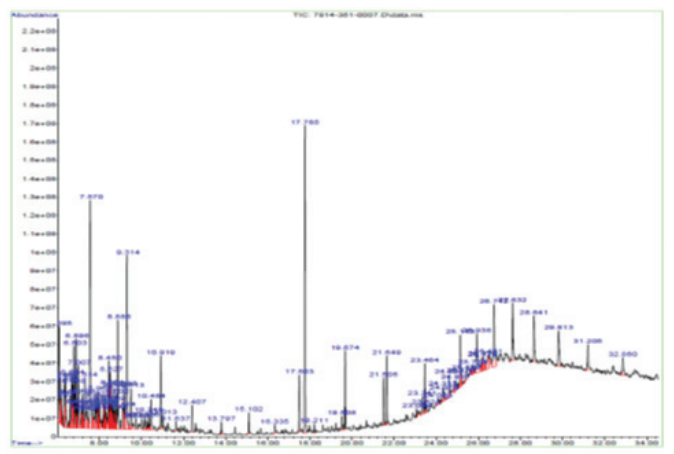


Figure 2: GC-MS Chromatogram of n-hexane extract of stem bark of *S. crataegoides*.

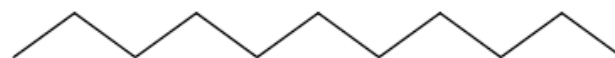


Figure 3: Undecane.

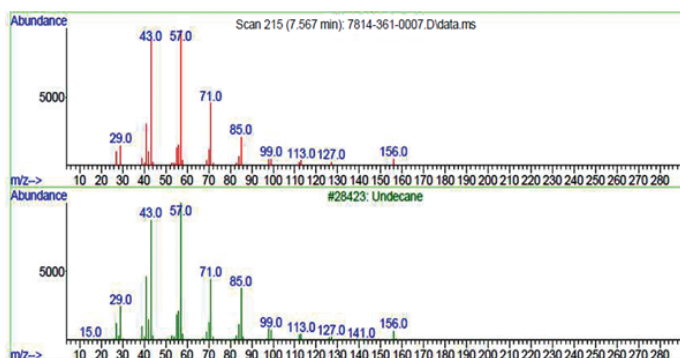


Figure 4: GC-MS Spectra of Undecane (Retention time -7.567).

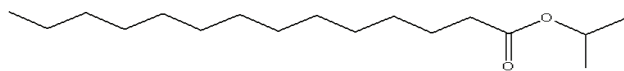


Figure 5: Isopropyl myristate.

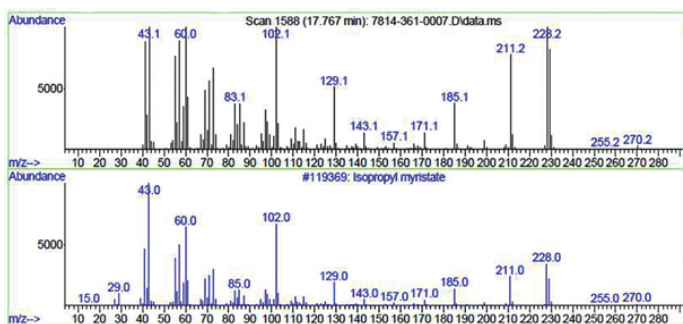


Figure 6: GC-MS Spectra of Isopropyl myristate (Retention time -17.767).

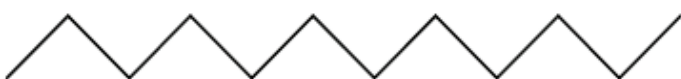


Figure 7: Dodecane.

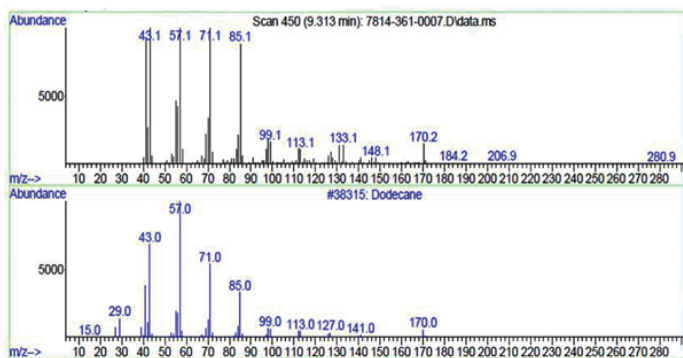


Figure 8: GC-MS Spectra of Dodecane (Retention time – 9.313).

be in small quantities. All the above 57 compounds were identified and reported for the first time from the stem bark of the plant.

## CONCLUSION

In the present study fifty seven chemical constituents have been identified from n-hexane extract of the stem bark of *S. crataegoides* by Gas Chromatogram Mass Spectrometry (GC-MS) analysis. The presence of various phytochemicals contributes to the medicinal activity of the plant.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ABBREVIATIONS USED

AR: Analytical Reagent; GC-MS: Gas chromatography and Mass spectroscopy; SI: Super Impossibility;  $t_R$ : Retention time.

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