International Journal of Chemical Science
Online ISSN: 2523-2843, Print ISSN: 2523-6075
Impact Factor: RJIF 5.22
www.chemicaljournals.com
Volume 1; Issue 2; November 2017; Page No. 07-08

Phytochemical studies of six *Salacia* species using liquid chromatography mass spectroscopic analysis

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Abstract
Comparative phytochemical studies were carried out in six Salacia species such as *Salacia agasthiamalana* (SA), *S. beddomei* (SB), *S. fruticosa* (SF), *S. macrosperma* (SM), *S. malabarica* (SMa) and *S. vellaniana* (SV). Major active compounds such as Salacianone, Salacinol, Kotalanol and Dulcitol were identified in various species using LC/MS/Ms analysis.

Keywords: *salacia*, LC MS, MS/MS

1. Introduction
Salacia L. (Celastraceae) commonly known as *Pitika* in Sanskrit is a tropical genus comprising of ca 150 species of which 21 have been reported from India and seven from Kerala [1]. The genus was formerly placed under the Hippocrateaceae, but is considered under the major family Celastraceae at present as the former Hippocrateaceae is now considered to be nested within the present Celastraceae. Root of *Salacia* spp. (*S. chinensis*, *S. reticulata*, *S. oblonga*) is one of the preferred drug sources for treating diabetes in the indigenous systems of medicine and decoction is useful in amenorrhoea, dysmenorrhoea and venereal diseases, rheumatism, gonorrhoea and asthma. The root is also used for the treatment of haemorrhoid, inflammation, leucorrhoea, leprosy, skin diseases, hyperhydrosis, hepatopathy, dyspepsia, flatulence and spermatorrhoea [2]. Many class of secondary metabolites such as alkaloids, terpenoids, flavonoids, phenolics were reported in various Salacia species [3, 4]. In the present study, six Salacia species such as *S. agasthiamalana*, *S. beddomei*, *S. fruticosa*, *S. macrosperma*, *S. malabarica* and *S. vellaniana* were screened phytochemicaly for their comparative evaluation of major compounds using LC/MS analysis.

2. Materials and Methods
2.1 Chemical and Solvents
Acetonitrile (LCMS grade) and distilled water for LCMS were obtained from Burdick & Jackson, USA. All other chemicals were of standard analytical grade from Merck India.

2.2 Plant Material
The Roots of Six species of *Salacia* were collected from different parts of Western Ghat area of South India. The specimens were identified by one of the authors (PSU), and voucher specimens of them are on file in KFRI Herbarium.

2.3 LC-MS sample preparation
Pulverized sample powders were accurately weighed (2g) and extracted with 80% of methanol under reflux for 120 min. The extraction was repeated twice. After centrifugation of the extracts at 3000 rpm for 5 min, the supernatants were combined and diluted to 100 ml with the extraction solvent. The solution was filtered through a syringe filter (0.45 µm) and an aliquot of 1 µl was subjected to LC-MS analysis. Chemicals, distilled water and Ion pair reagents were purchased from Sigma Aldrich.

2.4 LC-MS Analysis
The extracts were dissolved in LC/MS grade acetonitrile and filtered through PVDF membrane (0.45 µm) and these filtrates were subjected to LC-MS analysis. LC-ESI-MS analyses were performed using a Shimadzu LC-IT-TOF mass spectrometer equipped with an ESI interface. The LC-MS mass spectrometer was operated in the negative ion mode, scanning from *m/z* 100 to 2000. The chromatographic separation was performed on an Inertsil ODS-3 column (3-µm particle size, 2.1-mm i.d. 9 100 mm, GL Sciences Inc., Japan) operated at 40°C. The mobile phase was consisted of acetonitrile and water (78:22, v/v) and was delivered at a flow rate of 0.2 mL/min (Muraoka *et al*., 2010). The injection volume was 1 µl. The mass spectrometer was operated at negative mode with selected ion monitoring (SIM). Under SIM mode, deprotonated molecular ions ($[M-H]^-$) for each compound were observed

3. Results and discussion
3.1 LC- MS analysis
The analysis was performed by LC-MS in negative polarity mode (Muraoka *et al*., 2010). The mass fragmentation was performed by collision-induced dissociation (CID) in hexapole collision cell by varying the collision energy. The spectrum measurement has been achieved by Ballistic Ion Extraction (BIE) technology. The structural identification was carried by comparison of their retention times and mass fragments with previously reported mass fragmentation patterns.

3.2 LC-MS/MS analysis

The root extract of six species of *Salacia* presented different ion peaks on total ion chromatogram. The total ion chromatograms obtained in negative polarity mode were extracted to obtain base peak chromatogram (BPC).The base peak chromatogram showed 7 masses; out of these five molecular ions were further fragmented using MS/MS analysis (Table 1). The ion with R_t 5.8 showed a mass *m/z* (M-H) 181 yielded a fragment with *m/z* 167 which was identified as dulcitol on the basis of mass fragmentation pattern [5, 6, 7]. This was found in *Salacia fruticosa* and *S. vellaniana.* The compound dulcitol is common to Hippocrateaceae and Celastraceae family. The BPC indicated a molecular ion with *m/z* 440 which was fragmented with two major characteristic ions at *m/z* 330 and *m/z* 205 due to the cleavages of the C ring in a lupine skelton [8]. The presence of Salacianol at R_t 8.0 (*m/z* 330) was identified with the previously reported results [6]. The presence of Salacianol was confirmed in all the species. The ion at *m/z* 438 indicated Salacianone [9]. The major fragment ions produced by MS/MS analysis were *m/z* 232, 218, and 205 which characterize the cleavages of C ring in the lupine skeletons from which subsequent loss of methyl radicals were observed. The ion with R_t 5.8 14.8 showed a mass *m/z* 423 presented a major fragment 326. The compound was identified as kotalanol [6] and was found in *S. beddomei*, *S. fruticosa*, *S. macrosperma.*

Table 1: LC MS/MS analysis of selected species of Salacia

S. No.	Compound	Molecular formula	m/z experimental	m/z calculated	Error	MS/MS	Present
1	Salacianone	$C_{30}H_{46}O_2$	438.0451	438.0124	0.0327	438	SA, SB, SF, SM, SMa, SV
2	Salacinol	$C_{30}H_{48}O_2$	440.0326	440.0265	0.0061	333	SA, SB, SF, SM, SMa, SV
3	Kotalanol	$C_{12}H_{24}O_{12}S_2$	423.0489	423.0215	0.0274	326	SF, SB, SM
4	Dulcitol	$C_6H_{14}O_6$	181.0354	181.0321	0.0033	167	SF, SV

4. Conclusion

Root of various Salacia species is one of the main ingredients in many Ayurvedic antidiabetic formulations. The therapeutic efficacy of the same is due to the phytochemicals present in it. The current study substantiates the use of various species as source of drug in herbal industry as the active compounds were identified in various species of *Salacia*. The LC/MS-MS analysis provided the important phytochemicals present in the different *Salacia* species.

5. Acknowledgements

The authors is grateful to Dr. C. T. Sulaiman, Senior Scientist, Centre for Medicinal Plants Research, Kottakkal, Kerala for encouragement and critical comments on the manuscript. Also thankful to University Grant Commission (UGC), Govt. of India, New Delhi for the financial support. We also thank to Principal, Sree Krishna College, Guruvayur, Kerala and the Director, Kerala Forest Research Institute, Peechi, Kerala for encouragements.

6. References

1. Ramamurthy K, Venu P. Salacia. In: Daniel, P. (Ed.) Flora of Kerala. Botanical Survey of India, Kolkata, 2005; 1:718-722.
2. Warrier PK, Nambiar VPK, Ramankutty C, Vasudevan R. Indian Medicinal Plants: A Compendium of 500 Species. 5th Vol., Orient Longman Ltd., Hyderabad. 1996, 47.
3. Sulaiman CT, Thushar KV. Satheesh George, Indira Balachandran. Phenolic characterisation of selected Salacia species using LC-ESI-MS/MS analysis, Natural Product Research: Formerly. Natural Product Letters. 2014; 28(13):1021-1024.
4. Dhanaji M, Ghadage Parthraj, Kshirsagar R, Sandeep R, Pai Jaykumar, Chavan J *et al.* Extraction efficiency, phytochemical profiles and antioxidative properties of different parts of Saptarangi (*Salacia chinensis* L.) – An important underutilized plant. Biochemistry and Biophysics Reports. 2017; 12:79-90.
5. Wang Y, Chen WS, Wu ZJ, Xi ZX, Chen W, Zhao GJ *et al.* Chemical constituents from Salacia amplifolia, Biochemical Systems and Ecology. 2011; 39:205-208.
6. Muraoka O, Morikawa T, Miyaka S, Akaki J, Ninomiya K, Yoshikawa M *et al.* Quantitave determination of potent α-glucosidase inhibitors, salacinol and kotalanol, in *Salacia* species using liquid chromatography-mass spectrometry. Journal of Pharmaceutical and Biomedical Analysis. 2010; 52:770-773.
7. Figueiredo JN, Raz B, Sequin U. Novel Quinone Methides from Salacia kraussii with in vitro Anti-malarial Activity. Journal of Natural Products. 1998; 61:718-723.
8. Budzikiewics H, Wilson JM, Djerassi C. Mass Spectrometry in Structural and Stereochemical Problems. XXXII. Pentacyclic Triterpenes, J. Am. Chem. Soc. 1963; 85(22):3688-3699.
9. Hisham A, Kumar GJ, Fujimoto Y, Hara N. Salacianone and Salacianol, two triterpenes from *Salacia beddomei.* Phytochemistry. 1995; 40(4):1227-1231.