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Phytochemical profiling of *Coscinium fenestratum (Gaertn.)* Colebr Cultivar, by Liquid chromatography-Mass spectrometry

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A B S T R A C T

Coscinium fenestratum (Gaertn.) Colebr, commonly called as daruharidra which belongs to Menispermaceae family is rich with bioactive secondary metabolites that might signify valuable leads in the production of new pharmaceutical agents. The metabolite accumulation in the plants varies with the environmental factors, expression level of enzymes, climatic conditions etc. To evaluate the difference of metabolite in the cultivated vine, the sample was analysed by High performance Liquid Chromatography-Mass Spectrometry (HPLC-MS). So, in this study, we choose cultivated *Coscinium fenestratum* (Gaertn.) Colebr, (daruharidra) as study object and leaf and stem tissues were selected as samples and the metabolite content was analysed by chromatographic method. HPLC-MS with the electrospray (ES) ionization chamber were very efficient in ionizing in the positive ion mode (ES+) and the analytes being heterocyclic compounds predominantly protonated and was determined based on its molecular weight, retention time and the available library database. Thus the compounds deciphered were berberine, jatrorrhizine, palmatine, tetrahydropalmatine, tetrahydroberberine, magnoflorine, isocorydine, glaucine an alkaloid related to protoberberine and aporphine group of alkaloids and ecdysterone a plant sterol compound were identified in both leaf and stem sample.

Introduction

Medicinal herbs are a great source of treasure in Indian sub continent and these botanicals are considered as a local heritage of global importance. India has a rich source of medicinal herbs and is considered as botanical garden of the world (Seth *et al.*, 2004). Nature

has blessed on us with a unique and diverse species of botanicals which have a medicinal value and is used to cure specific ailments. In most of the developing countries these herbs are used to treat primary health care because of cultural acceptability, natural origin, availability and compatibility to human health with fewer side effects. Currently there is a phenomenal increase in screening medicinal plants and its preparations as a safe alternative to conventional medicines. A number of medicinal plants and its herbal preparation are traditionally named as rasayana and it is used for over centuries in our Indian traditional healthcare systems (Scartezzini *et al.,* 2000; Warrier *et al.,* 1983). So the growing interest to explore phytochemical component paved a way for discovering various synthetic substances which were most commonly used in pharmaceutical, cosmetic and food industry. Studies related to phytochemicals have lead to the discovery of plant drugs like quinine, morphine, cocaine and reserpine to name a few which have helped in the production of anti-malarial, analgesic, anti-inflammatory, anti-diabetic, anti-bacterial, hypersensitive drugs etc which are widely used in medicine today (Ashalatha *et al.,* 2013; Nambiar *et al.,* 2000).

Coscinium fenestratum (Gaertn.) Colebr, which is popularly called as daruharidra (Moss.1983) is used in over 62 ayurvedic medicaments like Aswagandharishtam, Anuthailam, Khadirarishtam, Katakakhadiradi kashayam., etc (Kulip. 2003; Siwon, *et al.,* 1989; Tushar, *et al.,* 2008; Rai, *et al.,* 2013). It is used in treating the excessive bleeding which is observed during menstruation and piles. In case of snakebite poisoning, *Coscinium* and turmeric paste is applied (http://www.island.lk). (Agusta. 2003) reported that many traditional healers use the bark in their treatments and according to their belief fresh aqueous extract is more potential in curing certain ailments but due to non-availability of fresh bark, a decoction of bark is preserved and consumed every day morning (http://www.botanical.com). Leucorrhoea and other gynaecological issues are treated with *C. fenestratum* bark. The gandai region traditional healers apply the bark powder in treating eye infections both internally and externally. In internal treatment the combination of herb medicament is used and in external treatment the paste of bark powder with cow milk is applied.

Bio-Chemical screening is one of the most compatible approaches for the rapid detection of novel new plant constituents (J.L. Wolfender *et al.,* 1994). HPLC (High performance liquid chromatography) integrated with UV and mass spectrometry (LC/MS) have been proven to be effective in analyzing the crude plant extract. Particularly LC-MS used with different ionization system like electrospray (ES), thermospray (TSP) have proven to be very efficient in analyzing the early recognition of Saponins in S.madagascariensis and *P. dodecandra*. LC-MS has become one of the powerful analytical tools for identification and quantification of plant constituents, even in trace amounts. It integrates LC with mass spectrometry (MS) where LC separates the compounds sparingly on differences in the affinity for the stationary and mobile phase and quantitates the substances based on peak intensity and peak area and in contrary Mass Spectrometry offers highly sensitive detection technique that ionizes sample with various method based on their mass to charge ratios.

The purpose of this study is to analyse the metabolite present in cultivar of *Coscinium fenestratum (Gaertn.) Colebr,* (daruharidra), the leaf and stem tissues were selected as samples. The major secondary metabolite of the sample was analysed by LC-MS to verify the metabolite variation between the samples.

Materials and Methods

Plant Material

The *Coscinium fenestratum (Gaertn.) Colebr.,* stem and leaf sample were collected from FRLHT campus, Bangalore, Karnataka, India

(13.135^0 N Latitude and 77.5891^0 E longitude) and voucher herbarium specimen (No.120017 for C. *fenestratum*) was deposited in the Herbarium of Foundation of Revitalization of Local Health Traditions (FRLHT). The fresh plant materials (Fig: 1) collected was rinsed in water to remove the contamination, dried and then homogenized to coarse powder. The coarsely powdered sample was stored in an air tight bottle for further studies.

Plant extract preparation

50g of each air dried plant materials were extracted with 200ml methanol solvent using soxhlet apparatus. The coarsely powdered sample was filled in a thimble and placed in soxhlet apparatus and was subjected to continuous hot extraction. On completion of the extraction, the extract was filtered and distilled using distillation unit to remove the solvent completely. The obtained crude extracts were transferred to air tight container and it is stored for further studies.

LC-MS analysis

Chromatography/MS grade methanol, purchased from Sigma Chemical Co., was used in the preparation of methanolic extract. LC-MS grade methanol, acetonitrile, formic acid and all the reagents were of analytical grade. Ultrapure Milli-Q water was used for the analysis. All mobile phase solvents were filtered using 0.45µm nitrocellulose membrane.

The plant extract was dissolved in 3ml of the mobile phase-0.2% formic acid in methanol, centrifuged and filtered with 0.2-µm membrane (Merck Millipore) and injected into LC-MS for identification of the alkaloid.

Instrumentation

The Acquity-UPLC (H-class) instrument from Waters (Milford, MA, USA) equipped by degasser, auto sampler injector, quaternary pump, with a diode array detector (DAD) set with Acquity UPLC BEH-C18 column. The complete system was overall controlled by the MassLynx software, managing data collection and treatment system.

Chromatographic Conditions

Chromatographic segregation of the compounds was established with a Water Acquity SIR (Selected Ion Recording) method, the analytical column used was 2.1x50 mm UPLC BEH C18 column (Waters, USA) with1.7µm guard column, operated at 25^0C. The mobile phase, Solvent A consisted of 0.5% formic acid in H_2O and Solvent B: 0.2% formic acid in 90% methanol was supplied at a flow rate of 0.3mL/min under the gradient program as follows (Table.1).

The sample injection volume used was 5µl each time, with flow ramp rate of 0.45min, high pressure limit of 15000psi and seal wash period of 5.00 min. The metabolites eluted were monitored using the UPLC column effluent with source temperature 135^0C, desolvation gas flow of 650 L/hr and temperature at 350^0C. Identification of different alkaloids was done through characteristic absorption spectra (-max), retention time, mass characterization and available published literature.

Results and Discussion

To explore the different metabolites present in the stem and leaf of *C.fenestratum*, LC/MS was performed. The LC-MS chromatogram of methanolic extract of *C.fenestratum* and the retention time is shown in fig.2.

The alkaloids present in the methanolic extract showed a stronger signal response to the ES^+ (positive ion mode) compared to ES^- (negative ion mode). The MS ion-transitions were observed in

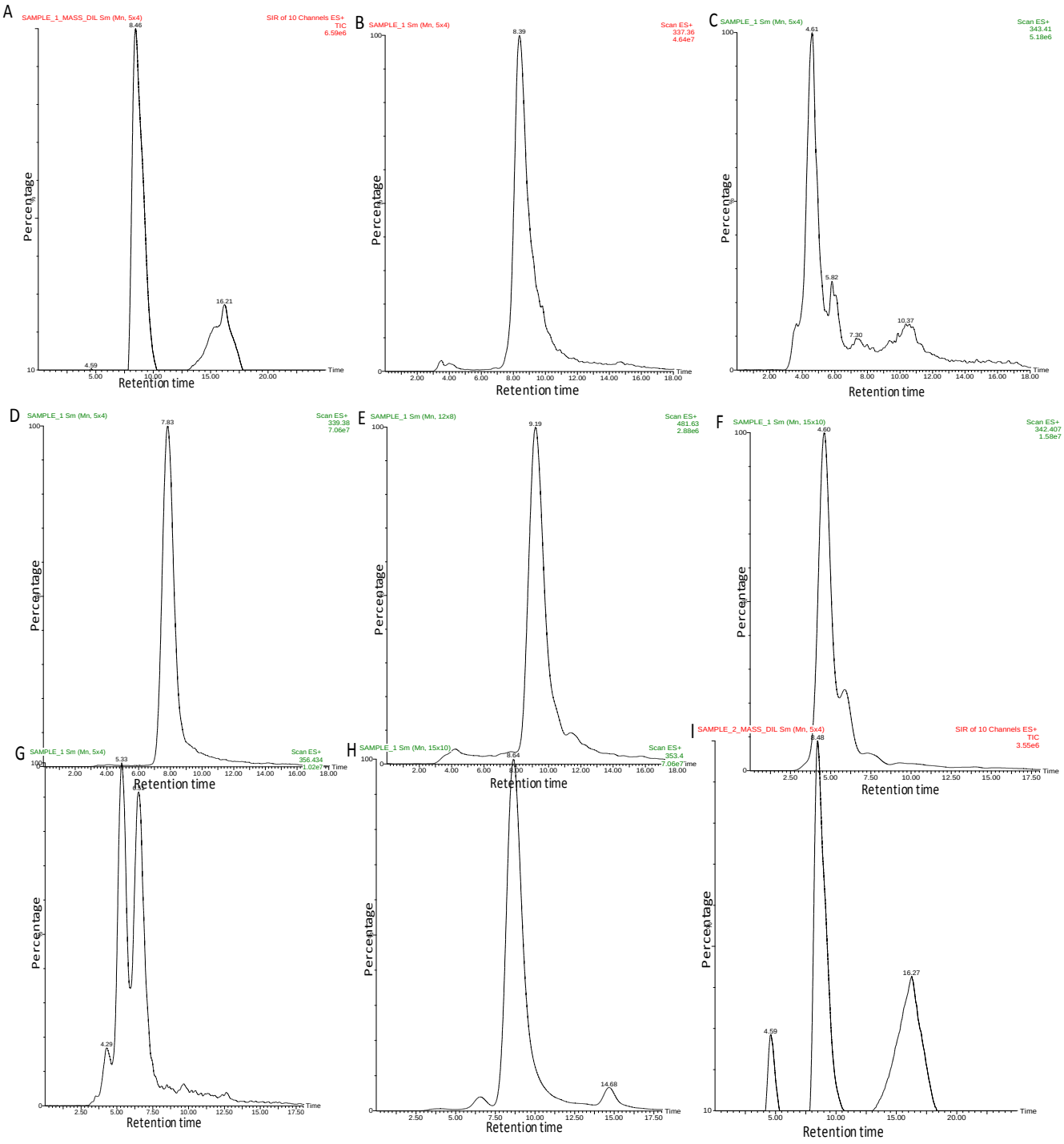
SIR (single ion reaction) mode to enhance the detection specificity of the sample. In the TIC (total ionization chromatography) spectra, the analytes being heterocyclic compounds predominantly protonated and is determined based on the molecular weight, retention time and the available library database the compounds were identified.

The LC-MS ES^+ TIC (Total Ion Count) of methanolic stem and leaf extract of *C.fenestratum*, Based on the molecular peak (m/z), retention time its empirical formula and compounds were deciphered and compounds detected are tabulated (Table.1). The components such as berberine, magnoflorine, isocorydine, glaucine, jatrorrhizine, palmatine were identified as few of the alkaloids present in the sample. These results were confirmed by previous observations (Akowuah *et al.*, 2014; Awantika *et al.*, 2016; Malhotra *et al.*, 1989; Pinho *et al.*, 1992; Rojsanga *et al.*, 2005); their studies with UPLC-ESI-MS/MS under MRM mode to detect alkaloids from different plant parts of *C. fenestratum* concluded the presence of eight bioactive compounds (protoberberine and aporphine alkaloids).

Phytochemical studies on stem and leaves of the plants also showed the steroid component ecdystreone (20E) apart from protoberberine and aporphine alkaloids. Similar observation were made by (Madhavan *et al.*, 2015) on phytochemical investigation which was carried out with the stem and leaves of *C. fenestratum*, inferred the presence of considerable amount of ecdysterone in the leaves (0.12%) and stem (0.22%). So, the results were in resemblance with previous study.

Though the plant sample was a cultivated vine, the environmental conditions were favourable for the plant to accumulate sufficient amount of secondary metabolite. The screening of the cultivated vine showed most of the active compounds which was reported earlier.

Figure.1 Leaves and stems of *C.fenestratum*


A
SAMPLE_1_MASS_DIL Sm (Mn, 5x4)
SIR of 10 Channels ES+
TIC
6.59e6
8.46
16.21
4.59
B
SAMPLE_1 Sm (Mn, 5x4)
Scan ES+
337.36
4.64e7
8.39
C
SAMPLE_1 Sm (Mn, 5x4)
Scan ES+
343.41
5.18e6
4.61
5.82
7.30
10.37
D
SAMPLE_1 Sm (Mn, 5x4)
Scan ES+
339.38
7.06e7
7.83
E
SAMPLE_1 Sm (Mn, 12x8)
Scan ES+
481.63
2.88e6
9.19
F
SAMPLE_1 Sm (Mn, 15x10)
Scan ES+
342.407
1.58e7
4.60
G
SAMPLE_1 Sm (Mn, 5x4)
Scan ES+
356.434
1.02e7
5.33
4.29
H
SAMPLE_1 Sm (Mn, 15x10)
Scan ES+
353.4
7.06e7
9.64
14.68
I
SAMPLE_2_MASS_DIL Sm (Mn, 5x4)
SIR of 10 Channels ES+
TIC
3.55e6
8.48
4.59
16.27
Percentage
Retention time
Time

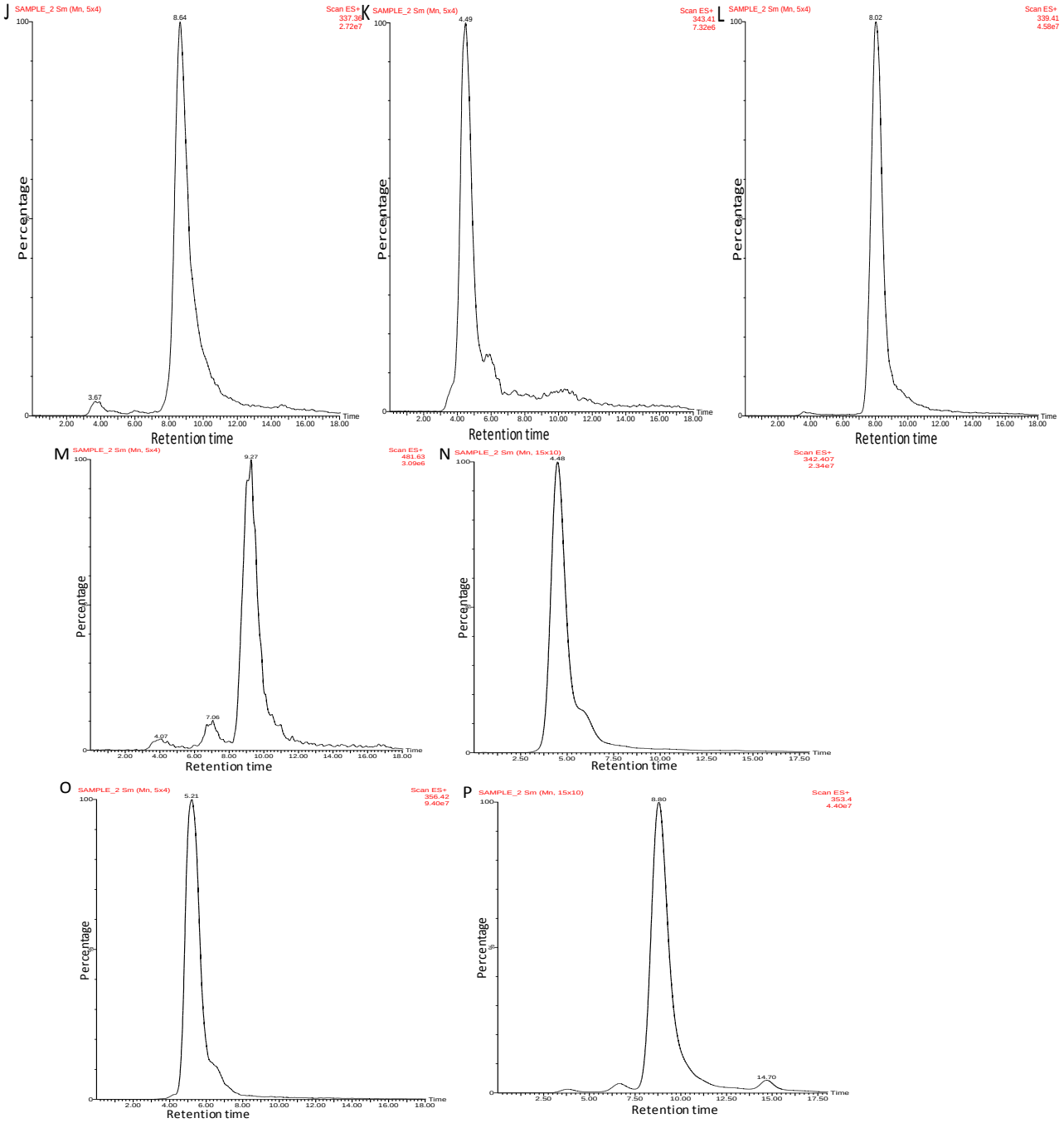


Figure.2 LC-MS chromatogram of methanolic extract of *C.fenestratum* stem (A-H) and leaf (I-P)
A. LC MS ES^+ TIC of *C.fenestratum* stem B. Chromatogram showing berberine C. Chromatogram showing magnoflorine D.Chromatogram showing jatrorrhizine E. Chromatogram showing ecdysterone. F. Chromatogram showing isocorydine G. Chromatogram showing glaucine H. Chromatogram showing palmatine I. LC MS ES^+ TIC of *C.fenestratum* stem J. Chromatogram showing berberine
K. Chromatogram showing magnoflorine L.Chromatogram showing jatrorrhizine M. Chromatogram showing ecdysterone N. Chromatogram showing isocorydine O. Chromatogram showing glaucine
P. Chromatogram showing palmatine

Table.1 Gradient program of LC/MS

Time in minutes	Flow rate mL/min	% Solvent A	% Solvent B	Curve
Initial	0.220	70.0	30.0	Initial
1.00	0.220	70.0	30.0	6
10.00	0.220	20.0	80.0	6
12.00	0.220	20.0	80.0	6
15.00	0.220	70.0	30.0	6
18.00	0.220	70.0	30.0	6

This investigation was carried out with an objective of deciphering the major metabolite in the cultivated vine of *C.fenestratum*. The methanolic extract of both leaf and stem sample were analysed by LC-MS/MS with electrospray ionisation method, could identify components such as berberine, magnoflorine, isocorydine, glaucine, jatrorrhizine, palmatine an alkaloid belonging to protoberberine and aporphine group of alkaloids and in addition could also identify ecdysterone a phytosterol compound in both the sample. The result thus showed that, the cultivated vine with the controlled climatic conditions also accumulated most of the secondary metabolite.

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