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High Resolution Liquid Chromatography Mass Spectrometry - A Tool for Structural Elucidation of Seed Metabolites of *Abrus Precatorius* (L.)

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Abstract:

Plant metabolites with therapeutic values provide enormous scope for novel drug leads. In this context several traditional medicinal plants are targeted for phytochemicals identification and characterization worldwide. The current investigation was undertaken to identify metabolites present in seeds of *Abrus precatorius* (L.), a medicinal plant with potent anti-inflammatory, antimicrobial, antioxidant, antifertility, and antispermatogenic activity. Ethanol extracted seed metabolites were identified by LC-ESI-Q-TOF-MS system equipped with a G4220B pump, G4226A auto sampler and G1316C, and a diode array detector. Metabolites profiling yielded large sets of data were analyzed by METLIN Mass Spectral Database. Metabolomics analysis revealed presence of Desethyletomidate, Butabarbital, 4-aminohippurate, 20α-Dihydroprogesterone glucuronide, Phenethylamine, Etomidate, Oxyquinoline, Norfloxacin, Phenylethylmalonamide (PEMA), and Dihydro streptomycin with typical plants metabolites. The masses of these uncommon metabolites were 216.08, 212.11, 222.09, 492.27, 121.09, 244.12, 145.05, 319.12, 206.10 and 567.28 respectively. These metabolites contribute to various drug preparations used to treat human ailments.

Keywords: LC-ESI-Q-TOF-MS, Phytochemical, *Abrus precatorius* (L.) seeds, Metabolites

Introduction

Phytoconstituents obtained from medicinal plants are effective for treating various human ailments. The traditional medicine practice is widespread in India and medicinal plant metabolites are successfully used to treat various communicable and non-communicable diseases[1,2]. Phytoconstituents of medicinal plants are admirably coping number of deadly diseases such as cancer, Hepatitis, AIDS etc. and considered safe future medicines[3]. The chemical exploration of these medicinalplants to discover hidden drug leads potential could be critical in the treatment of present and future human ailments.

Plants are the richest sources of natural compounds with diversifying pharmacological activities.These natural compounds can be characterized into primary and secondary metabolites. These chemical ingredients exhibit varied endogenous functions, specifically primary metabolites are directly involved in the growth and development of plants whereas secondary metabolites are known to protect plants from various abiotic and biotic stresses[4]. Various groups of secondary metabolites including phenolic compounds, flavanoids, steroids, triterpenoids, alkaloids, isoflavanoquinones, anthocyanins, toxalbumin are known[5,6].

Metabolomics isnon-selective, comprehensive analytical approach used to identify and quantify metabolites from biological samples. Metabolomics focuses on global detection of small molecules in sampleswith a molecular weight below 1500 Da[7]. Mass Spectrophotometry (MS) imaging is a label free technique and thus, can be used without prior knowledge of the analytes and can be detected simultaneously from biological samples[8]. Q-TOF LC/MS gives accurate mass measurements, high resolution and provides the elemental compositions of unknown peaks with more accuracy in complex matrices[9]. The metabolic fingerprinting from biological samples using Agilent 6550 iFunnel Q-TOF LC/MS System (6550 Q-TOF) has been reported earlier[10].

The aim of the current work was to establish metabolomic fingerprint and identification of major bioactive metabolites of *Abrus precatorius* (L.) by Agilent 6550 iFunnel Q-TOF LC/MS System (6550 Q-TOF). It belongs to Fabaceae family with wider distribution in India, ascending to an altitude of about 1050 m in the outer Himalayas. It is a woody twinning plant with characteristic toxic red seeds with black mark at the base[11].*Abrus precatorius* (L.) found to have potent anti-inflammatory, cytotoxic, antimicrobial and anti-diabetic activities and antispermatogenic activity[12,13].

Materials and Methods

Abrus precatorius (L.) seeds (100 grams, white, black and red seeds) were procured from local market of Aurangabad (MS) India. Seeds were thoroughly washed with distilled water and dried at room temperature (27 ºC). The dried seeds were crushed into fine powder using grinder mixer. Metabolites were solvent extracted by adding dried powder (20 g) in 300ml ethanol (Rankem, USA) using Soxhlet apparatus for 8 h. The resulting extract (10 g) filtered through Whatman filter paper no. 44 and evaporated to dryness at constant temperature and store at 4^{o}C for further use.

Identification of metabolites from ethanolic extract was carried out at SAIF, IIT, Bombay. Samples were analyzed on a LC-ESI-Q-TOF-MS (Agilent Technologies 6550i-Funnel) system equipped with a G4220B pump, G4226A auto sampler and G1316C, and a diode array detector (DAD). The elution solvent consisted of a gradient system of 0.1% formic acid in water (A) and acetonitrile (B) at a flow rate of 0.3 ml/min. The gradient system started with 95% A: 5% B reaching 5% A: 95% B in 50 min, then back to initial composition 95% A: 5% B in 10 min which was held at same composition for 5 min. The MS analysis was carried out by ESI positive ionization mode. MS source conditions were as follows: capillary voltage 3500 V, Gas temperature 250 ^{0}C, drying gas flow 13 L/min, sheath Gas temp 300, sheath Gas Flow 11, nebulizing gas pressure 35 (psig), fragment or 175 V, Skimmer 65 V, Octopole RF Peak 750 V, and mass range m/z 50–1000. The resolution was 40,000 FWHM. Metlin database was used to structure conformation.

Results and discussion

Metabolomics have become an indispensable tool for advancing our understanding of common and bioactive metabolites in plants. The ESI-Q-TOF-MS generated metabolomic chromatogram of ethanolic extract of *Abrus precatorius* (L.) is depicted in **Fig. 1**. Metabolites analysis revealed the presence of fatty acids, organic compounds, phenolics, alkaloids, amino pyrimidines, dipeptide and tripeptides (**Table 1**). The major abundant metabolites identified in the *Abrus precatorius* (L.) ethanol extract by ESI-QTOF-MS analysis were **(a)** Desethyletomidate, **(b)** Butabarbital, **(c)** 4-aminohippurate, **(d)** 20α-Dihydroprogesterone glucuronide, **(e)** Phenethylamine, **(f)** Etomidate, **(g)** Oxyquinoline, **(h)** Norfloxacin, **(i)** Phenylethylmalonamide (PEMA), **(j)** Dihydro streptomycin of mass 216.08, 212.11, 222.09, 492.27, 121.09, 244.12, 145.05, 319.12, 206.10 and 567.28 respectively (Figure 2a-j). The retention time, mass, molecular formula and the DB difference (ppm) of the major and minor metabolites were shown in **Table 1**.

These ten major metabolites discovered in *Abrus precatorius* (L.) are reported to have featured chemical and structural properties with therapeutic values as investigated from several sources earlier reviewed here. The findings of current study were compared with those earlier reports. Desethyletomidate (trade name, Etomidate): With IUPAC name 3-(1-phenylethyl) imidazole-4-carboxylic acid, it has molecular formula $C_{12}H_{12}N_2O_2$. It is a short-acting

intravenous anesthetic agent used for the induction of general anesthesia and sedation[14] for short procedures such as reduction of dislocated joints, tracheal intubation, and cardio version. It was developed at Janssen Pharmaceutical in 1964 and was introduced as an intravenous agent in 1972 in Europe and in 1983 in the United States[15] (Pubchem CID: 36339). Butabarbital (trade name Butisol): It is a prescription barbiturate sleep aid which makes it useful for certain applications such as treating severe insomnia and relieving anxiety before surgical procedures (Pubchem CID: 2479).

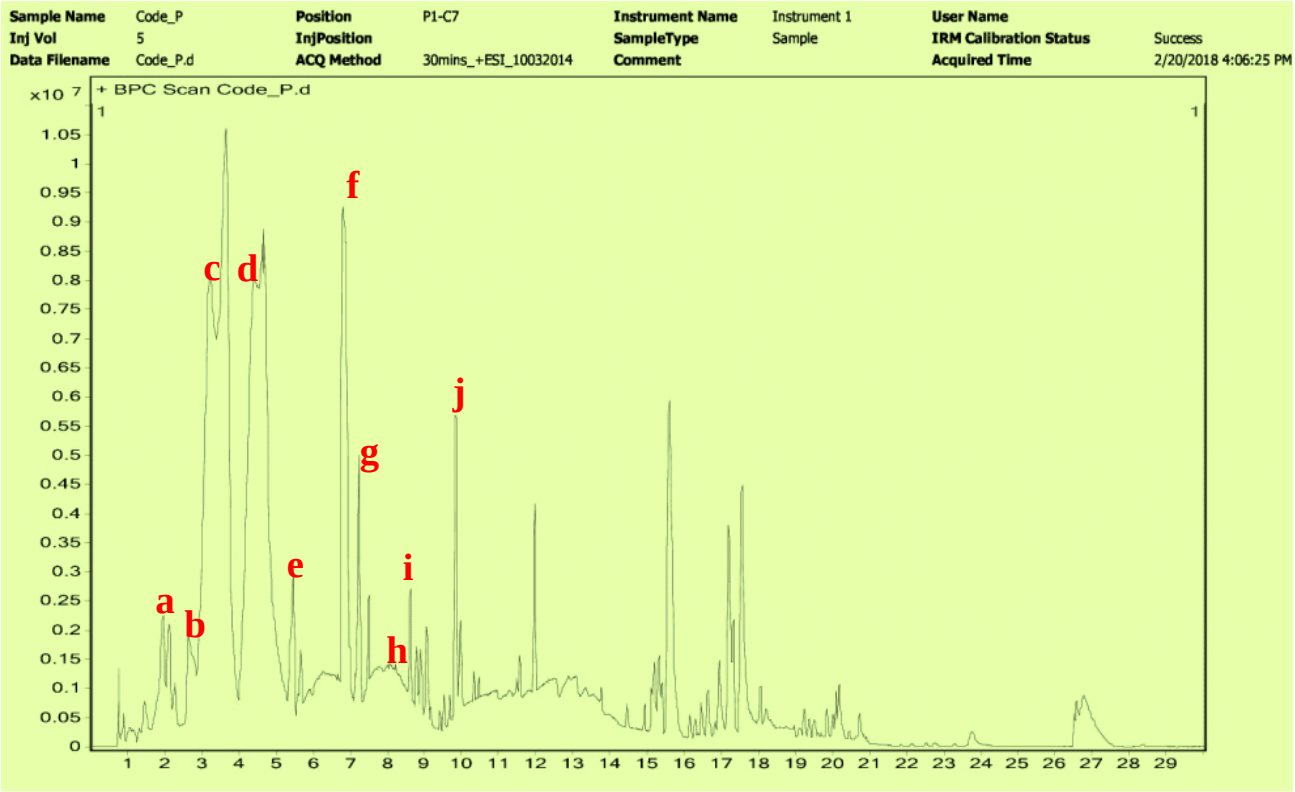

Fig. 1: Counts Vs. acquisition time (min.) chromatogram of ethanolic extract of *Abrus precatorius* (L.) by ESI-Q-TOF MS.

Table 1 Major abundant metabolites of ethanolic extract of *Abrus precatorius* (L.)

Sr. No.	Name	RT	Mass	Formula	M/Z	Ion	DB diff. (ppm)	Phytochemical constituents
a	Desethyletomidate	1.938	216.08	$C_{12}H_{12}N_2O_2$	217.09	$(M+H)^+$	3.08	Alkaloids
b	Butabarbital	1.940	212.11	$C_{10}H_{16}N_2O_3$	235.10	$(M+Na)^+$	-4.99	Alkaloids
c	4-aminohippurate	2.261	222.09	$C_{11}H_{14}N_2O_3$	223.10	$(M+H)^+$	4.82	Amino acids
d	20αDihydroprogesterone glucuronide	4.671	492.27	$C_{27}H_{40}O_8$	493.27	$(M+H)^+$	1.29	Triterpenoids
e	Phenethylamine	5.440	121.09	$C_8H_{11}N$	144.08	$(M+Na)^+$	-15.62	Proteins & Amino acids
f	Etomidate	7.141	244.12	$C_{14}H_{16}N_2O_2$	245.12	$(M+H)^+$	-4.42	Alkaloids
g	Oxyquinoline	7.195	145.05	C_9H_7NO	146.05	$(M+H)^+$	3.01	Flavonoids,
h	Norfloxacin	7.493	319.12	$C_{16}H_{18}FN_3O_3$	342.12	$(M+Na)^+$	-1.21	Flavonoids,
i	Phenylethylmalonamide	8.615	206.10	$C_{11}H_{14}N_2O_2$	229.09	$(M+Na)^+$	-9.67	Amino acids
j	Dihydro deoxy streptomycin	9.843	567.28	$C_{21}H_{41}N_7O_{11}$	568.29	$(M+H)^+$	-1.6	Triterpenoids

Aminohippuric acid or p-aminohippuric acid (PAH): It is a derivative of hippuric acid, it is a diagnostic agent useful in medical tests involving the kidney used in the measurement of renal plasma flow (Pubchem CID: 2148). 20α-Dihydroprogesterone (20α-DHP): It is a naturally occurring, endogenous progestogen (Pubchem CID: 8956). Phenethylamine (PEA): It is an organic compound, natural monoamine alkaloid and trace amine, which acts as a central nervous system stimulant in humans[16] (Pubchem CID: 1001). Etomidate (marketed as Amidate): It is a short-acting intravenous anesthetic agent used for the induction of general anesthesia and sedation[14]. Oxyquinoline: It exhibit antiseptic, disinfectant, and pesticide properties functioning as a transcription inhibitor (Pubchem CID: 1923). Norfloxacin: It is a synthetic antibacterial agent[17] (Nelson et al. 2007) used to treat urinary tract infections, gynecological infections, inflammation of the prostate gland, gonorrhea and bladder infection[18] (Pubchem CID: 4539). Phenylethylmalonamide (PEMA): It is an active metabolite of the anticonvulsant drug primidone[19] (Pubchem CID: 23611). Dihydrostreptomycin: It is derivative of streptomycin that has a bactericidal property. It's a semi-synthetic aminoglycoside antibiotic used in the treatment of tuberculosis (Pubchem CID: 439369).

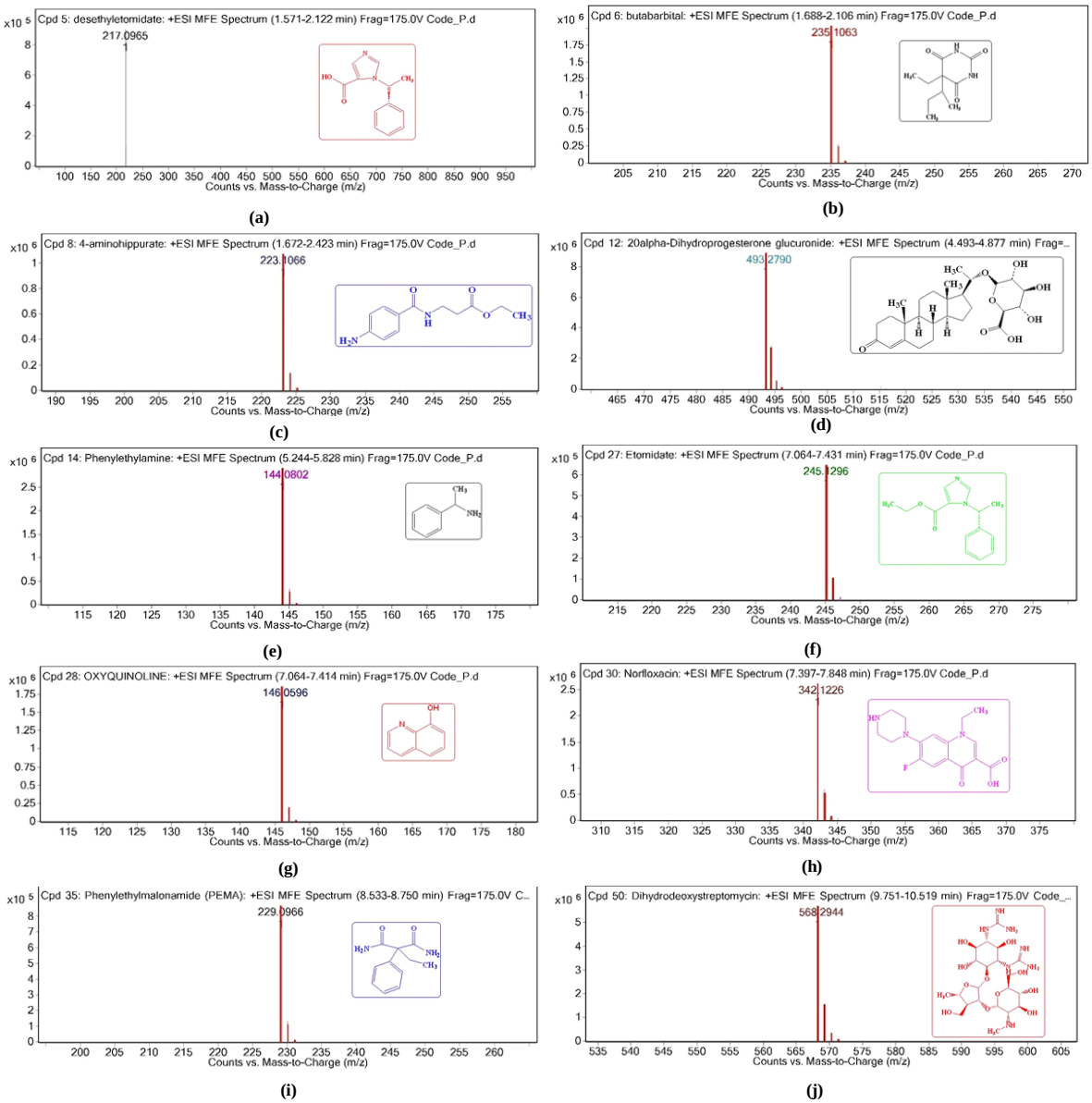

Fig.2 ESI-Q-TOF-MS spectra of major abundant metabolites in *Abrus precatorius* L. seeds (a) Desethyletomidate,

(b) Butabarbital, (c) 4-aminohippurate, (d) 20α-Dihydroprogesterone glucuronide, (e) Phenethylamine, (f) Etomidate, (g) Oxyquinoline, (h) Norfloxacin, (i) Phenylethylmalonamide(PEMA), (J) Dihydro deoxy streptomycin

Conclusion:

According to our knowledge, this is the first study reporting comprehensive metabolites profiling of ethanolic seed extract of *Abrus precatorius* (L.) by using high resolution mass spectrometry method. Our study suggested that *Abrus precatorius* (L.) seeds area rich source of important metabolites contributing phytopharmacology activity i.e. phytochemical study, antioxidant and antimicrobial activity.

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Conflict of interest statement

The authors declare no conflict of interest.

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