Identification of metabolites and Evaluation of the seeds of *Abrus precatorius (L.)* by high resolution mass spectrometry

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Abstract

Natural products from medicinal plants, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug leads because of unmatched availability of chemical diversity. The present study is performed to investigate the presence of phytochemicals for using selected medicinal plant such as *Abrus precatorius* (L.) Soxhlet apparatus was used for the organic solvent extraction. *Abrus precatorius* (L.) an important medicinal plant is enriched with remarkable highly potent anti-inflammatory, antimicrobial, antioxidant, antifertility, antispermatogenic activity. Metabolites are identified by high resolution mass spectrometry (HR-LCMS). These metabolites are found in various drug compounds used for drug industry as well as in Ayurveda, folk, Homeopathy, Sidha and Unani.

Introduction

Many plants are used as folk medicines to infectious diseases such as urinary tract infections, diarrhea, cutaneous abscesses, bronchitis and parasitic diseases (Yasunaka et al., 2005; Bhuiyan, Chowdhury & Begum, 2009; Zakaria et. al., 2010, Yob et. al., 2011). Due to the indiscriminate use of antibacterial drugs, the micro-organisms have developed resistance to many commercial antibiotics. Therefore, investigation of the chemical compounds within medicinal plants has become desirable (Ahmad, Mehmood & Mohammad, 1998). Plants are the richest sources of secondary metabolites with varying biological activities (Kumar, Manickavasakam & Mohan, 2016). These secondary metabolites are the important source with a variety of structural arrangements and properties (Pavitra & Vadivukarasi, 2012).

Abrus precatorius (L.) (Fabaceae) is distributed throughout India, ascending to an altitude of about 1050 m in the outer Himalayas. It is called Indian Wild Liquorice, Jequirity, Crab's Eye and Precatory Bean in English. *Abrus precatorius* is a woody twinning plant with characteristic toxic red seeds with black mark at the base (Mensah, Bonsu & Fleischer, 2011; Gotge, 2000). Several groups of secondary compounds have been found in this species, including alkaloids, steroids and other triterpenoids, isoflavanoquinones, anthocyanins, starch, tannin, toxalbumin (Lin, Yang & Chou, 2003; Shahat et al, 2003; Reddy et al, 2003), these major active compounds give highly potent anti-inflammatory, antimicrobial, antioxidant, antifertility, antispermatogenic activity (Das, Jain & Mishra, 2016). Antioxidants are important food additives which prolong food storage due to their protective effects against oxidative degradation of foods by free radicals. Phenolic compounds are secondary metabolites of plants which constitute most common and widespread groups. Several thousand compounds are identified with a large range of structure: monomeric, dimeric and polymeric phenolics among which over 8150 are flavonoids (Datta & Lal, 2012). For analyzed of this metabolites are present in *Abrus precatorius* (L.) by using high resolution mass spectrometry techniques, for the first time we have reported.

Mass Spectrophotometry (MS) imaging is a label free technique and thus, can be used without prior knowledge of the analyte. Due to this untargeted nature, hundreds of compounds can be detected simultaneously. The important advantages for the detailed investigation of metabolites from complex plant tissues (Balmer, Flors, Glauser & Mauch, 2013; Lee, Perdian, Song, Yeung & Nikolau, 2012). LC/TOF

(time of flight)-MS analysis gives accurate mass measurements, high resolution and provides the elemental compositions of unknown peaks with more accuracy in complex matrices (Ferrer et al, 2005; Segura-Carretero et al, 2008).

However, in India *Abrus precatorius* (L.) most important plant, were mainly seeds are used in Ayurveda. Hence present study is undertaken to characterize such type of compounds from *Abrus precatorius* (L.) seeds. The objective of the current study was focused to investigate the Phytochemical analysis and identification of metabolites by ESI-Q-TOF-MS from *Abrus precatorius* (L.).

Experimental

Seeds collection and preparation

Abrus precatorius (L.) is woody twinning plant. Various type of seeds variety is available, on the basis of seed colour i.e. white, black and red seeds with black mark at the base. We used red seeds with black mark at the base used for nanalysis. (Mensah, Bonsu & Fleischer, 2011; Gotge, 2000).

The sample *Abrus precatorius* (L.) seed were purchased from Ayurvedic medical shop, Shahaganj market, Aurangabad, Maharashtra, India. The purchased seeds were thoroughly washed with distilled water and dried seeds at room temperature. Completely dried seeds were crushed into fine powder by grinder mixer.

Extraction

Fine dried powder 20 gm of the *Abrus precatorius* (L.) seeds powder were extracted with ethanol 300 ml using soxhlet apparatus for 08 hrs and soxhlet was evaporated to dryness at constant temperature. The extracts were filtered through Whatman filter paper (number 44). Resulting extracts were n-hexane, chloroform, ethyl acetate and methanol. The ethanol extract exhibited showed the highest activity, Hence ethanol solvent used for seed extraction. The extract was evaporated to dryness and the resulting weight of extract was 10 gm (50 % yield of product). (stick and brown color) store at 4^0C for further use.

Phytochemical screening

Abrus precatorius (L.) seeds extract was subjected to qualitative chemical examination for the presence of alkaloids, flavonoids, carbohydrates, triterpenoids, glycosides and tannins according to standard procedure (Rahman, Alam, Chowdhury, Tha & Hasan, 2011). Each test was qualitatively expressed as negative (-) not present or positive (+) present; the intensity of characteristic color was expressed as (++) or (+++).

Equipment, chemical and reagents

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 6550 i-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 ^{O}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.

Chemical were procured from different reputed companies mentioned in bracket, Ethanol, methanol, ethyl acetate, chloroform (Rankem) all chemicals used in this study were of analytical grade.

Result And Discussion

Phytochemical screening

Ten phytochemicals, namely, carbohydrates, alkaloids, steroids and sterols, glycosides, triterpenoids, flavonoids, tannin and phenolic compounds, proteins and amino acids, fixed oil and Anthraquinone were assayed in ethanolic extracts of seed of *Abrus precatorius* (L.). The phytochemicals were detected in seed of *Abrus precatorius* (L.) very rich in Triterpenoids, tannin and phenols but low in Carbohydrates and steroids (Table 1).

Identification of metabolites by ESI-Q-TOF-MS

Metabolites analysis by ESI-Q-TOF-MS revealed the presence of fatty acids, organic compounds, phenolics, alkaloids, amino pyrimidines, dipeptide and tripeptides like important metabolites (Table 2a and 2b). 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 (Fig. 2). The retention time, mass, molecular formula and the DB difference (ppm) of the major and minor metabolites are shown in (Table 2a and 2b). The chromatogram spectra showed counts versus retention time (Fig. 1).

Conclusions

According to our knowledge this investigation is the first study reporting comprehensive metabolites profiling of ethanolic seed extraction of *Abrus precatorius* (L.) by using high resolution mass spectrometry method. This method by highly sensitive and used for rapid identification of metabolites. The finding of this study suggested that *Abrus precatorius* (L.) seeds is s source of important metabolites

contributing phytopharmacology activity i.e. phytochemical study, antioxidant and antimicrobial activity. It may prove value addition in drug industry as well as in Ayurveda, folk, Homeopathy, Sidha and Unani. However, during our study in addition to navel metabolites. Unexpectedly, medicinal compound was detected and they are used for the treatment of various diseases.

Declarations

Acknowledgements

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Conflicts of interest

The authors declare no conflict of interest.

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Tables

Tables 1 and 2 are available in the Supplementary Files.

Figures

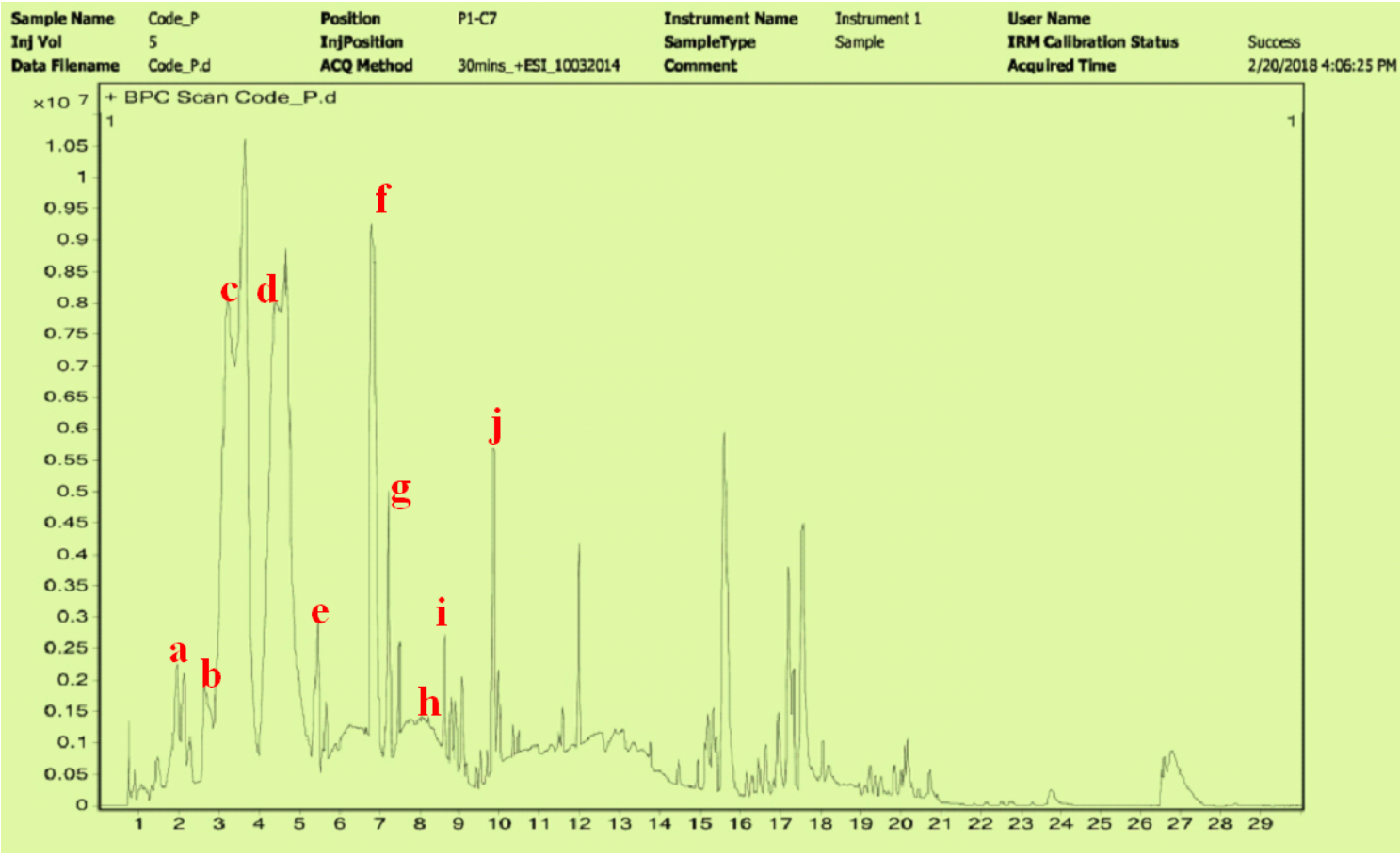

Figure 1

counts versus retention time (RT) ratio

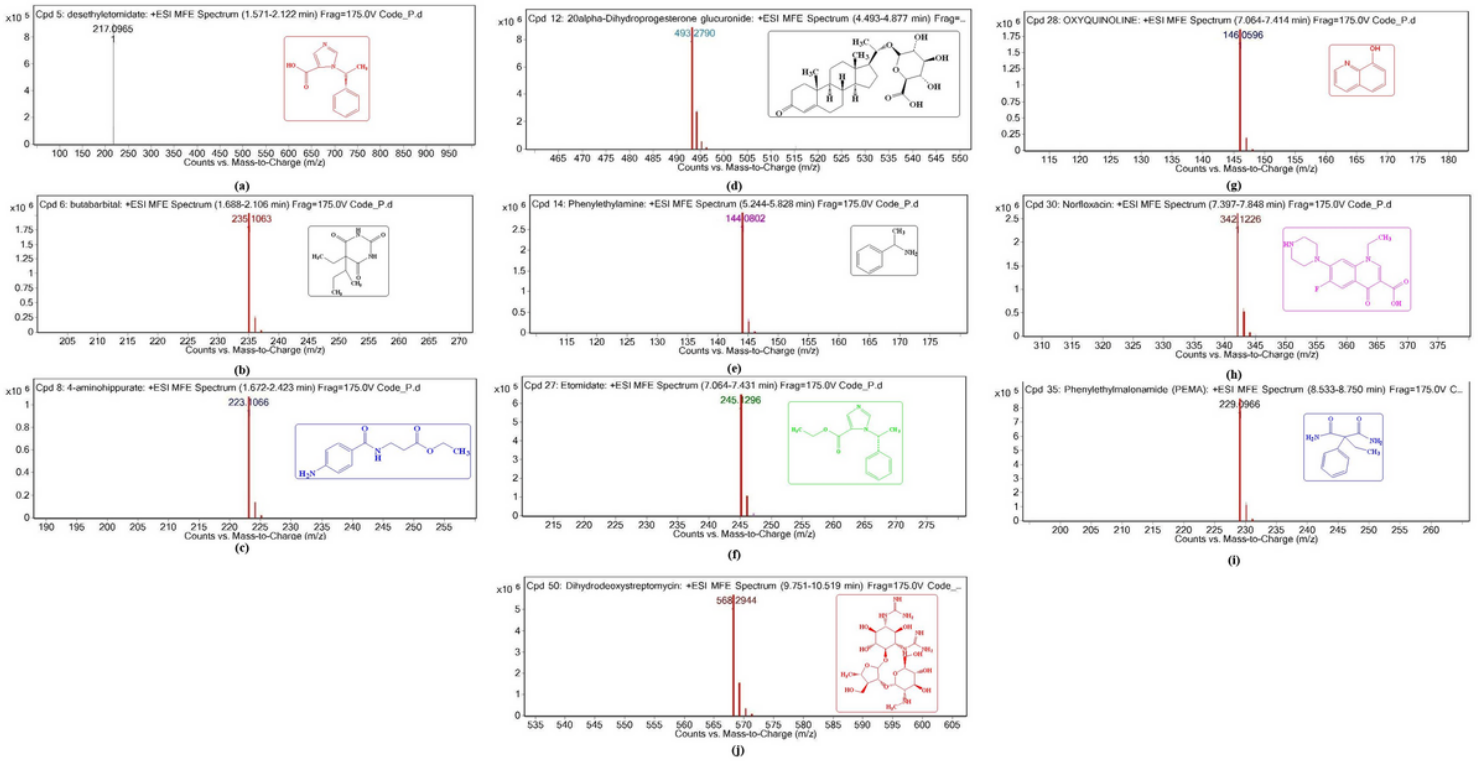

Figure 2

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) Dihydrostreptomycin (a) Desethyletomidate (marketed as Amidate) is a short-acting intravenous anesthetic agent used for the induction of general anesthesia and sedation (Vison & Bradbury, 2002) 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 (Bergen & Smith, 1998). (Pubchem CID: 36339). (b) Butabarbital (trade name Butisol) is a prescription barbiturate sleep aid. Butabarbital has a particularly fast onset of effects and short duration of action compared to other barbiturates, which makes it useful for certain applications such as treating severe insomnia and relieving anxiety before surgical procedures.(Pubchem CID: 2479). (c) Aminohippuric acid or para-aminohippuric acid (PAH), a derivative of hippuric acid, is a diagnostic agent useful in medical tests involving the kidney used in the measurement of renal plasma flow. It is an amide derivative of the amino acid glycine and para-aminobenzoic acid that is not naturally found in humans; it needs to be IV in fused before use diagnostically. PAH is useful for the measurement of renal plasma flow (Costanzo Linda, 2007). (Pubchem CID: 2148). (d) 20α-Dihydroprogesterone (20α-DHP), is a naturally occurring, endogenous progestogen (Beranic, Gobec & Rizner, 2011; Tony, Anthony, Knobil & Neill's, 2014; Marianne, 2009). It is a metabolite of progesterone, converted by the 20α-hydroxysteroid dehydrogenases AKR1C1 and AKR1C3, and although still active as a progestogen (Pasqualini & Chetrite, 2008), it has found to act as an aromatase inhibitor and to inhibit the production of estrogen in breast tissue in vitro. (Pubchem CID: 8956). (e) Phenethylamine (PEA), also known as β-

phenylethylamine (β-PEA) and 2-phenylethan-1-amine, is an organic compound, natural monoamine alkaloid, and trace amine which acts as a central nervous system stimulant in humans. Phenylethylamine functions as a monoaminergic neuromodulator and, to a lesser extent, a neurotransmitter in the human central nervous system (Sabelli et. al., 1976). (Pubchem CID: 1001). (f) Etomidate (marketed as Amidate) is a short-acting intravenous anesthetic agent used for the induction of general anesthesia and sedation (Vinson and Bradbury, 2002) for short procedures such as reduction of dislocated joints, tracheal intubation, andcardioversion. 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 (Bergen & Smith,1998). In emergency settings, etomidate is one of the most frequently used sedative hypnotic agents. It is used for conscious sedation (Di Liddo et. al, 2006; Miner, Danahy & Moch, 2007) and as a part of a rapid sequence induction to induce anesthesia (Sivilotti, Filbin, Murray, Slasor & Walls, 2003; Hohl et. al., 2010). It is used as an anesthetic agent since it has a rapid onset of action and a safe cardiovascular risk profile, and therefore is less likely to cause a significant drop in blood pressure than other induction agents (Zed, Abu-Laban & Harrison, 2006; Sokolove, Price & Okada, 2000). (Pubchem CID: 36339). (g) Oxyquinoline is an the complexes as well as the heterocycle itself exhibit antiseptic, disinfectant, and pesticide properties (Phillips, 1956), functioning as a transcription inhibitor. Its solution in alcohol is used in liquid bandages. It once was of interest as an anti-cancer drug (Shen, Wu & Chiu, 1999). (Pubchem CID: 1923). (h) Norfloxacin is a synthetic antibacterial agent (Nelson, Chiller, Powere & Angulo, 2007; Padeiskaia, 2003) that belongs to the class of fluoroquinolone antibiotics. It is used to treat urinary tract infections, gynecological infections, inflammation of the prostate gland, gonorrhea and bladder infection (Rafalsky, Andreeva, Rjabkova & Rafalsky, 2006). (Pubchem CID: 4539). (i) Phenylethylmalonamide (PEMA) is an active metabolite of the anticonvulsant drug primidone, although it is produced in a much lower concentration than phenobarbital, the other active metabolite (El-Masri, Portier, 1998) (Pubchem CID: 23611). (j) Dihydrostreptomycin is derivative of streptomycin that has a bactericidal property. It's a semi-synthetic aminoglycoside antibiotic used in the treatment of tuberculosis. It acts by irreversibly binding the S12 protein in the bacterial 30S ribosomal subunit, after being actively transported across the cell membrane, which interferes with the initiation complex between the mRNA and the bacterial ribosome. This leads to the synthesis of defective non-functional proteins, which results in the bacterial cell's death. (Pubchem CID: 439369).

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