

Original Research Article

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Alkaloids of the Ethnic Fruit Crop Khoonphal [*Haematocarpus validus* (Miers.) Bakh. f. ex Forman] from Andaman and Nicobar Islands

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ABSTRACT

Keywords

Haematocarpus validus, Khoonphal, HR LCMS, Alkaloids, Nonvolatile compounds.

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Haematocarpus validus (Miers.) Bakh. f. ex Forman is a woody perennial climber belonging to the family of Menispermaceae and found in the hot and humid tropics of Asian continent. It is a lesser known and rare ethnic fruit crop of high nutraceutical and traditional medicinal value with hepatoprotective and anti-inflammatory properties. There is no report on the secondary metabolites present in this plant and this is the first report on various alkaloids present in the leaf and fruit samples of this ethnomedicinal plant. In the present communication, we report six alkaloid compounds viz., Ecgonine (leaf); Sinomenine (leaf); Metanephrine (fruit), Reticuline (fruit), Ambelline (fruit), and choline (in both leaf and fruit) detected through HR LCMS analysis.

Introduction

The ‘moonseed family’ Menispermaceae consists of 75 genera and around 520 species (Watson and Dallwitz, 1992); many of the members are traditionally used for therapeutic purposes. The family contains a wide range of benzyloisoquinoline alkaloids and majority of these compounds are derived from the amino acid tyrosine. *Haematocarpus validus* is a member of the family Menispermaceae commonly known as ‘Blood fruit’ or ‘koonphal’ used in folklore medicine by ethnic groups of Meghalaya, Mizoram, Assam, Tripura, Andaman-Nicobar Islands (India), Bangladesh, Pakistan, Indonesia, Singapore and West Java. Fruits are rich in iron content and used for treating anaemic

conditions (Singh *et al.*, 2014; Rahim *et al.*, 2015) and for blood purification (Momin *et al.*, 2016). The tender shoots and leaves are used for curing hepatic disorders, root extract to relieve itching and pain (Bohra *et al.*, 2016). Even though this crop possesses immense nutritive and medicinal value, it occupies limited geographical locations and its potential uses are not yet explored. Furthermore, there are no scientific validation studies available to support the folklore knowledge on this underutilized fruit crop. In this context, it is necessary to unravel the metabolome of *H. validus* and the present investigation was designed to detect the non-volatile compounds present in the methanolic

fractions of the leaf and fruit extracts using HR LCMS analysis. This investigation provides information on alkaloid compounds of *H. validus* that support the traditional knowledge on the medicinal value of this plant.

Materials and Methods

Coarsely powdered leaf and fruit samples were extracted with methanol for the detection of compounds of non-volatile nature using HR LCMS facility provided by SAIF, IIT Bombay. HR LCMS was performed using 1290 Infinity UHPLC System of Agilent Technologies, USA (1260 infinity Nano HPLC with Chipcube, 6550 iFunnel Q-TOFs). The metabolites present in the methanolic extract were identified by comparing their retention indices (RI) and mass spectra fragmentation pattern with those on the stored library available in IIT, Bombay.

Results and Discussion

The present phytochemical analysis with the methanolic extract of leaf and fruit samples of *Haematocarpus validus* using HR LCMS detected three true heterocyclic isoquinoline alkaloids viz., sinomenine, (S)-reticuline, ambelline, one nonheterocyclic protoalkaloid metanephrin, one tropane group alkaloid ecgonine and one quaternary ammonium compound choline. The heterocyclic isoquinolin alkaloids viz., sinomenine, (S)-reticuline, and ambelline are derived from tyrosine. The protoalkaloid metanephrin is also the part of tyrosine metabolism. Out of the six compounds detected, choline was found in both leaf and fruit samples while ecgonine and sinomenine were detected in leaves; metanephrine, (S)-reticuline and ambelline were obtained from fruits (Table 1). The retention times (RT) and m/z values recorded were as follows: choline (leaf- RT

4.544, m/z 104.1068; fruit- RT 4.557, m/z 104.1063), sinomenine (RT 13.822; m/z 330.1685), (S)-reticuline (RT 15.993, m/z 330.1684), ambelline (RT14.089; m/z 314.1375), ecgonine (RT12.285; m/z168.1016). The fragmentation patterns of these compounds are presented in Figures 1-6. Details of these compounds are reported in the popular chemical databases of KEGG and PubChem. The KEGG id and Pubchem SID of the detected compounds are C00114, 3414 (choline); C09643, 11833 (sinomenine); C02105, 5187 ((S)-reticuline); C08517, 10710 (Ambelline); C10858, 13041 (ecgonine); C05588, 7913 (L Metanephrine) respectively.

The family Menispermaceae is rich in benzyloisoquinoline alkaloids and many of them are used as medicinal drugs. The central intermediate in biosynthesis of these alkaloids are (S)-reticuline (Springob and Kutchan, 2009) and it was previously reported in many plants of Menispermaceae family (Dasgupta *et al.*, 1979; Abbasoglu *et al.*, 1991; Likhitwitayawuid *et al.*, 1993; Nawai *et al.*, 1999). (S)-reticuline is used in the preparation of drugs against cancer and malaria (Minami *et al.*, 2008) and it also possesses cardiovascular effect (Medeiros *et al.*, 2009).

Sinomenine (cocculin), an anti-rheumatic alkaloid reported earlier from *Sinomenium acutum* (Zhao *et al.*, 2012) belonging to the same family, is detected in the leaf of *H. validus*. Ambelline is found generally in Amaryllidaceae plants that possess antimicrobial, anti-inflammatory, anti-cancer and antioxidant properties (Nair *et al.*, 2012). Ecgonine is a tropane alkaloid and used as a mild vasodilator and topical anesthetic. Metanephrine is a protoalkaloid with rare occurrence in nature and assumed to have sympathomimetic properties. Previously Keller *et al.*, (1973) isolated this compound from the cactus, *Coryphantha macromeris*.

Table.1 Heterocyclic, non-heterocyclic and pseudoalkaloid compounds detected in the leaf and fruit samples of *H. validus*

Alkaloids	Formula	Class of compound	Found in	Biological significance
Choline	C ₅ H ₁₄ NO	Quarternary ammonium compound	L, F	Hepatoprotective activity (Zeisel and da Costa, 2009; Idrus <i>et al.</i> , 2017)
Ecgonine	C ₉ H ₁₅ NO ₃	Tropane alkaloid	L	Mild vasodilator (Hoffman <i>et al.</i> , 2004)
Sinomenine	C ₁₉ H ₂₃ NO ₄	Isoquinoline alkaloid	L	Anti-inflammatory; anti rheumatic alkaloid (Zhao <i>et al.</i> , 2012)
Metanephrine	C ₁₀ H ₁₅ NO ₃	Protoalkaloid,	F	Sympathomimetic properties (Keller <i>et al.</i> , 1973)
(S)-Reticuline	C ₁₉ H ₂₃ NO ₄	Isoquinoline alkaloid	F	Cardiovascular effect (Medeiros <i>et al.</i> , 2009).
Ambelline	C ₁₈ H ₂₁ NO ₅	Isoquinoline alkaloid	F	Antimicrobial, anti-inflammatory, anticancer and antioxidant properties (Nair <i>et al.</i> , 2012)

L – Leaf sample; F – Fruit sample

Fig.1 Choline from fruit sample

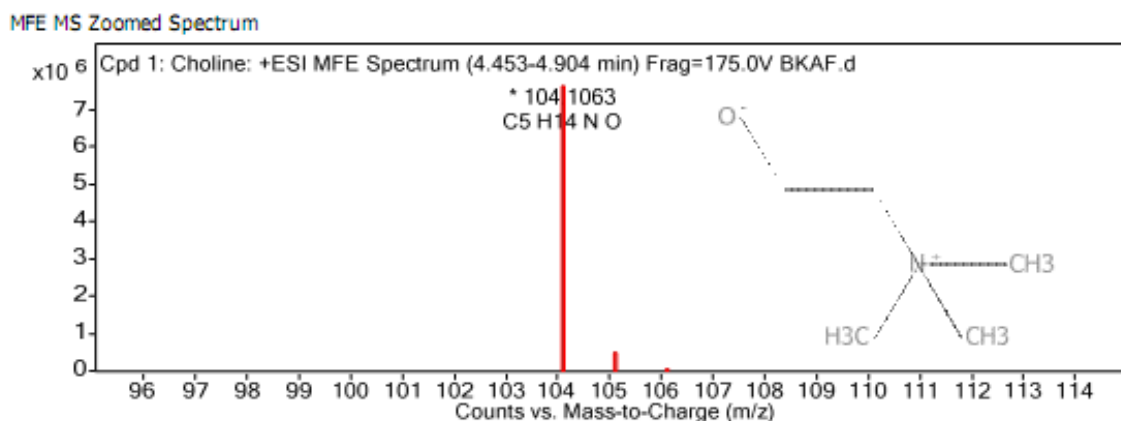


Fig.2 Ecgonine from leaf sample

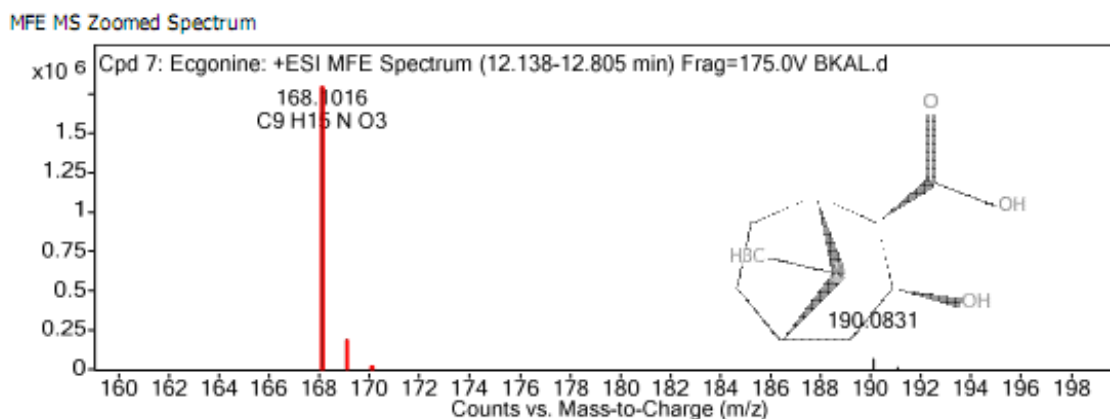


Fig.3 Sinomenine from leaf sample

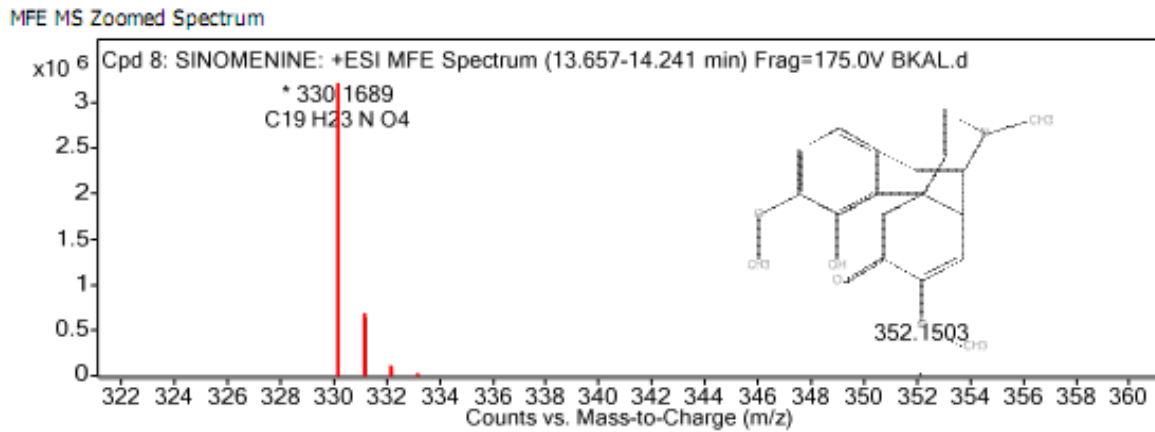


Fig.4 Metanephrine from fruit sample

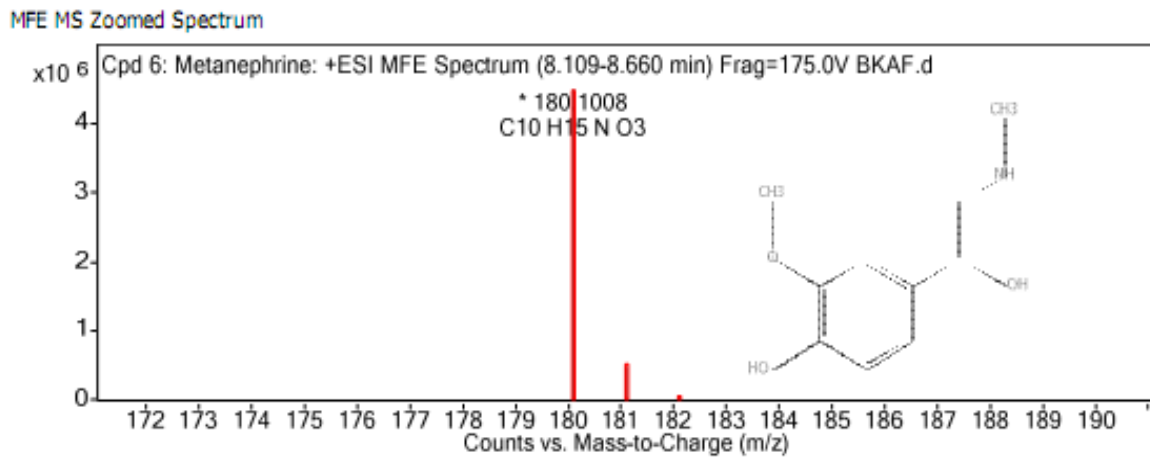


Fig.5 (S)-Reticuline from fruit sample

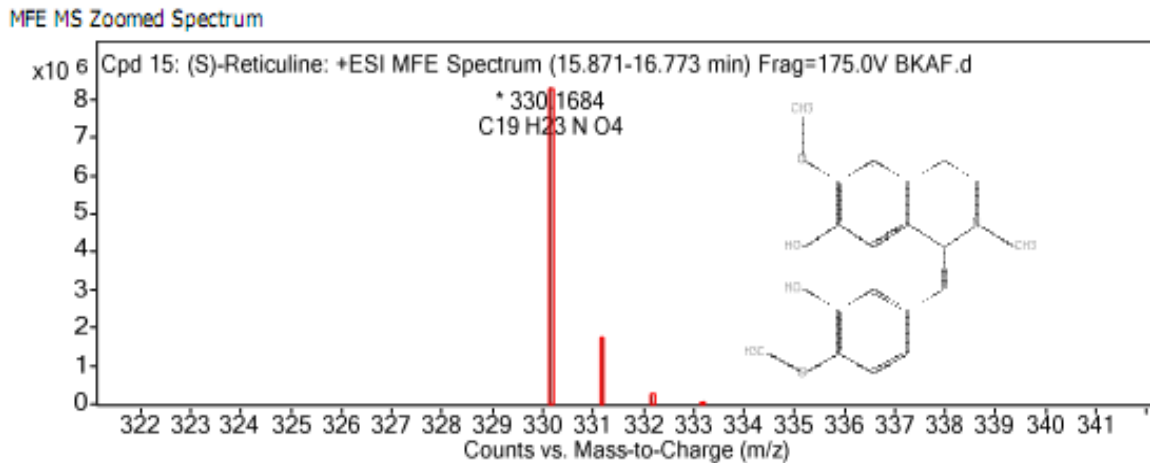
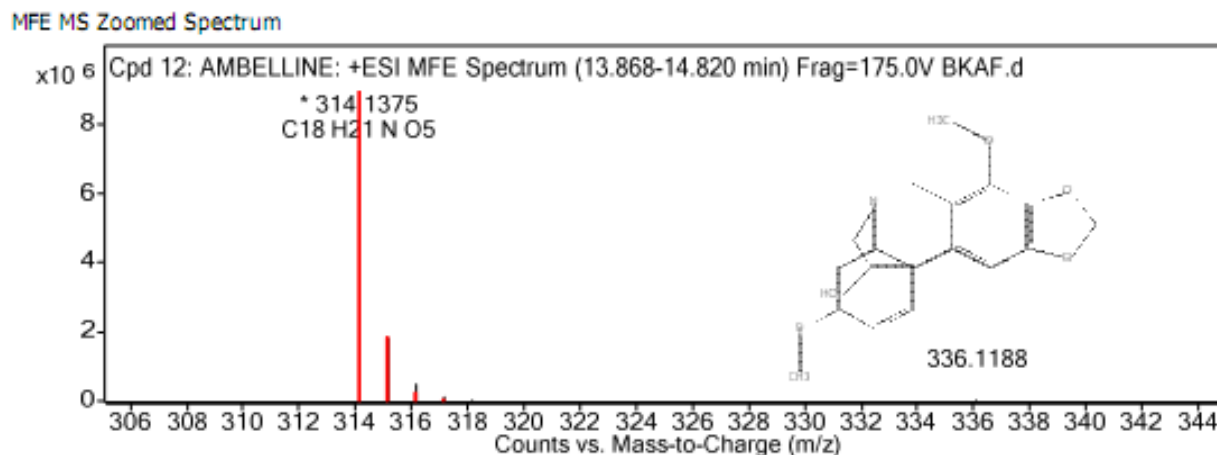


Fig.6 Ambelline from fruit sample



Choline is a quaternary ammonium compound with nutritional importance and used for liver health (Zeisel, 1992; Zeisel and da Costa, 2009; Idrus *et al.*, 2017) and it was reported previously from Menispermaceae plants including *Tinospora cordifolia*.

In conclusion, the current HR LCMS analysis of the lesser known medicinally important fruit crop *Haematocarpus validus* revealed the presence of six alkaloid compounds in the methanolic fractions of leaf and fruit extracts.

The detected alkaloid compounds include Sinomenine, (S)-Reticuline, Ambelline, Metanephrine, Ecgonine and Choline show biological significance in terms of hepatoprotective, anti-inflammatory, anti-rheumatic, cardiovascular, antimicrobial, anticancer and antioxidant properties.

The therapeutic potential of these alkaloid compounds support the folklore knowledge regarding this ethnomedicinal plant. Considering the enormous medicinal prospects as well as its rare and vulnerable status, this crop needs conservation, domestication and propagation in the humid and hot tropics of Asian continent for its better utilization.

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