Gas Chromatography-Mass Spectrometry (GC-MS) analysis of alkaloids isolated from *Epipremnum aureum* (Linden and Andre) Bunting

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Abstract

Recent advances in the use of GC coupled to MS have allowed a chemically guided isolation of uncommon and bioactive alkaloids. The present study was aimed to focus on the extraction and screening of alkaloids from *Epipremnum aureum* (Linden and Andre) Bunting. It has been observed that the plant is very rich in alkaloids and the modified method employed for the extraction of alkaloid is efficient and selective, where the interference of other secondary metabolites is negligible. The identification of each compound was made through gas chromatography-mass spectrometry (GC-MS). A total of twenty six structurally different alkaloids were identified for the first time from this plant. *E. aureum* is highly rich in alkaloids and twenty six different alkaloids were characterized. The present study may help in the field of natural products' chemistry and pharmaceuticals as well as drug discovery science and technology.

Keywords: Araceae, alkaloids, isolation, gas chromatography-mass spectrometry, *Epipremnum aureum*

Introduction

Epipremnum aureum (Linden and Andre) Bunting belongs to the family Araceae, commonly known as Pothos. It is widely distributed in Malaysia and Solomon Islands [1]. The plants possess many pharmacological activities such as antibacterial, antifungal, calming effect and relaxation [2,3]. It is capable of removing indoor air pollutants. The phytochemical studies report that the plant contains flavonoids, alkaloids, polyphenols, saponins and steroids [4].

Plants produce various secondary metabolites of which alkaloids function as a very important class of defense compounds. They usually have pharmacological effects and are used in medicines or as recreational drugs. Alkaloids are very useful pharmaceutical agents because of their biological activities [5,6] such as antimicrobial [7], antioxidant [8], analgesic potential and anti-inflammatory activities [9].

E. aureum also possesses same activities as those of alkaloids. Consequently, it is quite reasonable to attribute that those biological activities are due to the presence of alkaloids. Moreover, due to its usage as a raw plant there may be some toxic substances mostly present in plants for their defense [10], which may be harmful to human health. Because, generally beside other bioactive constituents; few alkaloids are toxic to health [11-13]; therefore, there must be a proper profile for the alkaloids that are present in this plant. Previously, no work has been reported as a systematic study regarding the alkaloid profile of *E. aureum* except preliminary confirmation of alkaloids via phytochemical studies [4]. Thus, in the present study it was aimed to focus on the screening of alkaloids in *E. aureum* plant among the active constituents using gas chromatography-mass spectrometry.

Methods

Chemicals

In this study, ethyl acetate, petroleum ether, diethyl ether, chloroform (analytical grade) and methanol (HPLC grade) were purchased from Merck chemicals. Ammonium hydroxide, sulphuric acid and ammonium sulfate from Sigma-Aldrich were used.

Plant material

Plant material was collected in July 2014 from Jaipur city of Rajasthan, India. The plant species was identified and authenticated by the Botanical Survey of India, Jodhpur, India, as *Epipremnum aureum* (Linden and Andre) Bunting. Collected plant material was washed, air dried and used for further studies.

Isolation of alkaloid

A powdered plant material (50 g) was treated with 15 ml of NH_4OH (25%) at room temperature and solvent extraction was performed with 300 ml of ethyl acetate for 72 h. The extract was filtered and air dried at 40°C. The residue was dissolved in H_2O and acidified with H_2SO_4 to pH 3-4. It was extracted with petroleum ether and diethyl ether and adjusted the pH of aqueous phase to 9-10 with NH_4OH (25%). It was extracted with chloroform and washed with distilled water. Crude alkaloids was concentrated to dryness under reduced pressure and dried over sodium sulfate [14]. The residue obtained were dissolved in methanol and subjected to GC-MS analysis.

Characterization using Gas Chromatography- Mass Spectrometry

Characterization of alkaloids was done by Gas Chromatography- Mass Spectrometry. GC-MS were recorded in a GCMS-2010 Shimadzu instrument operating in EI mode at 70ev. A Restek-5MS column (30m x 0.25mm x 0.25µm) was used. The oven temperature program was 100^0 to 250^0C at 5^0C min^{-1} and held for 5 min at 250^0C and from 250^0C to 280^0C at 10^0C min^{-1} and held for 10 min at 280^0C. The injector temperature was 250^0C with normal injection mode. The flow rate of carrier gas helium was 1.21ml min^{-1}. The identification of alkaloids was confirmed by comparing the mass spectral data with those of authentic compounds and with data obtained from the literature.

Results and discussion

In our study alkaloids extraction was carried out and it is the first time that a systematic analysis of alkaloids present in the leaves of *Epipremnum aureum* is reported. Most of the alkaloids were extracted with minor quantity of other constituents by modified extraction method [14]. Further identification of alkaloids was made by GC-MS without any derivatization (Figure 1). Total of twenty six alkaloids were identified of which fifteen compounds namely **(1)** 2-Pyrazolin-5-one,3,4,4-Trimethyl-5-pyrazolone, **(2)** Methyl 5-Hydroxy-1-(4-Methylbenzoyl)-5Phenyl, **(3)** 4,5-Dihydro-1H-Pyrazole-3-Carboxylate, **(4)** 5,6-methylenedioxy-Isoquinoline, **(5)** 5-Oxo-pyrrolidine-2-carboxylic acid methyl ester, **(6)** 2- Methoxy-5-methylphenol, **(7)** 3-(1-Methyl-2-pyrrolidinyl)-2-pyridinylamine, **(8)** 4-Methoxypyrrolo[2,3-d]pyrimidine, **(9)** 2-Methyl-5H-pyrrolo[3,4-b]pyridine-5,7(6H)-dione, **(10)** 6-Ethoxy-9H-purine, **(11)** N-Phenylaniline, **(12)** Viridiflorol, **(13)** Spiro[2,3-Dihydro-1-Methylindol-2-one-3,3'-[2-(4-Methoxyphenyl)]Pyrrolidine], **(14)** 3,4Dichloro[1,6]Naphthyridine, **(15)** 6-(Dimethylnitroryl)-4,4-diphenyl-3-heptanone (C21H27NO2) were characterized on the basis of their fragmentation pattern, m/z value, retention time and basic alkaloid structure [10, 15-19] (Table no 1). Eleven alkaloids namely **(16)** Nicotine, **(17)** Nornicotine, **(18)** Anabasine, **(19)** Catharanthine, **(20)** Salinorin A, **(21)** Salinorin B, **(22)** Salinorin C, **(23)** Octadecanamide, **(24)** Dihydro-oxo-demethoxy haemanthamine, **(25)** Oxoassoanine, **(26)** Crinane-3e-ol were characterized on the basis of similar fragmentation pattern with standard alkaloids (Table 2) [19-22]. This unique behavior of possessing various types of alkaloids present in this ornamental plant pothos makes it more valuable in the field of medical chemistry. As it has been already confirmed that the extract of *E. aureum* leaves is rich in alkaloids has antitermite potentiality [2, 23]. Similarly, presence of nicotine and anabasine in this plant makes it suitable for the isolation of insecticidal compounds [24].

Conclusion

It could be concluded that *E. aureum* is highly rich in alkaloids and diversity in their structures was observed. All twenty six alkaloids are reported for the first time from this plant. The chromatographic method used in this study is reproducible and efficient to separate all alkaloids in a single run and well resolved peaks are observed. The extraction method used in this study is efficient to extract alkaloids with minor quantity of other compounds.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

AM carried out the experiments and analysis, NS participated in interpretation of results, drafted the manuscript and also helped in the experiments and analysis. AK carried out the characterization of compounds. All authors have read and approved the final manuscript.

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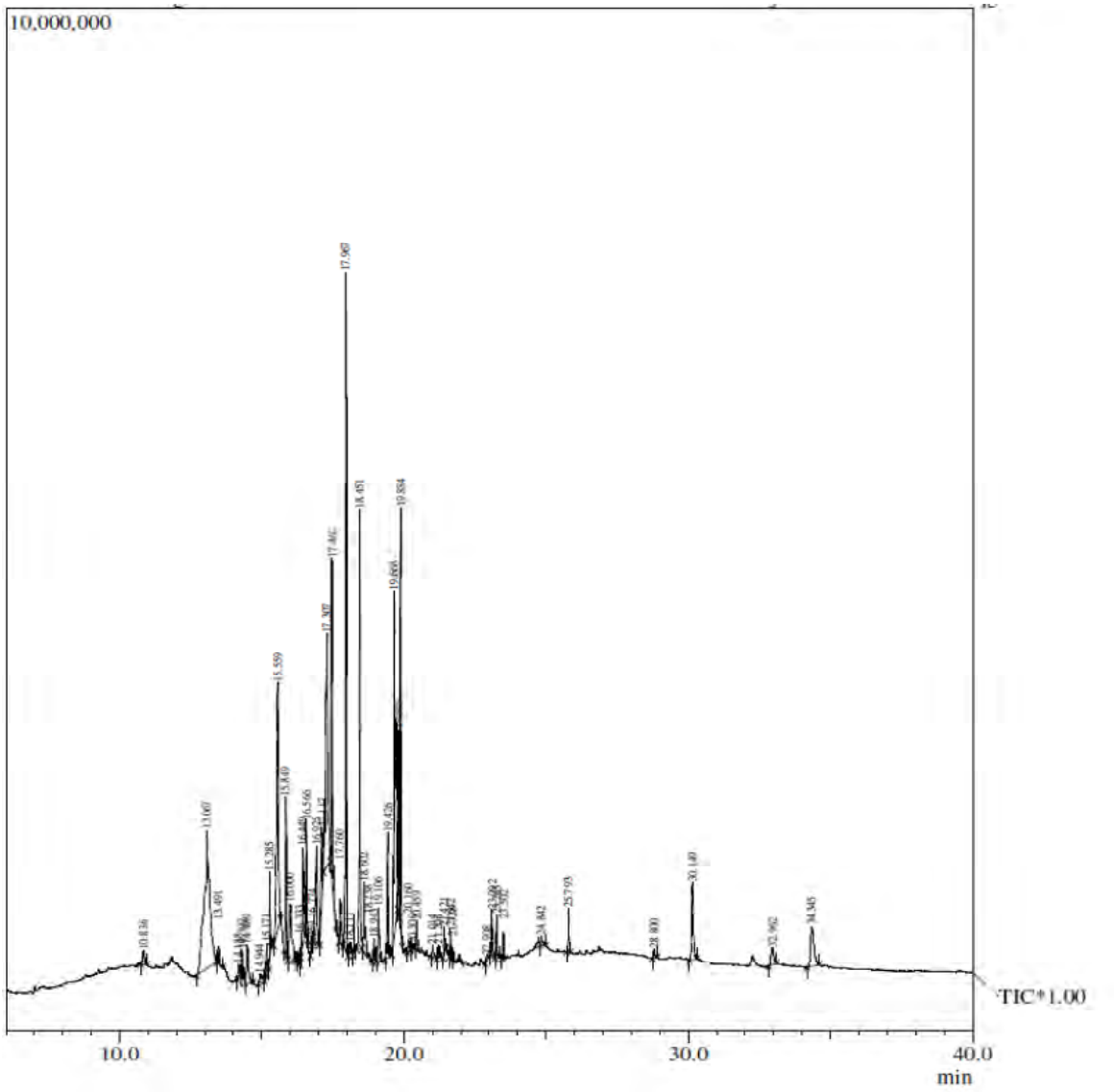
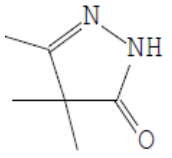
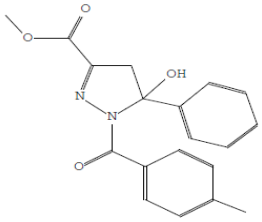
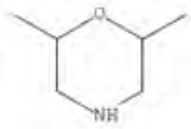
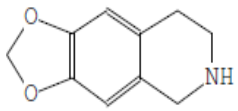
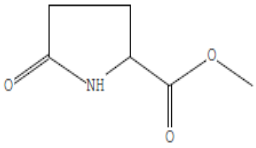
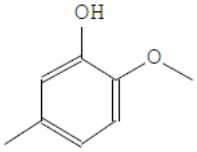
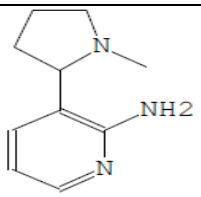
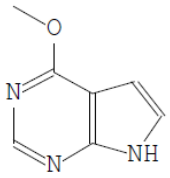
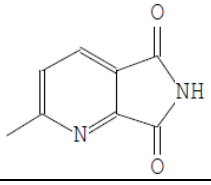
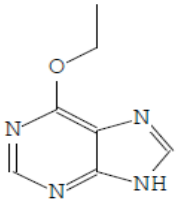
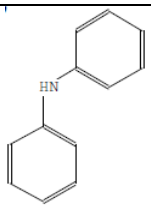
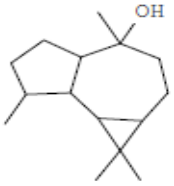
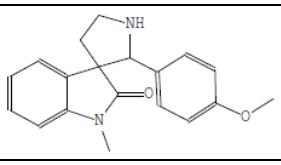


Fig. 1 Gas chromatogram comprised of total alkaloids identified from the vegetative shoot of *Epipremnum aureum.*

Table 1 Alkaloids of *Epipremnum aureum* characterized through GC-MS with their retention time and structure

S. No.	Compound	Retention time (min)	Structure
1.	2-Pyrazolin-5-one,3,4,4-Trimethyl-5-pyrazolone	6.892	
2.	Methyl 5-Hydroxy-1-(4-Methylbenzoyl)-5Phenyl, 4,5-Dihydro-1H-Pyrazole-3-Carboxylate	8.817	
3.	2,6-Dimethylmorpholine	11.225	

4.	5,6-methylenedioxy-Isoquinoline	12.050	
5.	5-Oxo-pyrrolidine-2-carboxylic acid methyl ester	12.1	
6.	2- Methoxy-5-methylphenol	12.192	
7.	3-(1-Methyl-2-pyrrolidinyl)-2-pyridinylamine	13.225	
8.	4-Methoxypyrrolo[2,3-d]pyrimidine	13.583	
9.	2-Methyl-5H-pyrrolo[3,4-b]pyridine-5,7(6H)-dione	14.333	
10.	6-Ethoxy-9H-purine	14.525	
11.	N-Phenylaniline	14.942	
12.	Viridiflorol	15.575	
13.	Spiro[2,3-Dihydro-1-Methylindol-2-one-3,3'-[2-(4-Methoxyphenyl)]Pyrrolidine]	22.483	

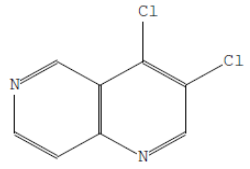
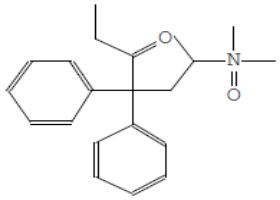
14.	3,4Dichloro[1,6]Naphthyridine	24.425	
15.	6-(Dimethylnitroryl)-4,4-diphenyl-3-heptanone (C21H27NO2)	43.43	

Table 2 Characterization of alkaloids of *Epipremnum aureum* on the basis of their mass fragmentation pattern

S. No.	Compound	Standard m/z (a)	Obtained m/z (b)	Approx. Ratio of b/a (Exact ratio)	% similarity with respect to standard (exact)
16	Nicotine	84, 78	84, 78	2/2	100 %
17	Nornicotine	148, 119, 78, 70, 40, 29	148, 119, 78, 40, 29	5/6	83.33%
18	Anabasine	162, 84, 78	162, 84, 78	3/3	100%
19	Catharanthine	337, 173, 144	337, 173, 144	3/3	100%
20	Salinorin A	433, 373, 355, 341, 337, 323, 313, 309, 295, 285, 267	433, 377, 323, 309, 285, 271	6/11 (4/11)	54.54% (36.36%)
21	Salinorin B	391, 373, 355, 341, 337, 323, 313, 309, 295, 285, 267	391, 377, 323, 309, 285, 271	6/11 (4/11)	54.54% (36.36%)
22	Salinorin C	475, 457, 415, 355, 337, 323, 261	475, 458, 414, 355, 341, 327, 265	7/7 (2/7)	100% (28.57%)
23	Octadecanamide	281, 126, 112, 98, 72, 59	281, 123, 112, 98, 71, 59	6/6 (4/6)	100% (66.66%)
24	Dihydro-oxo-demethoxy haemanthamine	271, 243, 214, 186, 115	271, 243, 213, 189, 113	5/5 (2/5)	100% (40%)
25	Oxoassoanine	281, 266, 250, 238	281, 267, 249, 237	4/4 (1/4)	100% (25%)
26	Crinane-3e-ol	273, 256, 229, 201, 185, 115	273, 255, 231, 199, 185, 115	6/6 (3/6)	100% (50%)