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Identification of Atropine and Scopolamine from *Datura* L. Plant and Market Samples by GC-MS

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Datura plant which generally grows as weed, is also used for medicinal purposes. The plant is narcotic and used to treat skin diseases and rheumatic pain. Atropine and scopolamine are the main alkaloids in the plant. Atropine is antimuscuranic whereas, scopolamine affects central nervous system. In the present study, the presence of atropine and scopolamine were verified in leaves and seeds of *Datura stramonium* and *D. metel*. *Argemone mexicana* which is another weed is also generally identified as 'Datturi' and a popular chewing product called 'pan masala' were also subjected to GC-MS analysis. Hexane extracts did not show the presence of atropine and scopolamine. However, ethyl acetate and methanol extracts of both species of *Datura* elucidated the presence of alkaloids. *Argemone mexicana* and 'Pan masala' did not show the presence of atropine and scopolamine. This confirms that *Argemone mexicana*, is not an alternate to *Datura* and commercial 'Pan masala' does not contains the alkaloids.

Keywords: *Datura*, *Argemone mexicana*, Pan masala, Atropine, Scopolamine, GC-MS.

INTRODUCTION

Plants are used for different purposes like food, fodder and medicine. Medicinal plants are collected from forests, open fields and road side also. Till today many people use different plants for various disease treatments. Though *Datura* plant is considered as weed, it is collected from open fields and road side for trade [1]. *Datura* plants are distributed from tropical to temperate zones throughout the world. *Datura stramonium* and *D. metel* grow up to 1m, divaricate branches, produce bell shaped solitary flowers. Fruits of *Datura metel* and *D. innoxia* are globose spiny and dehisces irregularly but *D. stramonium* and *D. ferox* are ovate and dehisce with four regular valves [2]. In certain markets, small black seeds are sold in the name of *Datura* which could be either from *D. ferox* or *D. stramonium* or mixture. Even *Argemone mexicana,* which is also identified as 'Datturi' plant in some parts of Karnataka. 'Datturi' or 'Dathura' is supplied to 'pan' manufacturing companies [3]. Traditionally leaf juice is applied on wounds by healers in Nepal. Crushed plant juice is used to treat rheumatic joints. Seed and leaf extracts are used to treat skin diseases [4]. The plant has narcotic, acrid, antispasmodic, intoxicant and emetic properties. The alkaloid, nicotine content is responsible for addiction in human beings [5].

Some of the biologically important active compounds in *Datura stramonium* are the alkaloids atropine and scopolamine. Atropine has been used in treating Parkinson's disease, peptic ulcers and bronchial asthma [6]. Atropine is an anti-muscarinic agent, which interrupts few sympathetic parasympathetic innervations. It blocks both central and peripheral muscarinic acetylcholine receptors and its action lasts approximately 4 h. Scopolamine is used to prevent motion sickness and can be absorbed transdermally. Adverse effects include blurred vision, anxiety and insomnia. It is central nervous system depressant and it has an elimination half-life of 8 h [7]. The death of Caucasian male was confirmed, when atropine and scopolamine were found in the stomach and its contents, which contained *Datura* seeds [8].

By considering the above facts, it is realized that authentication of the sample is necessary to identify the presence of alkaloid content. GC-MS is a powerful technique for the separation and identification of complex mixture of tropane alkaloids and other compounds which are not detected by other methods [9]. Twenty nine tropane alkaloids were identified in roots, leaves and seeds of *D. stramonium* [10]. The characteristic alkaloids of *D. ceraticaula* are tropanol esters of a range of acids. Hyoscyamine was the main alkaloid found in plant organs [11].

Diploid and tetraploid hairy root cultures of *D. stramonium* showed significant differences in alkaloid spectrum and accumulation. Except hyoscyamine and atropine, the percentage

contributions of alkaloids, were higher in the total alkaloid mixture of tetraploid hairy roots [11]. Screening of *Datura metel* flowers for bioactive components showed highest percentage of *cis*-2-nitro-4-t-butylcyclohexane [12]. Eight alkaloid compounds were identified in ethanolic extract of *D. stramonium* [13]. Nine new tropane alkaloids were identified in *D. Stramonium* by GC-MS by El Bazaoui *et al.* [14]. By considering the above mentioned facts, plant samples of *Datura stramonium*, *D. metel*, *Argemone mexicana* and commercial sample of 'Pan masala' (Pan masala is a mixture of betle leaf, lime, areca nut,tobaco and other spices. Which acts as mouth freshner) were subjected to GC-MS analysis to identify the alkaloid content in the samples.

EXPERIMENTAL

Leaves and seeds of *Datura stramonium* and *Datura metel* were collected from natural populations found in MM Hills and Bengaluru, India. Materials were dried in shade and powdered with blender. A known weight of the sample powder was used for the sequential extraction with the solvents namely; Hexane, Ethyl Acetate and Methanol. Extracts were subjected to agitation using sonicator and magnetic stirror. Extracts were concentraed to 1 mL by using Vaccum Rotary Evoporator and samples were maintained at 4 °C until analysis was carried out.

Confirmatory tests were conducted to identify the presence of alkaloids. TLC plates were spotted with hexane, ethyl acetate and methanol extracts. When acid reagent was sprayed on plate, orange to red spots appeared. Similarly, when Dragendroff's reagent was sprayed, spots appeared on the plate. Methanol extract showed bright spots in all the samples. GC-MS measurements were performed after Philipov *et al*. [15] and Berkov [11].

The concentrated hexane, ethyl acetate and methanol solvents of *Datura stramonium* and *D. metel* leaves and seeds, *Argemone mexicana* seeds and also commercial product like Pan masala 'Super gem' were used to analyze phytochemicals. GC-MS analysis of the extracts was performed using, Model Agilent. The clolumn used for the anlysis is Agilent 19091S-433: HP5MS HP-5MS 5 % Phenyl Methyl Silox 325 °C: 30 m × 250 µm × 0.25 µm. The oven temperature programmed from 40 °C with an increase of 10 °C/min, to 300 °C/min. Total GC run time was 42.833 min. The interpretation of the mass spectrum was carried out using the National Institute Standards and Technology (NIST) Ver.14.

RESULTS AND DISCUSSION

The study on the active components of *Datura stramonium, Datura metel, Argemon mexicana* and Pan masala samples in hexane, ethyl acetate and methanol extracts revealed many major and minor peaks. GC-MS analysis showed the presence of many components like ethyl benzene, naphthalene, tetracosane, phytol, cyclopropane, eicosanoic acid, *etc*. Atropine and scopolamine were identified only in ethyl acetate and methanol extracts (Fig. 1-8). Alkaloid name with retention time (RT) and percentage are presented in Table-1. Atropine and scopolamine were found, only in seed and leaf extracts of *Datura stramonium* and *Datura metel* but not in *Argemon mexicana* and Pan masala samples.

Conclusion

The GC-MS analysis of leaf and seed samples indicates the presence of atropine and scopolamine. This clearly reveals

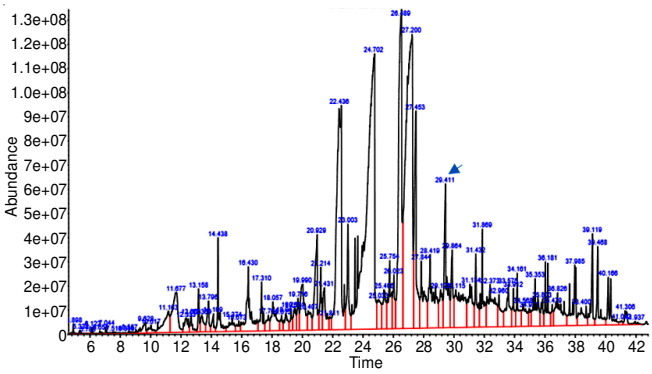

Fig. 1. Chromatogram of *D. stramonium* leaf sample indicating scopolamine

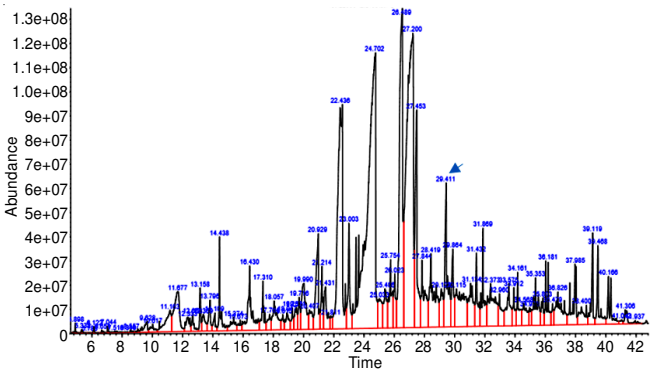

Fig. 2. Chromatogram of *D. stramonium* seed sample indicating scopolamine

TABLE-1
GAS CHROMATOGRAPHY-MASS SPECTROSCOPY ANALYSIS

Sample	Ethyle acetate extract			Methanol extract		
	Peak area (%)	RT	Alkaloid	Peak area (%)	RT	Alkaloid
D. stramonium (Leaf)	2.39	29.410	Scopolamine	1.13 2.43	27.904 29.24	Atropine Scopolamine
D. stramonium (Seed)	0.91	28.965	Atropine	0.52 3.86	21.888 29.139	Atropine Scopolamine
D. metel (Leaf)	6.34	29.491	Scopolamine	0.74 5.01	27.989 29.292	Atropine Scopolamine
D. metel (Seed)	2.16	28.998	Atropine	16.80 2.79	27.608 29.095	Atropine Scopolamine
Argemone mexicana (Seed)			–			–
Pan masala			–			–

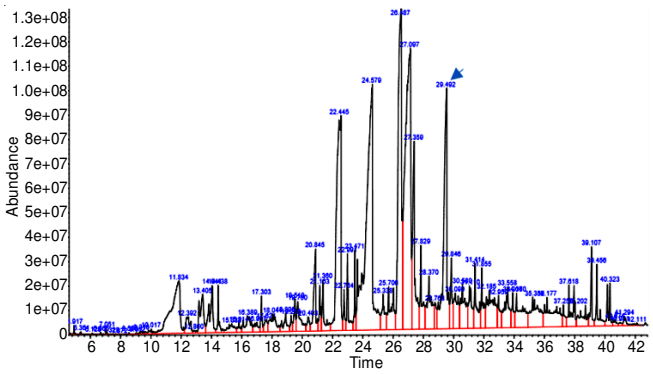

Fig. 3. Chromatogram of *D. metel* leaf sample indicating scopolamine

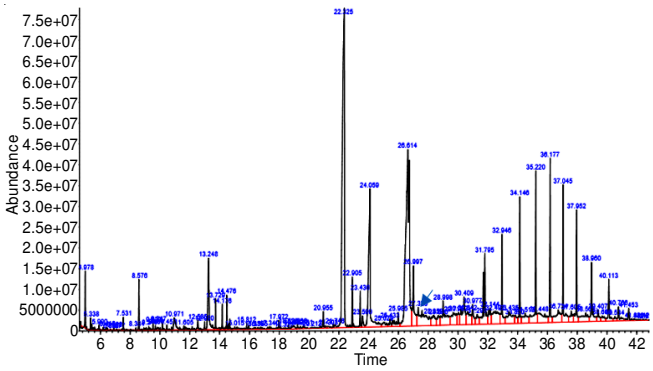

Fig. 4. Chromatogram of *D. metel* leaf sample indicating atropine and scopolamine

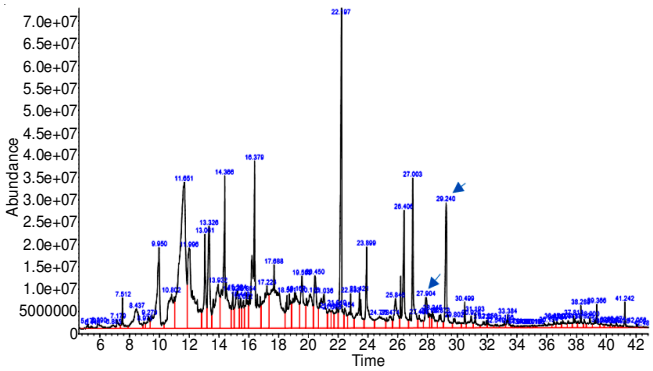

Fig. 5. Chromatogram of *D. stramonium* leaf sample indicating atropine and scopolamine

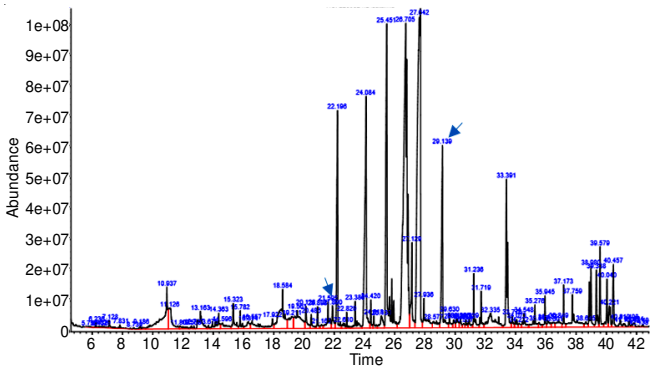

Fig. 6. Chromatogram of *D. stramonium* seed sample showing atropine and scopolamine

that though *Argemone mexicana* seeds resemble *D. stramonium* seeds which is called as 'Datturi', cannot be used as alternate to *Datura* in any case, if the therapeutic use is related to atropine or scopolamine. Similarly, it indicates that commercial product Pan masala does not contains the above said alkaloids.

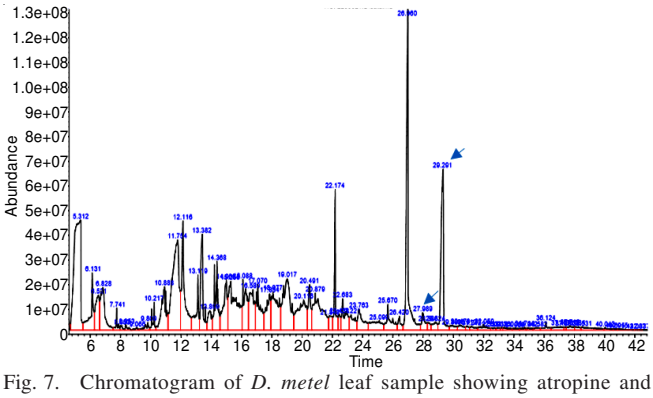

Fig. 7. Chromatogram of *D. metel* leaf sample showing atropine and scopolamine

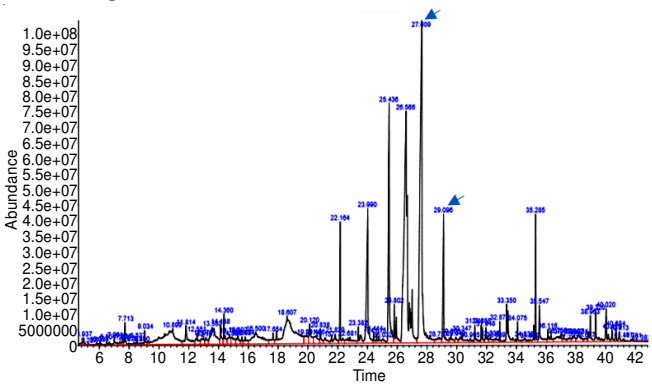

Fig. 8. Chromatogram of *D. metel* seed sample indicating atropine and scopolamine

The studies also indicated the presence of many other phytochemicals which are not under the purview of discussion in this paper. Hence further studies are needed to be worked out to identify and understand the application of other phytochemicals in drug delivery and pharmaceutical fields.

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