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Research Article

PHTYTOCHEMICAL AND GAS CHROMATOGRAPHY MASS SPECTROMETRY (GC-MS) ANALYSIS OF *Datura stramonium* L. FLOWERS METHANOL EXRTRACT

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Abstract

Natural products have been, and will continue to be a rich source of new drugs against many diseases like Ageing, Coronary heart disease, Alzheimer's disease, Neurodegenerative disorders, Atherosclerosis, Cataracts, cancer and Inflammation. The present study was aimed to examine the phytochemical constituents present in *Datura stramonium* L. flowers. The preliminary phytochemical constituents were qualitatively analyzed using the methanol. This analysis confirmed the presence of various secondary metabolites like alkaloids, flavonoids, glycosides, phenol, steroids, tannin and terpenoids. The Gas Chromatography Mass Spectrometry (GC-MS) analysis of *Datura stramonium* revealed the presence of eight bioactive compounds. The results of the present study will enhance the traditional usage of *Datura stramonium* flowers which possesses several known and unknown bioactive compounds.

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1. Introduction

Phytochemicals are a group of non-nutrient bioactive compounds naturally found in plant parts such as flowers, leaves, fruits, roots, barks, spices and medicinal plants. In humans, numerous phytochemicals have been found to be protective and preventive against many degenerative diseases such as in Ageing, Coronary heart disease, Alzheimer's disease, Neurodegenerative disorders, Atherosclerosis, Cataracts and Inflammation (Pacome *et al.,* 2014). Since ancient times, people have been exploring the nature particularly plants in search of new drugs. This has resulted in the use of large number of medicinal plants with curative properties to treat various diseases. Nearly, 80 % of the world's population relies on traditional medicines for primary health care, most of which involve the use of plant extracts. In India, almost 95 % of the prescriptions were plant based in the traditional systems of Unani, Ayurveda, Homeopathy and Siddha. The study of plants continues principally for the discovery of various novel pharmaceutically active secondary metabolites (Savithramma *et al.,* 2011) which has pharmacologically active substances showing antifungal, antibacterial and anticancer activities. The crude extracts and active pure compounds isolated from plants species used in herbal and traditional remedies. Now, it is essential to isolate, identify and characterize the new phytochemical components of medicinal plants for the treatment of different diseases (Hossain *et al.,* 2013).

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Datura stramonium belonging to family Solanaceae and it includes 85 genera and about 2,800 species in the world. There are approximately 25 different species of *Datura* throughout the world and they are often called as Jimson weed or 'Thornapple' (Rai *et al.*, 2013). Gas Chromatography Mass Spectroscopy (GC-MS), a hyphenated system which is a very compatible technique and the most commonly used technique for the identification and quantification purpose. The unknown organic compounds in a complex mixture can be determined by interpretation and also by matching the spectra with reference spectra (Thomas *et al.*, 2013). The present work was carried out to identify some of the phytochemical components present in the methanol extract of the flowers parts of *Datura stramonium* by GC-MS technique to ascertain the medicinal properties of the plant.

2. Materials and Methods

Collection of plant material

The flowers of *Datura stramonium* were collected from Tirupattur, Vellore district, Tamil Nadu, India (Figure – 1). The fresh samples were washed with tap water, rinsed with distilled water and blotted gently between the folds of the filter paper which was then chopped in small pieces and dried in the shade on laboratory. The samples were then ground to a powder using a blender. The powdered samples were stored in sealed plastic bags and kept inside the cupboards at room temperature.

Figure – 1: *Datura stramonium* flower

Preparation of methanol extract of *D. stramonium* flowers (MEDsF)

Twenty gram of powdered flowers of *D. stramonium* was extracted with 150 ml of methanol at 50 – 60 °C in a Soxhlet apparatus for 6 hours. Two different flower extracts were transferred to a petri plates. The obtained extracts were kept in refrigerator at 4 °C for further studies.

Preliminary phytochemical analysis of methanol extracts of *D. stramonium* flowers

The preliminary phytochemical analysis were carried out by the following the methods of Gibbs (1974); Odebiyi and Sofowora (1978); Trease and Evans (1985); Sofowara (1993); Siddiqui and Ali (1997); Evans (1997); Trease and Evans (2002); Wolfe and Liu (2003).

Test for Alkaloids

Small amount of solvent free extract was dissolved in diluted hydrochloric acid and 2 ml of this extract was mixed with 0.1ml of Mayer's reagent. Formation of white precipitate shows the presence of alkaloids (Evans, 1997).

Test for Flavonoids

A pinch of the extract was dissolved in 5 ml of distilled water. Then, 10 % of Sodium hydroxide was prepared and mixed with the extract. Formation of yellow colour which disappears by the addition of diluted hydrochloric acid shows the presence of flavonoids (Trease and Evans, 2002).

Test for Glycosides

Two ml of the sample was dissolved in 2 ml of chloroform and then 2 ml of acetic acid was added. The solution was cooled well in ice. Sulphuric acid was then added carefully. A colour change from violet to blue to green indicates the presence of a steroidal nucleus that is a glycone portion of glycoside (Trease and Evans, 1985).

Total Phenolic content

The mixture was prepared dissolving 0.1mg of sample in 1 ml of methanol. From the mixture, 20 µl was taken and 180 µl of water was added. Then, 0.5ml of Folin's phenol reagent, 0.5 ml of water and 1 ml of 7.5 % sodium carbonate was added to the mixture. Then, it was kept for 2

hours for incubation. Absorbance was read at 720 nm by spectrophotometer. Gallic acid was used as phenol standard and expressed as Gallic acid equivalent (Wolfe and Liu, 2003).

Test for Saponins

A pinch of the extract was dissolved in 1 ml of distilled water. It was warmed in the heating mantle for 2 min at 60 °C. Then, 0.5 ml of distilled water was added to it and shaken well. Appearance of froth on the top layer shows the presence of saponins (Sofowara, 1993).

Test for Steroids

A red colour produced in the lower chloroform layer when 2 ml of organic extract was dissolved in 2 ml of chloroform and 2 ml concentrated sulphuric acid was added in it, indicates the presence of steroid, development of a greenish colour when 2 ml of the organic extract was dissolved in 2 ml of chloroform and treated with sulphuric and acetic acid indicates the presence of steroids (Gibbs, 1974).

Test for Tannins

Two ml of the sample was stirred with 2 ml of distilled water and few drops of Ferric chloride solution were added. Formation of green precipitate was indication of presence of tannins (Odebiyi and Sofowora, 1978).

Test for Terpenoids

A small amount of extract was dissolved in 1 ml of chloroform and 1 ml of concentrated sulphuric acid and formation of reddish brown coloration confirms the presence of terpenoids (Sofowara, 1993).

Gas Chromatography

An Agilent 6890 Gas Chromatograph was equipped with a straight deactivated 2 mm direct injector liner and a 15 m Alltech EC-5 column (250 µ I.D., 0.25 µ film thickness). A split injection was used for sample introduction and the split ratio was set to 10:1. The oven temperature program was programmed to start at 35 °C, hold for 2 minutes, then ramp at 20 °C per minute to 30 °C and hold for 5 minutes. The helium carrier gas was set to 2 ml/minute flow rate (constant flow mode).

Mass Spectrometry

A JEOL GC mate II bench top double-focusing magnetic sector mass spectrometer operating in electron ionization (EI) mode with TSS-2000[1] software was used for all analyses. Low-resolution mass spectra were acquired at a resolving power of 1000 (20 % height definition) and scanning from m/z 25 to m/z 700 at 0.3 seconds per scan with a 0.2 second inter-scan delay. High resolution mass spectra were acquired at a resolving power of 5000 (20 % height definition) and scanning the magnet from m/z 65 to m/z 750 at 1 second per scan.

Mass spectrometry library search

Identification of the components of the purified compound was matching their recorded spectra with the data bank mass spectra of NIST library V 11 provided by the instruments software.

3. RESULTS AND DISCUSSION

The phytochemical constituents of MEDsF

Photochemistry is the branch of chemistry which deals with the isolation and characterization of the available primary and secondary metabolites in plants using the modern techniques like GC-MS. The secondary metabolites plays an important role as antioxidants and anticancer agents.

The preliminary phytochemical analysis

In the present investigation, preliminary phytochemical screening has been done in MEDsF. The extracts showed the presence of phytochemical constituents namely, alkaloids, flavonoids, glycosides, phenol, steroids, tannin and terpenoids and the results of the present findings were tabulated in Table – 1.

Table – 1: Preliminary phytochemical screening of methanolic extract of *D. stramonium* Flower

Phytochemical constituents	MEDsF
Alkaloids	+
Flavonoids	+
Glycosides	+
Phenol	+
Saponin	-
Steroids	+
Tannin	+
Terpenoids	+

+ → Present and -→Absent

The Gas-Chromatography Mass Spectroscopy (GC-MS)

Gas Chromatography Mass Spectroscopy (GC-MS) is an analytical method that combines the features of Gas Chromatography and Mass Spectrometry to identify different substances with in a test sample.

GC-MS analysis of MEDsF

The major constituents of MEDsF were analyzed using GC-MS analysis. The GC-MS analysis revealed several peaks for MEDsF identified by matching the peaks with national Institute of Standards and Technology Spectral Library. The eight phytochemical constituents present in MEDsF was identified by GC-MS are the compounds are listed in Table - 2 and Figure – 2.

Table – 2: Phytochemical constituents of MEDsF identified by GC-MS analysis

S. No	Retention Time	Name of the Compounds	Molecular Formula	Molecular Weight	Peak area (%)
1.	18.13	9,12,15-Octadecatrienoic acid, 2,3–bis[(trimethylsilyl)oxy]propyl ester [zzz]	$C_{20}H_{38}O_2$	310	0.97
2.	15.37	Cyclopropanedodecanoic acid, 2-octyl, methyl ester	$C_{27}H_{52}O_4Si_2$	496	0.52
3.	16.32	Cyclopropanebutanoic acid, 2-[[2-[[2-[[2-pentylcyclopropyl]methyl]cycloproyl]methyl] cyclopropyl]methyl]-,methyl ester	$C_{25}H_{42}O_2$	374	0.60
4.	16.67	Acetic acid, 17-acetoxy-3-hydroxyimino-4,4,13-trimethyl-hexadecahydrocyclopenta[a]phenanthren-10-ylmethyl ester	$C_{25}H_{39}NO_5$	433	0.64
5.	18.02	Oleic acid, eicosyl ester	$C_{38}H_{74}O_2$	562	0.71
6.	18.20	16-octadecenoic acid, methyl nester	$C_{19}H_{36}O_2$	292	0.79
7.	18.60	[E]-9-octadecenoic acid ethyl ester	$C_{20}H_{38}O_2$	310	0.99
8.	18.83	Heptadecanoic acid, 15-methyl-,ethyl ester	$C_{20}H_{40}O_2$	312	0.98

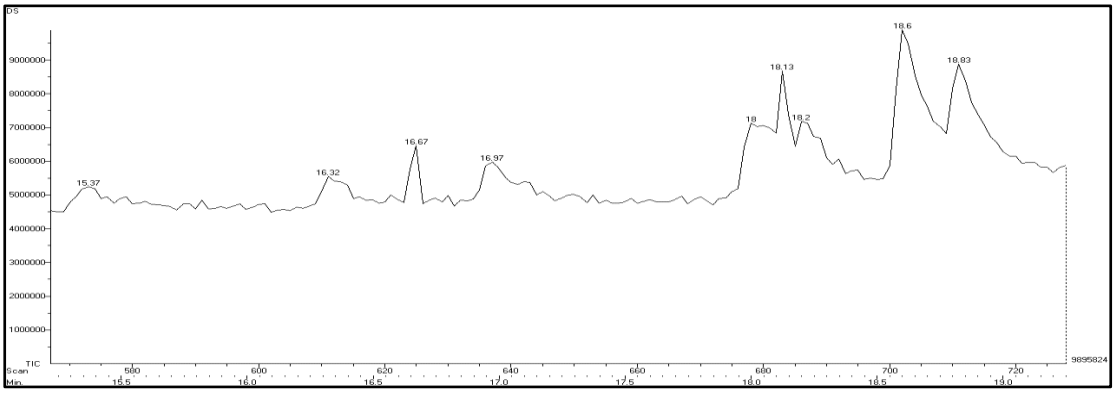


Figure - 2: GC-MS chromatogram of MEDsF

Mass Spectrum and structure of phytocomponents identified by GC-MS in the MEDsF

Figure –3
Compound - 1
9,12,15-Octadecatrienoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester, [zzz]

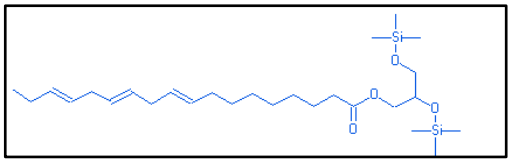


Figure - 4
Compound - 2
Cyclopropanedodecanoic acid, 2-octyl, methyl ester

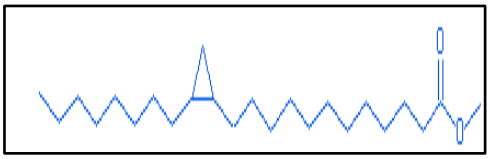


Figure - 4
Compound - 3
Cyclopropanebutanoic acid, 2-[[2-[[2-[[2-pentylcyclopropyl] methyl] cycloproyl] methyl] cyclopropyl] methyl] -,methyl ester

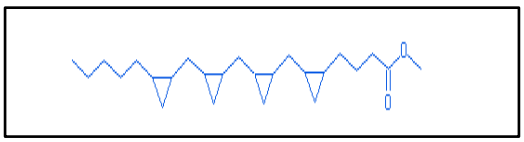


Figure –5
Compound - 4
Acetic acid, 17-acetoxy-3-hydroxyimino-4,4,13 – trimethyl – hexadecahydrocyclopenta [a] phenanthren – 10 - ylmethyl ester

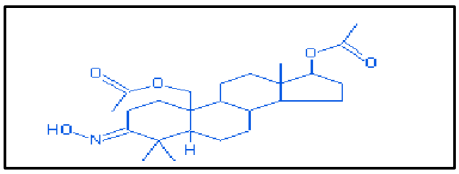


Figure – 6
Compound - 5
Oleic acid, eicosyl ester

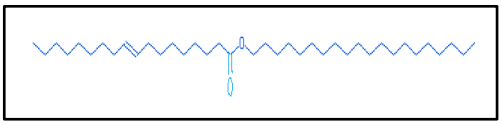


Figure – 7
Compound - 6
16-octadecenoic acid, methyl ester

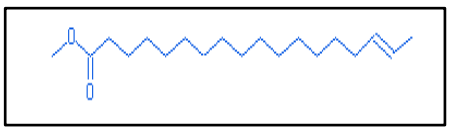


Figure - 8
Compound - 7
[E]-9-octadecenoic acid ethyl ester

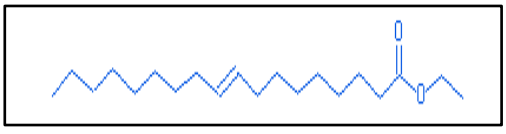
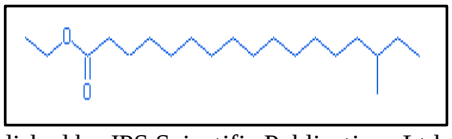


Figure –9
Compound - 8
Heptadecanoic acid, 15-methyl-ethyl ester



In the present study, the GC-MS analysis of methanolic extract of *Datura stramonium* showed the presence of 8 compounds which possess several known and unknown bioactive compounds. Selvamangai and Bhaskar (2012) also reported that the GC-MS analysis of the methanolic extract of *E. triplinerve* whole showed the presence of ten compounds. In terms of percentage amounts hexadecanoic acid, tetradecanoic acid and octadecanoic acid were predominant in the extract. These three major compounds have all shown to have hypocholesterolemic activity, antioxidant and lubricating activity. Anticancer and antiproliferative are shown by tetradecanoic acid and 2,6,10,-trimethyl,14-ethylen-14-pentadecne, while 1-hexyl-1-nitrocyclohexane and 1,14-tetradecanediol other compounds show antimicrobial and anti-inflammatory activities.

4. Conclusion

From the present study, it was concluded that the methanolic extract of the *Datura stramonium* L. flowers are rich in various secondary metabolites like alkaloids, flavonoids, glycosides, phenol, steroids, tannin and terpenoids. The Gas Chromatography Mass Spectrometry (GC-MS) analysis of *Datura stramonium* revealed the presence of eight bioactive compounds. The results of the present study will enhance the traditional usage of *Datura stramonium* flowers which possesses several known and unknown bioactive compounds.

5. References

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