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GC-MS Analysis of Bio-active Compounds in Ethanolic Extract of Leaf and Stem of *Asclepias curassavica* L.

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ABSTRACT

Background: The presence of phytochemical constitutes has been reported from species of the family *Asclepiadaceae*. No reports exist on the phytochemical components and biological activity of leaf and stem of *Asclepias curassavica* L. **Objective:** The present study was designed to determine the bioactive compounds in the leaf and stem ethanolic extract of *Asclepias curassavica* L. **Materials and Methods:** Phytochemical screening of ethanolic extract of leaf and stem revealed the presence of some bio-active components. Gas chromatography-mass spectrometry (GC-MS) analysis of the leaf and stem ethanolic extract of *Asclepias curassavica* L. was performed on a GC-MS equipment. **Results:** The phytochemical tests showed the presence of cardiac glycosides, phenols, saponins, steroids, tannins, and protein/amino acids in ethanolic extract of *Asclepias curassavica* L.. The GC-MS analysis has shown the presence of different phytochemical compounds in the ethanolic extract of *Asclepias curassavica* L. A total of 18 and 7 compounds were identified in leaf and stem ethanolic extract composition. **Conclusion:** From the results, it is evident that *Asclepias curassavica* L. contains various phytocomponents and is recommended as a plant of phytopharmaceutical importance.

Key words: GC-MS analysis, *Asclepias curassavica* L., Phytochemical screening, Ethanol extract, Phytochemical analysis, Bioactive compound etc.

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INTRODUCTION

Use of plants as a source of medicine has been inherited and is an important component of the health care system. India is the largest producer of medicinal herbs and is appropriately called the botanical garden of the world.[1] Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases.[2] Phytochemicals are the chemicals that present naturally in plants. Now- a-days these phytochemicals become more popular due to their countless medicinal uses. Phytochemicals play a vital role against number of diseases such as asthma, arthritis, cancer etc. Unlike pharmaceutical chemicals these phytochemicals do not have any side effects. Since the phytochemicals cure diseases without causing any harm to human beings these can also be considered as "man-friendly medicines".[3]

Asclepias curassavica (*Asclepiadaceae*) is one such plant, which has very high medicinal property. It is a species of an evergreen perennial plant in the milkweed family, commonly called as milk weed. It is used in the treatment of swelling, bruises, wounds, skin ulcers and chronic cough. It has a specific action on the lungs, making it a valuable medicinal herb in all chest complaints and in the treatment of many lung diseases. Internally, it is used to treat diarrhoea, dysentery and chronic rheumatism.[4]

In recent years GC-MS studies have been increasingly applied for the analysis of medicinal plants as this technique has proved to be a valuable method for the analysis of non-polar components and volatile essential oil, fatty acids, lipids and alkaloids.[5]

Literature review on the Leaf and stem part of plant in investigation has shown that, there are no published reports worldwide related to the possible chemical components of Leaf and stem of *Asclepias curassavica* L. So, the present study was aimed to investigate the possible chemical components by first preparing the ethanolic extract and separation and identification of the compounds by subjecting it to GC-MS analysis.

MATERIALS AND METHODS

Collection of plant material

The plant was collected from Pune in the month of June, identified and authenticated from St. Xavier's College, Mumbai.

Preparation of extract

100 g of plant powdered material was successively extracted by using Soxhlet extractor and placed in a central compartment of Soxhlet assembly, 650 ml of solvent was placed in a lower compartment and a reflux condenser is attached above the central compartment. The vapour passes through the side-arm up into the reflux condenser. Here the vapour liquified and drips into the plant material to be extracted. The warm solvent percolates through the material band the extracts gradually collects in the central compartment. Once the height of the extract reached the top of the siphon, the entire liquid flowed through this and back to the lower solvent container. The process is then repeated. The extract collected in the lower vessel, gradually becoming more and more concentrated. The vapour rising from the heated extract is pure solvent vapour and so the liquid dripping into the material from the condenser is essentially pure solvent, though derived from the extract. After complete extraction the lower vessel was removed, solvent recovered and the extract is concentrated and percentage yield was calculated.[6]

Preliminary phytochemical screening

The extract obtained from the powdered leaf and stem of Asclepias curassavica L. were subjected to phytochemical teste to determine the presence of active secondary metabolites using standard procedure. This extract were filter through fine filter paper into test tube and used for further studies. Preliminary phytochemical analysis was carried out using standard qualitative methods.[7-9] The crude extracts obtained were qualitatively tested for the presence of various phytochemical constituents using standard protocols.[10,11] The extractive values were calculated by standard methods. The concentrated crude extracts obtained were weighed and dissolved in the respective solvent used for the extraction (1g / 100 ml, w/v).

Qualitative analysis

Test for identification of Steroids

Dissolve the extract in chloroform and add equal volume of concentrated sulphuric acid. Formation of bluish red to cherry colour in chloroform layer and green fluorescence in the acid layer represents the steroidal components in the tested extract.

Test for Glycosides

1gm of powdered drug is extracted with 10 ml of 70% alcohol for 2 minutes, filtered, add to the filtrate, 10 ml of water and 0.5 ml of strong solution of lead acetate and filtered and the filtrate is shaken with 5 ml of chloroform. The chloroform layers were separated in a porcelain dish and remove the solvent by gentle evaporation. Dissolve the cooled residue in 3 ml of glacial acetic acid containing 2 drops of 5% ferric chloride solution. Carefully transfer this solution to the surface of 2 ml of concentrated sulphuric acid. A reddish brown layer forms at the junction of the two liquids and the upper layer slowly becomes bluish green, darkening with standing.

Test for Tannins and Phenolic compounds

a) Take the little quantity of test solution and mixed with basic lead acetate solution. Formation of white precipitates indicates the presence of tannins.

b) To 1 ml of the extract, add ferric chloride solution, formation of a dark blue or greenish black color product shows the presence of tannins.

c) The little quantity of the extract is treated with potassium ferric cyanide and ammonia solution. A deep red color indicates the presence of tannins.

d) To the test extract, add strong potassium dichromate solution, a yellow color precipitate indicates the presence of tannins and phenolic compounds.

Test for Saponins

Take small quantity of alcoholic and aqueous extract separately and add 20 ml of distilled water and shake in a graduated cylinder for 15 min lengthwise. No layer of foam indicates the absence of saponins.

Test for Protein and Amino Acids

Add two drops of freshly prepared 0.2% ninhydrin reagent (0.1% solution in n-butanol) to the small quantity of extract solution and heat. Development of blue color reveals the presence of proteins, peptides, or amino acids.

GC-MS (Gas Chromatography-Mass Spectrometry) analysis

The phytochemical investigation of ethanolic extract was performed on a GC-MS equipment from IIT SAIF, Mumbai. Injection volume was 1 µl. Samples dissolved in ethanol were run fully at a range of 40m,-650 m/z and the results were compared by using NIST library search programme.

RESULTS

Present study contributes valuable information of bioactive compound in *Asclepias curassavica* L. Qualitative analysis of plant extract was carried out for Alkaloids, flavonoids, glycosides, phenols, saponins, steroids, tannins, terpenoids and protein/amino acids.

Preliminary phytochemical study revealed that ethanolic extract of *Asclepias curassavica* L. contains glycosides, phenols, saponins, steroids, tannins, terpenoids and protein/amino acids. Alkaloids and flavonoids were absent in the leaf and stem ethanolic extract, as summarized in [Table 1].

The results pertaining to GC-MS analysis of the ethanolic Leaf and stem extract of *Asclepias curassavica* L. lead to the identification of a number of compounds. These compounds were identified through mass spectrometry attached with GC. The various components present in *Asclepias curassavica* L. were detected by the GC-MS are shown in [Table 2 and 3]. Benzene, 1,1'-(1,2-dimethyl-1,2-ethanediyl) bis-(R*,S*), 1,2-propanediol, 3-benzyloxy-1,2-diacetyl, 1,3,5-triphenyl-1,5-pentanedione, 2-Butene, 1,4-diol-1,4-diphenyl, Benzeneethanamine, N-methyl-3-nitro-N-(2-phenyl), 2-phenethyl- -phenylpropionate, 2-Butene, 1,4-diol-1,4-diphenyl, 2-Butene, 1,4-diol-1,4-diphenyl, 2-Butene, 1,4-diol-1,4-diphenyl, Benzene, 1-(dichloromethyl)-2-methyl, Benzene, 1-(dichloromethyl)-2-methyl, 2,4,6- Triphenyl-1,3-dioxane, 2-Butene, 1,4-diol-1,4-diphenyl, 2,4,6- Triphenyl-1,3-dioxane, 2-Butene, 1,4-diol-1,4-diphenyl, 2-Butene, 1,4-diol-1,4-diphenyl, 2-Butene, 1,4-diol-1,4-diphenyl, 2-Butene, 1,4-diol-1,4-diphenyl, 9,12,15-Octadecatrienoic acid,2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy] methyl] ethyl ester, (zzz), 1-Monolinoleoylglycerol trimethylsilyl ether, d- Mannitol, 1-decylsulfonyl, 9,12,15-Octadecatrienoic acid, - 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy] methyl] ethyl ester, (zzz), 9,12,15-Octadecatrienoic acid, - 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy] methyl] ethyl ester, (zzz), 9,12,15-Octadecatrienoic acid, - 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy] methyl] ethyl ester, (zzz), 9-Octadecenoic acid (Z), phenylmethyl ester were present in ethanolic extract of leaf and stem of *Asclepias curassavica* L.

Table 1: Preliminary phytochemical evaluation of ethanolic extract of leaf of *Asclepias curassavica* L.

Phytochemical constituents	Test/Reagent	Leaf	Stem
Alkaloids	Dragendroffs	-	-
	Mayer s test	-	-
	Hagers	-	-
Flavonoids	Shinoda's test	-	-
Steroids	Salkowski test	+	+
Glycosides test	Kellar killani test	+	+
Phenols	$FeCl_3$ test	+	+
	Potassium Dichromate	+	+
Tannins	Lead acetate test	+	+
	Potassium Dichromate	+	+
Saponin	Froth test	+	-
Proteins and Amino acids	Ninhydrin test	+	+

+ = Present; - = Absent.

Table 2: Compounds identified in the ethanolic extract of leaf of *Asclepias curassavica* L.

Sr. No.	Name of compound	RT	Formula	Molecular weight
1	Benzene, 1,1'-(1,2-dimethyl-1,2-ethanediyl) bis-(R*, S*)	24.25	$C_{16}H_{18}$	210
2	1,2-propanediol, 3-benzyloxy-1,2-diacetyl	25.44	$C_{14}H_{18}O_5$	266
3	1,3,5-triphenyl-1,5-pentanedione	25.95	$C_{23}H_{20}O_2$	328
4	2-Butene, 1,4-diol-1,4-diphenyl	26.34	$C_{16}H_{16}O_2$	240
5	Benzeneethanamine, N-methyl-3-nitro-N-(2-phenyl)	27.19	$C_{17}H_{20}N_2O_2$	284
6	2-phenethyl--phenylpropionate	27.68	$C_{17}H_{18}O_2$	254
7	2-Butene, 1,4-diol-1,4-diphenyl	28.10	$C_{16}H_{16}O_2$	240
8	2-Butene, 1,4-diol-1,4-diphenyl	28.70	$C_{16}H_{16}O_2$	240
9	2-Butene, 1,4-diol-1,4-diphenyl	29.31	$C_{16}H_{16}O_2$	240
10	Benzene, 1-(dichloromethyl)-2-methyl	29.95	$C_8H_8Cl_2$	174
11	Benzene, 1-(dichloromethyl)-2-methyl	30.20	$C_8H_8Cl_2$	174
12	2,4,6- Triphenyl-1,3-dioxane	30.82	$C_{22}H_{20}O_2$	316
13	2-Butene, 1,4-diol-1,4-diphenyl	31.39	$C_{16}H_{16}O_2$	240
14	2,4,6- Triphenyl-1,3-dioxane	31.63	$C_{22}H_{20}O_2$	316
15	2-Butene, 1,4-diol-1,4-diphenyl	32.11	$C_{16}H_{16}O_2$	240
16	2-Butene, 1,4-diol-1,4-diphenyl	32.54	$C_{16}H_{16}O_2$	240
17	2-Butene, 1,4-diol-1,4-diphenyl	32.93	$C_{16}H_{16}O_2$	240
18	2-Butene, 1,4-diol-1,4-diphenyl	32.80	$C_{16}H_{16}O_2$	240

The GC-MS spectrum confirmed the presence of various components with different retention times as illustrated in [Figure 1,2]. The mass spectrometer analyses the compounds eluted at different times to identify the nature and structure of the compounds. The large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios. These mass spectra are fingerprint of that compound which can be identified from the data library. The present study helps to predict the formula and structure of 25 biomolecules. Further investigation may lead to isolation of bio-active compounds and their structural elucidation and screening of pharmacological activity will be helpful for further drug development.

Table 3: Compounds identified in the ethanolic extract of Stem of *Asclepias curassavica* L.

Sr. No.	Name of compound	RT	Formula	Molecular weight
1	9,12,15-Octadecatrienoic acid, - 2-[(trimethylsilyl) oxy]-1-[[(trimethylsilyl)oxy] methyl] ethyl ester, (zzz)	30.05	$C_{27}H_{52}O4Si_2$	496
2	1-Monolinoleoylglycerol trimethylsilyl ether	30.99	$C_{27}H_{54}O_4Si_2$	498
3	d- Mannitol, 1-decylsulfonyl	31.30	$C_{16}H_{34}O_7S$	370
4	9,12,15-Octadecatrienoic acid, - 2-[(trimethylsilyl) oxy]-1-[[(trimethylsilyl)oxy] methyl] ethyl ester, (zzz)	31.71	$C_{27}H_{52}O_4Si_2$	496
5	9,12,15-Octadecatrienoic acid, - 2-[(trimethylsilyl) oxy]-1-[[(trimethylsilyl)oxy] methyl] ethyl ester, (zzz)	32.10	$C_{27}H_{52}O_4Si_2$	496
6	9,12,15-Octadecatrienoic acid, - 2-[(trimethylsilyl) oxy]-1-[[(trimethylsilyl)oxy] methyl] ethyl ester, (zzz)	31.61	$C_{27}H_{52}O_4Si_2$	496
7	9-Octadecenoic acid (Z), phenylmethyl ester	33.01	$C_{25}H_{40}O_2$	372

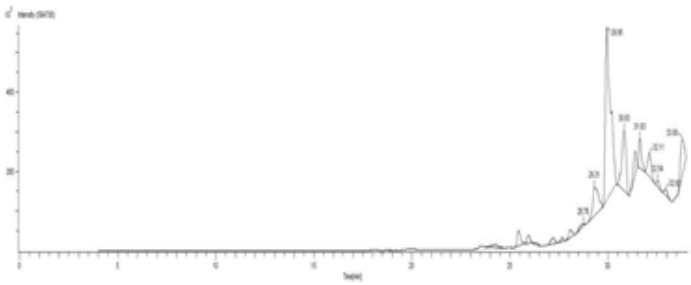

Figure 1: GC-MS chromatogram of ethanolic Leaf extract of *Asclepias curassavica* L.

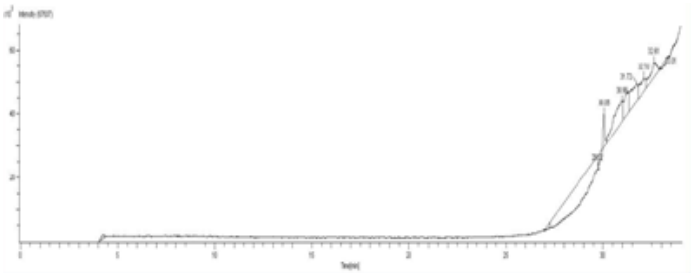

Figure 2: GC-MS chromatogram of ethanolic Stem extract of *Asclepias curassavica* L.

DISCUSSION

The analysis and extraction of plant material play an important role in the development, upgrading, and quality control of herbal formulations. Studying of medicinal plants helps to cure humans and animals from various diseases.

Gas chromatography coupled mass spectrometry (GC-MS) is an analytical method that combines the features of gas chromatography and mass

spectrometry to identify different substances within a test sample. The spectra of unknown compounds were compared with spectra of known compounds stored in identification of compounds was confirmed based on the active principle, Molecular Weight (MW), Concentration (%), Retention Time (RT).

The GC-MS analysis of various compounds from *Asclepias curassavica* L. leaf and stem extracts was performed using shimadzu capillary GC-quadrupole MS system QP 5000 with two fused silica capillary Coolum DB-5 and typical total ion chromatograms (TIC) of each sample were given in Figure 1 and 2 respectively. The comparison of the mass spectrums with the data base gave more than 90% match as well as confirmatory compound structure match. The GCMS analysis of the concentrated ethanol extract resulted many compounds which have diverse use. Compounds having anti-inflammatory, antibacterial, antifungal, anticancer activity properties have been identified.

9,12- Octadecadienoic acid shows property of antimicrobial, antioxidant, anticancer, hypercholesterolemic, antiulcerogenic, lubricant, nematicide, anti-inflammatory, antiandrogenic and other activities.[12]

Benzene, 1,1'-(1,2-dimethyl-1,2-ethanediyl) bis-(R*,S*) has antioxidant and anticholinesterase activity. Benzene, 1-(dichloromethyl)-2-methyl as a antimicrobial agent.

1-Monolinoleoylglycerol trimethylsilyl ether shows Antimicrobial Antioxidant Antiinflammatory Antiarthritic Antiasthma, Diuretic activity.[13]

The presence of various bioactive compounds justifies the use of the whole plant recommended for phytopharmaceutical importance.

CONCLUSION

The presence of various bio-active compounds detected after GC-MS analysis using the ethanolic extract of leaf and stem of *Asclepias curassavica* L justifies the use of plant for various elements by traditional practitioner. However, isolation of individual phytochemical constituent and subjecting it to the biological activity will definitely yield fruitful results and will open a new area of investigation of individual components and their pharmacological potency. From these results, it is concluded that *Asclepias curassavica* L. possesses various bio-active compounds which are pharmaceutically important. There is great scope for isolation of these bioactive compounds for further study and the plant can be subjected to in depth pharmacological study.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

g: Gram; **ml:** Millilitre; **w/v:** Weight by volume; **%:** Percentage; **min:** Minute.

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