ORIGINAL ARTICLE

Gas chromatography-mass spectroscopy analysis of bioactive compounds in the whole plant parts of ethanolic extract of *Asclepias Curassavica* L.

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Abstract

Objective: The present study was carried out to cultivate *Asclepias curassavica* and explore the phytoconstituents of ethanolic extract of *A. curassavica* whole part using gas chromatography-mass spectroscopy (GC-MS). This research may be useful for constituent(s)-based pharmacological activity. **Materials and Methods:** 5 g of powdered plant material was taken in a spotless, level container and soaked in 80 ml of ethanol, and extraction was done by hot extraction method. The glass container with extract was sealed and kept aside for few hours. It was then filtered through Whatman filter paper No.42. Ethanolic extract of *A. curassavica* was further undertaken for GC-MS analysis. **Result:** The ethanolic extract of the whole plant of *A. curassavica* revealed the presence of 49 diverse phytochemical compounds. Several of these phytoconstituents are reported to possess pharmacological potential. **Conclusion:** *A. curassavica* L. has been successfully cultivated, authenticated, and used for the identification of its organic chemical components by GC-MS. Pesticide residues may have an adverse effect on the food chain, and the concentration should be within permissible range for safe human consumption of any herbal drug product. *A. curassavica* has been observed as a tremendous source of many important phytochemicals, especially glycosidal, alkaloids, steroidal, and terpenoidal compounds. There were no pesticidal residues found in the tested plant drug. Therefore, it may be explored for the production of natural medicinal formulations in pharmaceutical drug industries including anticancer drugs, antibacterial, antimicrobial, antiasthmatics, urine acidifiers, and antimicrobial on account of its potential antioxidant activity.

Key words: *Asclepias curassavica*, ethanolic extract, gas chromatography-mass spectroscopy, phyto-compounds

INTRODUCTION

Each and every plant contains a certain specific type of chemical compounds, which are produced during the normal growth and development of the plant. These compounds are generally referred to as "phytochemicals" or sometimes also known as "secondary metabolites". Herbal medicines have been use since the dawn of civilization, to maintain health and to treat diseases. The WHO estimates that about three-quarters of the world's population currently use herbs and other forms of traditional medicines to treat their diseases.[1] Medicinal plants have had an essential role in human background and civilization. The two main objectives of using plants as a therapeutic agent are either to isolate a bioactive phytoconstituent for direct use as drug e.g. digoxin, morphine, vinblastine, reserpine, etc. or to synthesize some novel bioactive compound by using phytoconstituent as a precursor e.g. metformin, nabilone, verapamil, etc.[2] *Asclepias curassavica* Linn. [*Tropical* Milkweed; Figure 1] is an erect, evergreen shrub belonging to the family Asclepiadaceae which possess a vast range of medicinal activities, introduced as an ornamental plant in India, where it has become naturalized in many parts of the country at lower elevations in waste places and on a roadside.[3] The ethanolic extract of the whole plant of

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A. curassavica is reported to induce many pharmacological activities including analgesic, antipyretic, anticancer, antimicrobial, cardiovascular, anti-inflammatory, cytotoxic activity, antioxidant activity, uterine stimulant effect, and many other pharmacological activities.[4] Taxonomical classification of this plant was mentioned in Table 1.

Gas chromatography-mass spectroscopy (GC-MS) is a conscientious and high-principled technique has been adapted precisely to evaluate the different constituents present in the plant extract along with their molecular structure. It is having two considerable advantages for using GC-MS to analyze the phytoconstituents present in the herbal drugs. First, GC-MS is having superior separation ability with the capillary column, which can produce a chemical fingerprint with higher precision and accuracy; second, quantitative data of the herbs studied could be given by GC-MS with the coupled mass spectral database, and it will be enormously valuable for the further study for investigating the correlation between phytoconstituents in the phyto-medicinals and its biological activity.[5] GC-MS is very helpful for identifying the pure compounds which are present in minute quantity, i.e., <1 mg. However, there is no information available on the detailed analysis using GC-MS and bioactive constituents of this plant, and hence, it was planned to take up detailed investigation on ethanol extract of the whole plant of *A. curassavica* for the isolation of active biomolecules and its pharmacological activities from the potent constituent.

Table 1: Taxonomical classification of *Asclepias curassavica*

Taxonomical classification	
Kingdom	Plantae
Division	Angiosperms
Order	Gentianales
Family	Asclepiadaceae
Genus	*Asclepias*
Species	*Curassavica*

Figure 1: The plant *Asclepias curassavica* L.

METHODS

Collection and authentication of the whole plant

The proposed plant for research study was collected from Patanjali (Haridwar), India. The collected plant was botanically authenticated from the National Institute of Science Communication and Information Resources (Delhi) and cultivated in Yamuna Nagar. The collected plants were washed 3–4 times with tap water and then with autoclaved distilled water, then dried at low temperature, and used for further investigation.

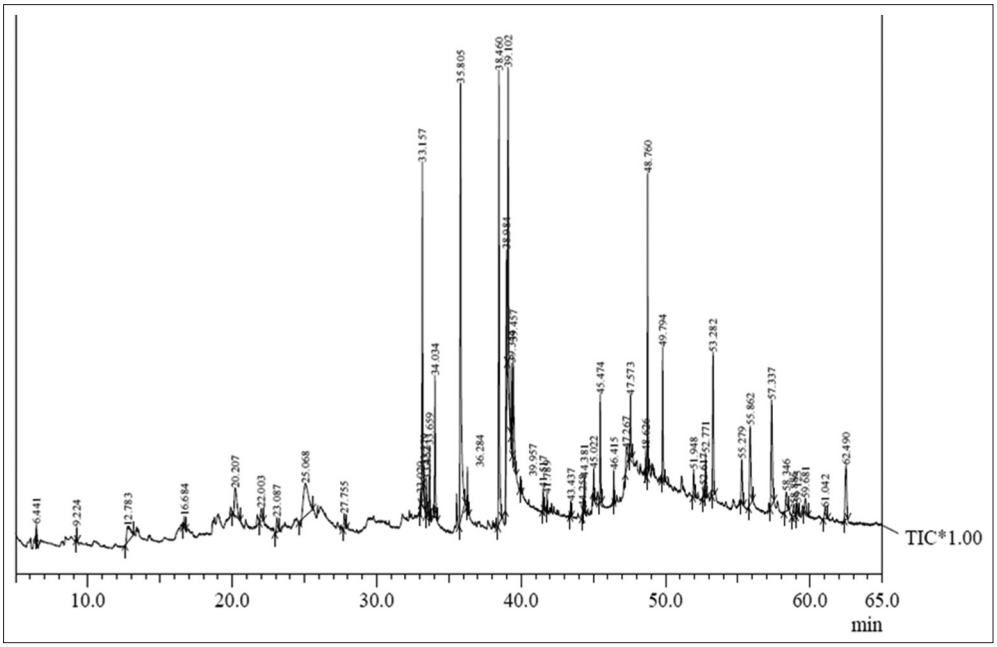

Figure 2: Total ion chromatogram of the entire plant parts of the ethanolic extract of *Asclepias curassavica*

Table 2: Phytoconstituents present in *A. curassavica* as identified using GC-MS

RT	Name of the compound	MF	MW	% of peak area
6.441	1-Butoxy-1-ethoxyethane	$C_8H_{18}O_2$	146	0.29
9.224	1,1-Diethoxy-3-methylbutane	$C_9H_{20}O_2$	160	0.22
12.783	(2E)-2-Methyl-2-hexenoic acid	$C_7H_{12}O_2$	128	1.64
16.684	5-methyl-2-(1-methylethyl)-	$C_{10}H_{20}O$	156	0.23
20.207	3,3-Diacetyl-2,3,4,5-tetrahydro-2-oxofuran	$C_8H_{10}O_4$	170	2.14
22.003	1,3,4-Eugenol	$C_{10}H_{12}O_2$	164	0.65
23.087	Methyl 2-[acetyl (methyl) amino]-2-deoxy-3,4,6-triI-O-methylhexopyranoside	$C_{13}H_{25}NO_6$	291	0.62
25.068	Cytidine	$C_9H_{13}N_3O_5$	243	4.60
27.755	1,2-Benzenedicarboxylic acid	$C_{12}H_{14}O_4$	222	0.45
33.020	(2E)-3,7,11,15-Tetramethyl-2-hexadecene	C_20H_40	280	0.25
33.157	Neophytadiene	$C_{20}H_{38}$	278	6.30
33.279	(2E)-3,7,11,15-Tetramethyl-2-hexadecene	$C_{20}H_{40}$	280	0.42
33.452	Card-20 (22)-enolide	$C_{36}H_{54}O_{14}$	710	0.70
33.659	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	1.33
34.034	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	2.87
35.805	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	14.31
36.284	Heptadecanoic acid	$C_{19}H_{38}O_2$	298	0.76
38.460	2-Hexadecen-1-ol	$C_{20}H_{40}O$	296	11.14
38.984	9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$	280	4.40
39.102	9,12,15-Octadecatrienoic acid	$C_{18}H_{30}O_2$	278	7.45
39.344	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308	1.32
39.457	9,12,15-Octadecatrienoic acid	$C_{18}H_{30}O_2$	280	2.92
39.957	Heptadecanoic acid	$C_{19}H_{38}O_2$	298	0.23
41.517	Octanoic acid	$C_{12}H_{25}NO_2$	215	0.42
41.789	Heneicosane	$C_{21}H_{44}$	296	0.25
43.437	Heneicosane	$C_{21}H_{44}$	296	0.25
44.258	3-Cyclopentylpropionic acid	$C_{12}H_{23}NO_2$	213	0.17
44.381	3-Cyclopentylpropionic acid	$C_{12}H_{23}NO_2$	213	0.71
45.022	Heneicosane	$C_{21}H_{44}$	296	0.42
45.474	1,2-Benzenedicarboxylic acid	$C_{12}H_{14}O_4$	222	2.12
46.415	Heneicosane	$C_{21}H_{44}$	296	0.44
47.267	Cymarin	$C_{30}H_{44}O_9$	548	1.12
47.573	2-Methylhexacosane	$C_{27}H_{56}$	380	1.28
48.626	Heneicosane	$C_{21}H_{44}$	296	0.27
48.760	Squalene	$C_{30}H_{50}$	410	4.51
49.794	Dotriacontane	$C_{32}H_{66}$	450	2.56
51.948	gamma.-Tocopherol	$C_{28}H_{48}O_2$	416	0.79
52.617	3-Bromocholest-5-ene	$C_{27}H_{45}$	448	0.16
52.771	Dotriacontane	$C_{32}H_{66}$	450	0.91
53.282	Vitamin E	$C_{29}H_{50}O_2$	430	3.95
55.279	Ergost-5-en-3-ol	$C_{28}H_{48}O$	400	1.54
55.862	Stigmasta-5,22-dien-3-ol	$C_{29}H_{48}O$	412	3.21
57.337	Stigmast-5-en-3-ol	$C_{29}H_{50}O$	414	4.23
58.346	24-Norursa-3,12-diene	$C_{29}H_{46}$	394	0.86

(Contd...)

Table 2: *Continued*

RT	Name of the compound	MF	MW	% of peak area
58.886	(E)-3-Methyl-5-((1R,4aR,8aR)-5,5,8a-trimethyl-2-methylenedecahydronaphthalen-1-yl) pent-2-en-1-ol	$C_{20}H_{34}O$	290	0.52
59.125	22-Stigmasten-3-one	$C_{29}H_{48}O$	412	0.43
59.681	Methyl Commate D	$C_{31}H_{50}O_4$	486	0.67
61.042	Cholest-4-en-3-one	$C_{27}H_{44}O$	384	0.48
62.490	1-(1,5-Dimethyl-4-hexenyl)-3A,6,6,12A-tetramethyltetradecahydro-1H-cyclopenta[A] cyclopropa[E]	$C_{32}H_{52}O_2$	468	2.49

RT: Retention time, MF: Molecular formula, MW: Molecular weight, *A. curassavica*: *Asclepias curassavica*, GC-MS: Gas chromatography-mass spectroscopy

Table 3: Nature and biological properties of *A. curassavica* phytoconstituents by GC-MS analysis

Name of the compound	Nature and pharmacological potential	References
Dotriacontane	Antimicrobial, antioxidant, antispasmodic	[6]
n-Hexadecanoic acid	Palmitic acid (saturated fatty acid) and antioxidant, hypocholesterolemic, nematicide, pesticide, anti-androgenic, flavor, hemolytic, 5-alpha-reductase inhibitor	[6,9,10]
9,12-Octadecadienoic acid	Linoleic acid and anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, antihistaminic antieczemic, antiacne, 5-alpha-reductase inhibitor, antiandrogenic, antiarthritic, anticoronary	[7,13,17]
Squalene	Triterpene (saturated fatty acid) and anti-inflammatory, antibacterial, antitumor, immunostimulant, chemopreventive, cancer preventive, lipoxygenase inhibitor, pesticide, antioxidant	[8,11,16]
Stigmasterol	Steroid and antioxidant, hypoglycemic and thyroid inhibiting properties, precursor of progesterone, antimicrobial, anticancer, antiarthritic, antiasthma, anti-inflammatory, diuretic	[8,10,14]
Cytidine	Nucleoside molecule and glutamatergic antidepressant drug	[9]
22-Stigmasten-3-one	Steroid and antimicrobial, anticancer, antiarthritic, anti-asthma, diuretic	[10,18]
Stigmast-5-en-3-ol	Phytosterols and reduce blood level of glucose, hypercholesterolemia, anti-inflammatory, antipyretic, antiarthritic, antiulcer	[11,16]
Neophytadiene	Hydrocarbons and antipyretic, analgesic, anti-inflammatory, antimicrobial, antioxidant	[11,15]
9,12,15-Octadecatrienoic acid	Unsaturated fatty acid and anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic, antieczemic, anticancer, 5-alpha-reductase inhibitor, antiandrogenic, anti-arthritic, anti-coronary, insectifuge	[11,22,27]
2-Methylhexacosane	Antimicrobial, decrease blood cholesterol	[12]
1,2-Benzenedicarboxylic acid	Colorless crystalline acid used in the synthesis of dyes and perfumes, neurodegenerative disorders	[13]
Card-20 (22)-enolide	Glycoside compound and cardiac stimulant	[14]
Ergost-5-en-3-ol	Anticancer, antioxidant, antitumor, anti-inflammatory, hypocholesterolemic	[14,20]
Methyl Commate D	Antimicrobial, anti-inflammatory	[15,29]

(Contd...)

Table 3: *Continued*

Name of the compound	Nature and pharmacological potential	References
Vitamin E	Lipid and antiaging, anti-Alzheimer, antidermatitic, antidiabetic, antioxidant, antitumor, cancerpreventive, hypocholesterolemic, immunostimulant, antiaging, analgesic, antidiabetic, anti-inflammatory, antioxidant, antidermatitic, antileukemic, antitumor, anticancer, hepatoprotective, hypocholesterolemic, antiulcerogenic, vasodilator, antispasmodic, antibronchitic, anticoronary	[16,20,21]
Heneicosane	Antibacterial, antimicrobial, antiasthmatics, urine acidifiers, antimicrobial	[19]
gamma.-Tocopherol	Vitamin compound and anticancer, antioxidant, antitumor, anti-inflammatory, hypocholesterolemic, cardioprotective	[20,28]
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Terpene alcohol and anti-inflammatory, antimicrobial, flavoring agent, cancer-preventive, anti-diuretic, antioxidant	[22,25,26]
Cymarin	Cardiotonic cardiac glycoside and cardiac arrhythmic, congestive heart failure	[24]
2-Hexadecen-1-ol	Phytol and antimicrobial	[27]

A. curassavica: *Asclepias curassavica*, GC-MS: Gas chromatography-mass spectroscopy

Sample preparation for GC-MS

Fresh plants were collected and then dried at room temperature and then pulverized to obtain a coarse powder in a grinder. 5 g of powdered plant material was taken in a spotless, level flask and soaked in 80 ml of ethanol, and extraction was done by hot extraction method. The flask with extract was preserved and kept apart for few hours. It was then filtered using Whatman filter paper No. 42 to attain a clear extract concentrated up to 5 ml. Then, this ethanolic extract was undertaken for the GC-MS investigation.

GC-MS analysis

Ethanolic extract of entire parts of *A. curassavica* Linn. was analyzed by GC-MS technique for the detection of the components present in the extract. GC-MS analysis of some of the potent constituents present in the extracts was performed at "Jawahar Lal National University," Delhi, India. GC analysis of the ethanolic extract was done using a GC-MS (Model; QP 2010 series, Shimadzu, Tokyo, Japan) equipped with a Rxi-5MS fused silica capillary column (5% diphenyl/95% dimethyl polysiloxane) and AOC-20i+s (autosampler) of 0.25 mm diameter, 30 m length, and 0.25 µm film thickness. The sample size of 2 µl was injected through the injector. The inert gas helium was used as carrier gas. The MS was taken at 70 eV of ionization energy. Column flow was1.21 mL/min and total flow was 16.3 ml/min. Flow control with linear velocity was 39.9 cm/s. Initial temperature of the oven was 50°C, 250°C for 5 min, ramp 22 min to 280°C, hold 69.98 min, ACQ Mode Scan range: 40 m/z to 700 m/z and a scan interval of 0.50 s, 260°C with a split ratio of 10:0. Total running time of GC-MS was 65 min. The relative % amount of each component was expressed as a percentage with peak area normalization.

Identification of components

In the MS Program, National Institute Standard and Technology (NIST) Version 14.0, Wiley 8.0 library database of NIST having more than 62000 patterns was used for identifying the chemical components. The mass spectrum of unknown phytochemicals was compared with the spectrum of known compounds stored in the NIST library. Measurement of peak areas and data processing of test sample were carried out.

RESULTS

In the present study, GC-MS chromatogram shows the presence of 49 different peaks which confirm the presence of 49 compounds with their respective RT [Figure 2] in the ethanolic extract of the whole plant of *A. curassavica.* The spectra of the compounds were matched with Wiley 8.0 and NIST libraries. The identified compounds, their retention time (RT), molecular weight, molecular formulae, and percentage composition (%area) are given in Table 2. The individual fragmentation of major components is illustrated in Figure 3. Some of the identified components possess biological activities which are listed in Table 3 with their molecular structure. The prevailing compounds in ethanol extract were (2E)-3,7,11,15-tetramethyl-2-hexadecen-1-ol, card-20(22)-enolide, 9,12-octadecadienoic acid, heneicosane, methyl Commate D, cymarin, 2-methylhexacosane, gamma.-tocopherol, Ergost-5-en-3-ol, 22-stigmasten-3-one, n-hexadecanoic acid (14.31% with RT 35.805), 2-hexadecen-1-ol(11.14% with RT 38.460), 9,12,15-octadecatrienoic

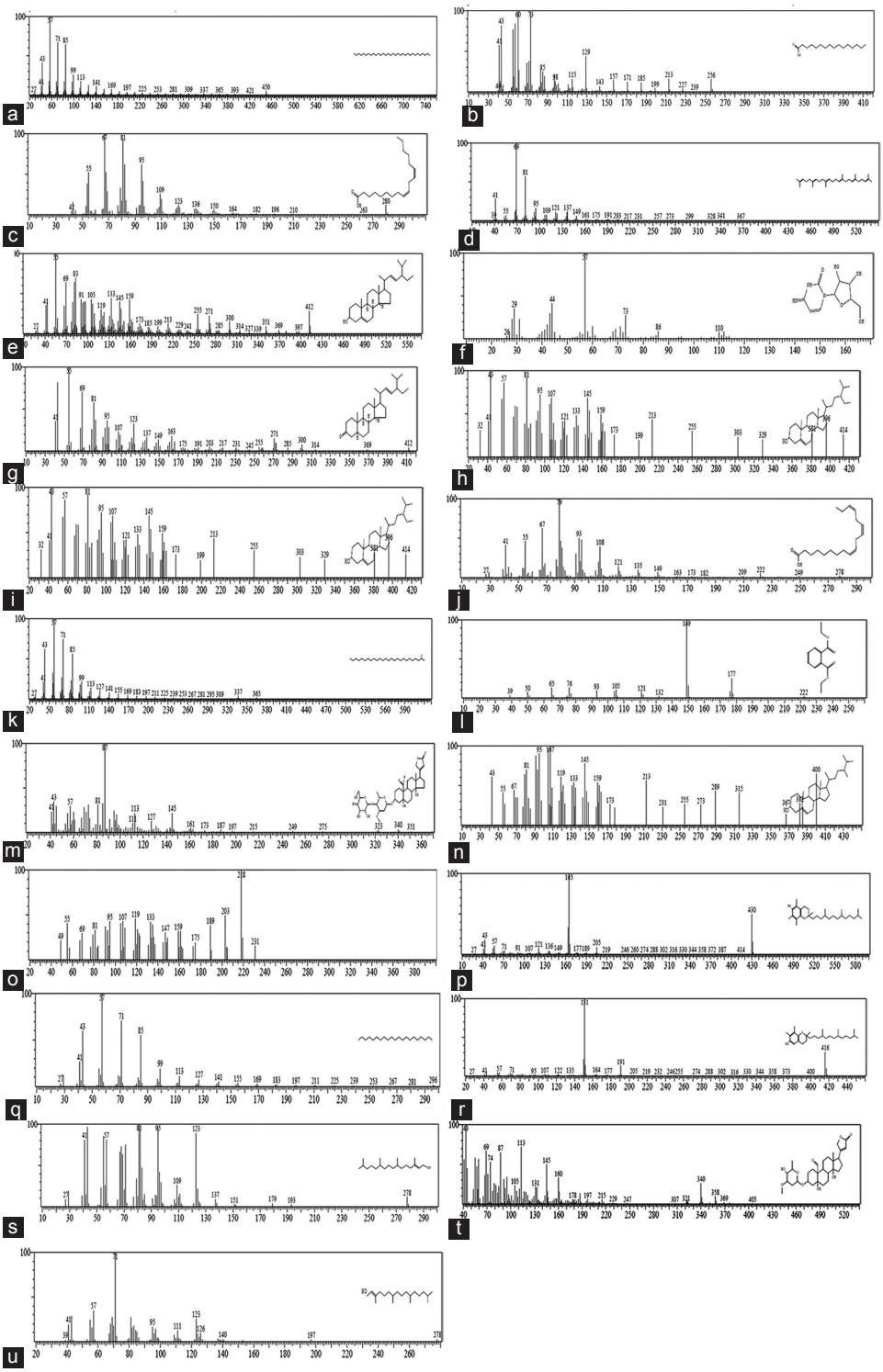

Figure 3: (a) Mass spectrum and molecular structures of dotriacontane. (b) Mass spectrum and molecular structures of n-hexadecanoic acid. (c) Mass spectrum and molecular structures of 9,12-octadecadienoic acid. (d) Mass spectrum and molecular structures of squalene. (e) Mass spectrum and molecular structures of stigmasterol. (f) Mass spectrum and molecular structures of cytidine. (g) Mass spectrum and molecular structures of 22-stigmasten-3-one. (h) Mass spectrum and molecular structures of stigmast-5-en-3-ol. (i) Mass spectrum and molecular structures of neophytadiene. (j) Mass spectrum and molecular structures of 9,12,15-octadecatrienoic acid. (k) Mass spectrum and molecular structures of 2-methylhexacosane. (l) Mass spectrum and molecular structures of 1,2-benzenedicarboxylic acid. (m) Mass spectrum and molecular structures of card-20(22)-enolide. (n) Mass spectrum and molecular structures of Ergost-5-en-3-ol. (o) Mass spectrum of methyl commate D. (p) Mass spectrum and molecular structures of Vitamin E. (q) Mass spectrum and molecular structures of heneicosane. (r) Mass spectrum and molecular structures of gamma. – Tocopherol. (s) Mass spectrum and molecular structures of 3,7,11,15-Tetramethyl-2-hexadecen-1-ol. (t) Mass spectrum and molecular structures of cymarin. (u) Mass spectrum and molecular structures of 2-hexadecen-1-ol

acid(7.45% with RT 39.102), neophytadiene (6.30% with RT 33.157), cytidine (4.60% with RT 25.068), squalene (4.51% with RT 48.760), stigmast-5-en-3-ol (4.23% with RT 57.337), Vitamin E (3.95% with RT 53.282), stigmasterol (3.21% with RT 55.862), dotriacontane (2.56% with RT 49.794), and 1,2-benzenedicarboxylic acid (2.12% with RT 45.474) in the whole plant extract. Several of these phytoconstituents are reported to possess pharmacological potential.

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 also facilitates to comprehend plant toxicity and also helps to protect human and animals from natural poisons. GC-MS plays a vital role in the analysis of unknown components of plant origin.

Dotriacontane has antimicrobial, antioxidant, antispasmodic, antibacterial, and antiviral. 9,12- Octadecadienoic acid has antimicrobial, antioxidant, anticancer, hypercholesterolemic, antiulcerogenic, lubricant, nematicide, anti-inflammatory, antiandrogenic and other activities. n-Hexadecanoic acid is an ester compound of fatty acid and possesses various activities such as antioxidant, anti-androgenic, flavor, hemolytic, hypocholesterolemic, nematicide, pesticide, and 5-alpha-reductase inhibitor.

Squalene and Vitamin E both show antioxidant activity and prevent the propagation of free radical reaction but squalene is a triterpene in nature and Vitamin E is a lipid soluble fatty acid. Squalene is also an antitumor agent. Stigmasterol is used for antiasthma, diuretic, anti-inflammatory, hypocholesterolemic agent. The presence of various bioactive compounds justifies the use of the whole plant recommended for phytopharmaceutical importance.

CONCLUSION

A. curassavica Linn. has been successfully cultivated, authenticated, and used for the identification of its organic components by GC-MS. The observed relative concentrations of various compounds were calculated by the use of gas chromatogram. Pesticide residues may have an adverse effect on a food chain, and the concentration should be within permissible range for safe human consumption of any herbal drug product. As per the results regarding the determination of contaminants due to organochlorides and organophosphates compounds by GC-MS analysis, the tested whole plant was found to be free from pesticidal residues. *A. curassavica* has been observed as an excellent source of many important secondary metabolites, especially steroidal, glycosidal, and terpenoidal compounds. It may be explored for the production of natural medicinal formulations in pharmaceutical drug industries including anticancer drugs on account of its potential antioxidant activity.

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