Available online on 15.02.2022 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

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Research Article

To perform Gas chromatography and Mass spectroscopy (GC-MS) analysis of *Achyranthes aspera* L. leaf extract

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Article Info:

Article History:

Received 11 December 2021
Reviewed 13 January 2022
Accepted 17 January 2022
Published 15 February 2022

Cite this article as:

Prakash V, To perform Gas chromatography and Mass spectroscopy (GC-MS) analysis of *Achyranthes aspera* L. leaf extract, Journal of Drug Delivery and Therapeutics. 2022; 12(1-s):1-3

DOI: http://dx.doi.org/10.22270/jddt.v12i1-s.5299

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Abstract

To understand the significance of herbal drugs and the mechanism of their preparations, the knowledge of the bioactive chemical constituents of plants is required. The knowledge of the chemical constituents of plants would be valuable in discovering the actual potential/value of folkloric remedies. Gas chromatography and Mass spectroscopy (GC-MS) is considered as the most promising technique to identify or find out the exact chemical constituents in different plant extracts possessing medicinal activity. The current study was designed to find out the bio-active constituents present in methanol leaf extract of *Achyranthes aspera* by using Gas chromatography and Mass spectroscopy. GC-MS chromatograms of leaf extracts revealed the presence of numerous phyto-chemicals by showing different peaks of different heights. 12 major peaks were seen in methanol leaf extract of this plant by using this technique. The major chemical compounds/constituents were Phytol (21.99%), 9,12-Octadecadienoicacid (Z,Z) (13.74%) and 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z) (13.35%). The occurrence of these active compounds in *A. aspera* may be associated directly or indirectly with its therapeutic potential.

Keywords: *Achyranthes aspera*, methanol leaf extract, phytochemicals, Gas chromatography and Mass spectroscopy (GC-MS)

INTRODUCTION

Due to the development of new and sophisticated techniques, phyto-chemical studies have attracted the attention of scientists throughout the world. These techniques played a significant role in the search for additional resources/materials of raw material for pharmaceutical industries[1]. Knowledge of the chemical constituents or bio-active components of plants is desirable because such information will be of value for the synthesis of complex chemical substances.

One of the most popular methods of studying phytochemical composition of plant extract is Gas Chromatography and Mass Spectroscopy (GC-MS) which is a very compatible and the most commonly used technique for the identification and quantification purpose. The unknown organic compound in a complex mixture can be determined by interpretation and also by matching the spectra with the reference spectrum[2]. GC-MS method used for the analysis of prepared plant extracts can be used as an interesting tool for testing the amount of some active principles in different plants used in cosmetics, drugs, pharmaceutical or food industry. Because of its very good separation ability which can produce a chemical fingerprint of high quality, GC-MS technique has become a prevalent technique for the analysis of phyto-chemicals. Moreover, with the coupled mass spectroscopy and the corresponding mass spectra database, the qualitative and the quantitative composition information/knowledge of the herb under investigation could be provided by GC-MS which will be extremely useful for elucidating the relationship between chemical constituents in herbal medicine and its pharmacology in further research[3]. Therefore, GC-MS technique can be a valuable tool in natural product research assisting in the separation and identification of biologically active volatile organic compounds[4]. This method also finds application in certain other areas such as medical, environmental, chemical engineering and law-enforcement fields.

Approximately 122 biologically active compounds have been identified only from 94 species of different plants. A conservative estimate of the number of flowering plants occurring on the planet is around 2,50,000. Of these, only about 6% have been screened for biological activity and 15% have been evaluated phyto-chemically by different methods. Consistent findings should be carried out to discover a probable abundance of medicinal constituents in these plants/plant parts[5].

Achyranthes aspera L., an erect or procumbent, annual or perennial herb of about 1-2 m is found on road sides, field boundaries and waste places as a weed throughout India, tropical Asia and other parts of the world up to an altitude of 2100 m. It grows throughout the tropical and warmer regions of the world and is also widespread in Baluchistan, Ceylon, Tropical Asia, Africa, Australia, America and Northern Bangladesh[6,7].

Traditionally, *A. aspera* is used in indigenous system of medicine as emenagogue, anti-arthritic, anti-fertility, anti-phlegmatic, anti-periodic, purgative, laxative, ecbolic, abentifacient, anti-helminthic, aphrodisiac, antiviral, anti-plasmodic, anti-hypertensive, anticoagulant, antitumor agent etc.[8,9]. It is also effective in controling or treating cough, asthma, oedema, renal dropsy, fistula, scrofula, skin rash, nasal, infection, chronic malaria, impotence, fever, piles and snake bites[10,11]. Because of above mentioned useful properties, GC-MS analysis was carried out of this plant to find out the exact chemical constituents responsible for producing medicinal effects.

MATERIALS AND METHODS

Collection and processing of plant material

Fresh leaves of *Achyranthes aspera* L. were plucked, collected and were brought to the laboratory for further examinations. *A. aspera* leaves were washed thoroughly under tap water and then treated with 2% Mercuric chloride. Thereafter, the parts were cut into smaller pieces for quick drying. The plant material thus obtained after drying was crushed into fine powder with the help of pestle mortar and stored in air tight containers at room temperature.

GC-MS analysis

GC-MS analysis was carried out using thermo GC model TRACE 1300 and thermo MS model TSQ 8000 (triple quadrupole) equipment employing the following conditions/features: Thermo TG 5MS Column having dimensions of "30 m × 0.25 mm × 0.25 µm", operated in electron impact mode at 70 eV. Helium (99.99%) was used as carrier gas at a constant flow rate of 1 mL per minute with an injection volume of 1 µL. An injector temperature of 250°C and an ion-source temperature of 280°C were regularly employed. The Oven program consisted of maintaining the temperature at 60°C for 2 minutes followed by an increase to 250°C at the rate of 15°C/minute and maintaining this temperature of 250°C for a further 15 minutes duration. Samples or extracts which dissolved in methanol were run fully at a range of 50-1000 m/z. The mass spectra were detected in 26 minutes and the percentage of each chemical constituent was calculated by comparing the average peak area to the total area.

Identification of component/s

Interpretation of mass spectrum of GC-MS was done by using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST Library. The name, molecular weight and structure of the components of the test materials were ascertained finally.

RESULTS AND DISCUSSION

GC-MS analysis of leaves of *Achyranthes aspera* revealed the presence of 12 major peaks in methanol extract. Phytol (21.99%), 9,12-Octadecadienoicacid (Z,Z) (13.74%) and 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z) (13.35%) were among the major compounds of methanol leaf extract. Phytol is a key acyclic diterpene alcohol that is a precursor for vitamins E and K and generally used along with simple sugar or corn syrup as a hardener in candies. Varadharaj and Kuppan[12] studied *Achyranthes aspera* whole plant extract for its phytochemical constituents by using GC-MS analysis method and revealed the occurence of phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy (2.95%), dl-(2-fluorophenyl)-glycine (2.18%), hexadecanoic acid, ethyl ester (3.28%), phytol (22.13%) and 9,12-octadecadienoic acid (Z,Z) (12.74%) as the major chemical constituents.

Phytochemical examination of the seeds of *A. aspera* revealed the presence of α-L-rhamnopyranosyl-(14)-(β-Dglucopyranosyluronicacid)-(13)-oleanolic acid, α-L-rhamnopyranosyl-(14)-(β-Dglucopyranosyluronicacid)-(13)-oleanolic acid-28-O-β-D- glucopyranoside and α-L-rhamnopyranosyl-(14)-(β-Dglucopyranosyluronic acid)-(13)-oleanolic acid-28-O-β-Dglucopyranosyl-(14)-β-Dglucopyranoside[13]. Hariharan & Rangaswami[14] and Ali[15] reported the isolation & identification of Saponins A and B in the seeds of *A. aspera*. Hence, Saponin A was identified as D-glucuronic acid and Saponin B was identified as β-D-galactopyranosyl ester of D-glucuronic acid. Along with these chemical constituents, certain other constituents were also isolated such as oleanolic acid, amino acids and hentriacontane. Chemical constituents like 10-tricosanone, 10-octacosanone and 4-tritriacontanone were also found in this plant by Ram et al.[16]. Danial[17] also reported the presence of many compounds such as polysaccharides, ecdysterone (harmone), achyranthine, betaine (alkaliods), vanillic acid, syringic acid, p-coumaric acid (phenolic acids), saponin A, saponin B (saponins), protein and carbohydrates in *A. aspera*.

Table 1: GC-MS spectral analysis of methanol extract of *Achyranthes aspera*

Peak No.	Retention time	Name of compound	Molecular formula	Peak area %
1	8.64	Benzoic acid, 2,6-bis[(trimethylsilyl)oxy], trimethylsilyl ester	C16H30O4Si3	1.87
2	11.12	1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	$C_{14}H_{44}O_6Si_7$	1.13
3	13.35	Methyl p-methoxycinnamate	$C_{11}H_{12}O_3$	0.86
4	15.34	Hexasiloxane, tetradecamethyl-	$C_{14}H_{42}O_5Si_6$	0.85
5	17.37	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	1.09
6	18.17	9,12-Octadecadienoicacid (Z,Z)	$C_{18}H_{32}O_2$	13.74
7	18.64	Phthalic acid, butyl isohexyl ester	$C_{18}H_{26}O_4$	1.62
8	19.81	Methyl 9-cis, 11-transoctadecadienoate	$C_{19}H_{34}O_2$	5.21
9	19.88	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)	$C_{19}H_{32}O_2$	13.35
10	19.99	Phytol	$C_{20}H_{40}O$	21.99
11	21.91	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)	$C_{21}H_{36}O_4$	2.60
12	25.00	Heneicosanoic acid, 18-propyl, methyl ester	$C_{25}H_{50}O_2$	1.52

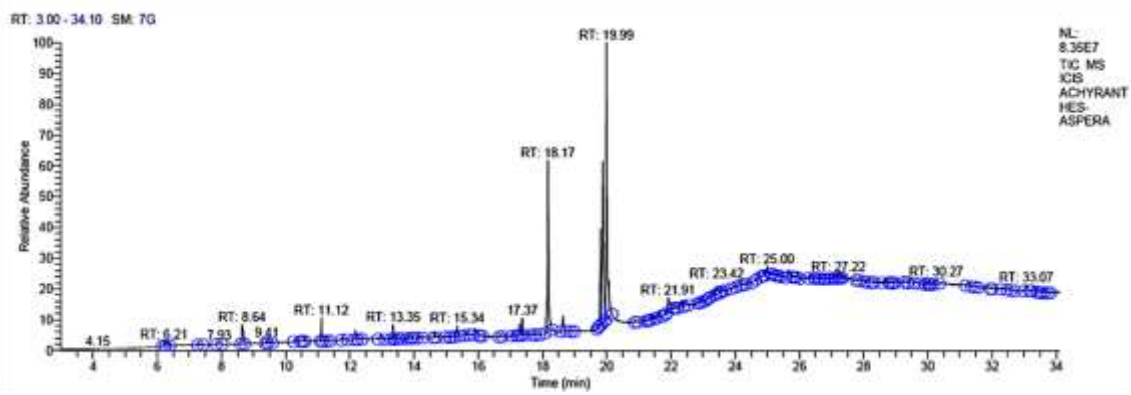

Figure 1: GC-MS chromatogram of methanol extract of *A. aspera*

CONCLUSIONS

The present study was carried out to find out the bio-active constituents present in methanol extract of *Achyranthes aspera* L. leaves by using Gas chromatography and Mass spectroscopy. GC-MS chromatograms of plant extracts revealed the presence of numerous phytochemicals by showing different peaks. GC-MS analysis of *A. aspera* revealed the presence of 12 major peaks in methanol leaf extract. The major chemical compounds were reported to be Phytol (21.99%), 9,12-Octadecadienoicacid (Z,Z) (13.74%) and 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z) (13.35%). The presence of these phyto-compounds in this plant may be directly or indirectly responsible for its valuable medicinal activities.

ACKNOWLEDGEMENT

Author greatly acknowledges the financial support provided by Indian Council of Medical Research (ICMR), New Delhi to conduct this work smoothly and efficiently.

CONFLICT OF INTERESTS

Author hereby declares no conflict of interest.

REFERENCES

1. Shakeri A, Hazeri N, Vlizadeh J, Ghasemi A, Tavallaei F, Photochemical screening, antimicrobial and antioxidant activity of *Anabasis aphylla* L. extracts, Kragujevac Journal of Science, 2012; 34:71-78.

2. Ronald HA, Gas Chromatography Mass Spectroscopy: Handbook of Instrumental Techniques for Analytical Chemistry, 1997; p. 609-611.

3. Jothy SL, Yeng C, Sasidharan S, Chromatographic and spectral fingerprinting of *Polyalthia longifolia*: A source of phytochemicals, Bioresource, 2013; 8:5102-5119. https://doi.org/10.15376/biores.8.4.5102-5119

4. Zhang Z, Li T, Wang D, Zhang L, Chen G, Study on the volatile profile characteristics of Oyster *Crassostrea gigas* during storage by a combination sampling method coupled with GC-MS, Food Chemistry, 2009; 1150-1157. https://doi.org/10.1016/j.foodchem.2008.12.099

5. Turker AU, Usta C, Biological screening of some Turkish medicinal plant extracts for antimicrobial and toxicity activities, Natural Product Research, 2008; 22:136-146. https://doi.org/10.1080/14786410701591663

6. Shafique S, Javaid A, Bajwa R, Shafiqe S, Biological control of Achyranthes aspera and *Xanthium strumarium* in Pakistan, Pakistan Journal of Botany, 2007; 39:2607-2610.

7. Akter A, Zuberi MI, Invasive alien species in Northern Bangladesh: Identification, inventory and impacts, International Journal of Biodiversity and Conservation, 2009; 1:129-134.

8. Selvanayagam ZE, Gnanavendan SG, Balakrishnan K, Rao RB, Anti-snake venom botanicals from ethnomedicine, Journal of Herbs, Spices and Medicinal Plants, 1994; 2:45-100. https://doi.org/10.1300/J044v02n04_06

9. Prakash V, Sagar A, Analysis of *Achyranthes aspera* L. extracts for their α- amylase and urease inhibitory activities, Journal of Drug Delivery and Therapeutics, 2021; 11(2):108-112. https://doi.org/10.22270/jddt.v11i2.4678

10. Singleton P, Bacteria in Biology, Biotechnology and Medicine. Fourth edition. New York, John Wiley and Sons Ltd. 1999.

11. Shukla R, Chakravarty M, Gautam MP, Indigenous medicine used for treatment of gynecological disorders by Tribals of Chhattisgarh, India, Journal of Medicinal Plants Research, 2008; 2:356-360.

12. Varadharaj V, Kuppan M, Identification and determination of bioactive phytochemical constituents from the hydro-alcoholic extract of *Achyranthes aspera* whole plant by gas chromatography-mass spectrometry analysis, Asian Journal of Pharmacy and Clinical Research, 2015; 8:125-129.

13. Sas K, Robotka H, Toldi J. Vecsei L, Mitochondrial, metabolic disturbances, oxidative stress and kynurenine system, with focus on neurodegenerative disorder, Journal of Neurological Sciences, 2007; 257:221-239. https://doi.org/10.1016/j.jns.2007.01.033

14. Hariharan V, Rangaswami S, Structure of saponins A and B from the seeds of *Achyranthes aspera*, Phytochemistry, 1970; 9:409-414. https://doi.org/10.1016/S0031-9422(00)85154-7

15. Ali M, Chemical investigations of *Achyranthes aspera* Linn., Oriental Journal of Chemistry, 1993; 9:84-85.

16. Ram P, Rastogi BN, Mehrotra M, Compendium of Indian medicinal plants. Central Drug Research Institute, Lucknow and National institute of science communication and information resources, New Delhi. 2004; 11:7-8.

17. Danial M, Medicinal Plants: Chemistry and properties. Oxford & IBH Publishing Co. Pvt. Ltd, New Delhi, India, 2006.