GC-MS ANALYSIS AND BIOACTIVE COMPONENTS OF FLOWERS OF *Bergenia ciliata*, A WEED OF ROCK CREVICES IN PAKISTAN

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DOI: https://doi.org/10.28941/pjwsr.v27i4.1012

ABSTRACT

Bergenia ciliata is a weed of hilly areas of Pakistan that generally grows in rock crevices. In the present study, flowers of this weed were collected from Murree, Pakistan. The dried flowers were extracted in methanol and subjected to GC-MS analysis that showed 7 compounds in it. The predominant compound was hexanedioic acid, bis(2-ethylhexyl) ester (48.88%) followed by γ-sitosterol (22.56%). Moderately occurring compound was cyclohexane, 1,3,5-triphenyl- (12.87%). The remaining compounds namely *n*-hexadecanoic acid (4.97%), pentadecanoic acid, 14-methyl-, methyl ester (3.77%), 9,12-octadecadienoic acid, methyl ester, (Z,Z)- (2.94%) and 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)- (2.92%) were categorized as less abundant compounds. Literature survey indicated that these compounds possess antimicrobial, larvicidal, anti-inflammatory, anticancer and/or antidiabetic properties.

Keywords: *Bergenia ciliata,* Bioactive compounds, Flower extract, Murree, Pakistan.

Citation: Ferdosi, M.F.H.; I.H. Khan; A. Javaid; H.M. Saeed; I. Butt and A. Munir. 2021. GC-MS Analysis and Bioactive Components of Flowers of *Bergenia ciliata*, A Weed of Rock Crevices in Pakistan. Pak. J. Weed Sci. Res., 27(4): 527-535.

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INTRODUCTION

Plants have been utilized for various purposes since early human civilization. Naturally originated plant-based products play an important role in the discovery process of drug (Ugboko *et al.,* 2020). These provide unlimited opportunities because of their unmatched chemical diversity with potential to develop innovative drugs (Anand *et al.,* 2019; Salmerón-Manzano *et al.,* 2020). Plants have a wide range of compounds that can be used for treatment of infectious as well as chronic diseases in traditional medicine system (Boy *et al.,* 2018; Khan and Javaid, 2020a; Javaid *et al.,* 2021). Chemically synthesized drugs have adverse effects therefore, these turned scientists' attention towards the ethnopharmacognosy (Zeidali *et al.,* 2021). Plant derived phytochemicals are broadly effective with much safer and less adverse effects. Many biological activities such as antimicrobial, anticancer, wound healing, antidiarrheal, analgesic, antioxidant have been found associated with the use of plants (Riaz *et al.,* 2008; Oladeji *et al.,* 2019; Javed *et al.,* 2021).

Bergenia ciliata belongs to family Saxifragaceae, is an evergreen small perennial weed plant that is commonly known as winter bergonia (Kumar *et al.,* 2020). It is mostly distributed in the cold and temperate regions of Pakistan, Nepal, Bhutan, India and some other countries (Ali *et al.,* 2020). It has been used in traditional ayurvedic medicine to treat several diseases including fever, diarrhea, pulmonary affections, cough, vomiting and menorrhagia (Ahmad *et al.,* 2018; Zafar *et al.,* 2019). *B. ciliata* contains a variety of substances belonging to terpenoids, flavonoids, sterols, glycosides and saponins (Hussain *et al.,* 2019). The major steps to use compounds of biologically importance from plant resources include extraction, pharmacological screening, isolation and identification (Oreopoulou *et al.,* 2019). The literature search on *B. ciliata* showed that very little work has been done on phytochemical analysis of flowers of this plant especially in Pakistan. Therefore, in the present study, *B. ciliata* flowers were extracted in methanol and its GC-MS analysis was done to explore its phytochemical profile.

MATERIALS AND METHODS

Collection of flowers

Flowers from healthy *B. ciliata* plants growing in a rock crevice at Hill Fruit Research Station, Sunny Bank, Murree, Pakistan were collected during March 2021 and packed in paper bags to avoid moisture and contaminants. These samples were shifted to the laboratory in Punjab University Lahore for further experimentation and analysis. Identification of the specimen was done by Dr. Faheem Arshad, Chairperson Department of Botany, University of Okara, Pakistan.

Fig. 1: *Bergenia ciliata* growing in crevices at Sunny Bank, Murree.

Preparation of Methanolic extracts

The flowers were kept in steel strays and dried thoroughly for 5 days at room temperature. To ensure complete moisture evaporation, the trays were placed at 35 °C in hot air oven for 24 h. Subsequently, the flowers were ground into fine powder with the help of a mortar and pestle. Thereafter, 10 g of fine powdered of *B. ciliata* flowers were soaked in 50 mL of methanol in a 250-mL conical flask and left for two weeks. The methanolic extract was separated from the dipped material by using muslin cloth and then filtered through double layered filter paper into a sterilized flask. Finally, 2 mm of extracted material was collected in a vial and subjected to GC-MS analysis for biochemical profiling of *B. ciliata*.

GC-MS analysis

Gas chromatography (model 7890B), Agilent Technologies, USA was used with the scan range of 50–500 m/z. The injection volume was 1 µL; model of the column used was DB 5MS along with the dimensions 30 m × 0.25 µm × 0.25 µm; the inlet temperature was 280 ºC; solvent delay time was 5 min and helium was used as a carrier gas with split less mode. The initial temperature of oven ramping was 80 ºC which was raised to 10 ºC more.

For mass spectroscopy, machine of model 5977A with MS search library having mass hunter/NIST 2017 version was used. The source and the quadrupole temperatures were 230 ºC and 150 ºC, respectively, with 50 min run time. Chemical compounds were identified by comparing their spectra with library NIST 2017. The compounds were arranged in an ascending order of their retention time. Further, the relative abundance was reported from respective peak areas. Structures of the identified compound were drawn by using ChemDraw Pro 8.0 software.

Literature survey

To figure out any previous reports on the bioactivity of compounds that were identified in the flower extract of *B. ciliata*, a comprehensive literature survey was done.

RESULTS AND DISCUSSION

GC-MS analysis indicated the presence of seven compounds in the methanolic flower extract of *B. ciliata* (Fig. 2). The predominant compound with 46.56% peak area was hexanedioic acid, bis(2-ethylhexyl) ester. γ-Sitosterol was the second most abundant compound with peak area of 21.06% followed by cyclohexane, 1,3,5-triphenyl- (12.07%). Other compounds included *n*-hexadecanoic acid (4.64%), pentadecanoic acid, 14-methyl-, methyl ester (3.52%), 9,12-octadecadienoic acid, methyl ester, (Z,Z)- (2.75%) and 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)- (2.73%) (Table 1). Their structures are shown in Fig. 3.

The predominant compound hexanedioic acid, bis(2-ethylhexyl) ester is a plasticizer derivative (Ghisari and Bonefeld-Jorgensen, 2009). However, it has been reported earlier from various living sources namely *Streptomyces* isolate TN262 (Elleuch *et al.,* 2010), stem of *Hugonia mystax* (Vimalavady and Kadavul, 2013), roots of *Stellera chamaejasme* (Xue-Na *et al.,* 2012), wood of *Populus tomentosa* and *P. lasiocarpa* (Peng *et al.,* 2017), and leaves of *Adenophyllum porophyllum* (Hernández-Ceja *et al.,* 2021). Its antifungal activity has been reported against *Fusarium* sp. (Elleuch *et al.,* 2010), *Monilinia fructicola* (Xue-Na *et al.,* 2012), *Lasiodiplodia pseudotheobromae* and *Colletotrichum gloeosporioides* (Hernández-Ceja *et al.,* 2021).

Table 1: Compounds identified in methanolic flower extract of *Bergenia ciliata* through GC-MS analysis.

Sr. No.	Names of compounds	Molecular formula	Molecular weight	Retention time (min)	Peak area (%)
1	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	270.45	14.367	3.77
2	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	14.785	4.97
3	9,12-Octadecadienoic acid, methyl ester, (Z,Z)-	$C_{19}H_{34}O_2$	294.47	16.009	2.94
4	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292.45	16.067	2.92
5	Hexanedioic acid, bis(2-ethylhexyl) ester	$C_{22}H_{42}O_4$	370.56	18.590	48.88
6	Cyclohexane, 1,3,5-triphenyl-	$C_{24}H_{24}$	312.4	19.156	12.87
7	γ-Sitosterol	$C_{29}H_{50}O$	414.70	25.434	22.56

Table 2: Bioactivity of components of methanolic leaf extract of quinoa.

Sr. No.	Names of compounds	Bioactivity	Reference
1	Pentadecanoic acid, 14-methyl-, methyl ester	Antifungal, antibacterial	Bashir *et al.* (2012)
2	*n*-Hexadecanoic acid	Anti-inflammatory, antioxidant, nematicidal, pesticidal, anti-androgenic flavor, mosquito larvicide, hemolytic	Kumar *et al.* (2010); Aparna *et al.* (2012)
3	9,12-Octadecadienoic acid, methyl ester, (Z,Z)-	Urine acidifier, anticancer, hypocholesterolemic, hepatoprotective	Duke (1992); Yu *et al.* (2005); Sermakkani and Thangapandian (2012)
4	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	Anti-inflammatory, anticancer, nematicidal, antihistaminic, anticoronary, insectifuge, Cardioprotective	Sermakkani and Thangapandian (2012); Godwin *et al.* (2015)
5	Hexanedioic acid, bis(2-ethylhexyl) ester	Antifungal	Elleuch *et al.* (2010); Xue-Na *et al.* (2012)
6	Cyclohexane, 1,3,5-triphenyl-	-	-
7	γ-Sitosterol	Anticancer, Antidiabetic	Balamurugan *et al.* (2011); Sundarraj *et al.* (2012)

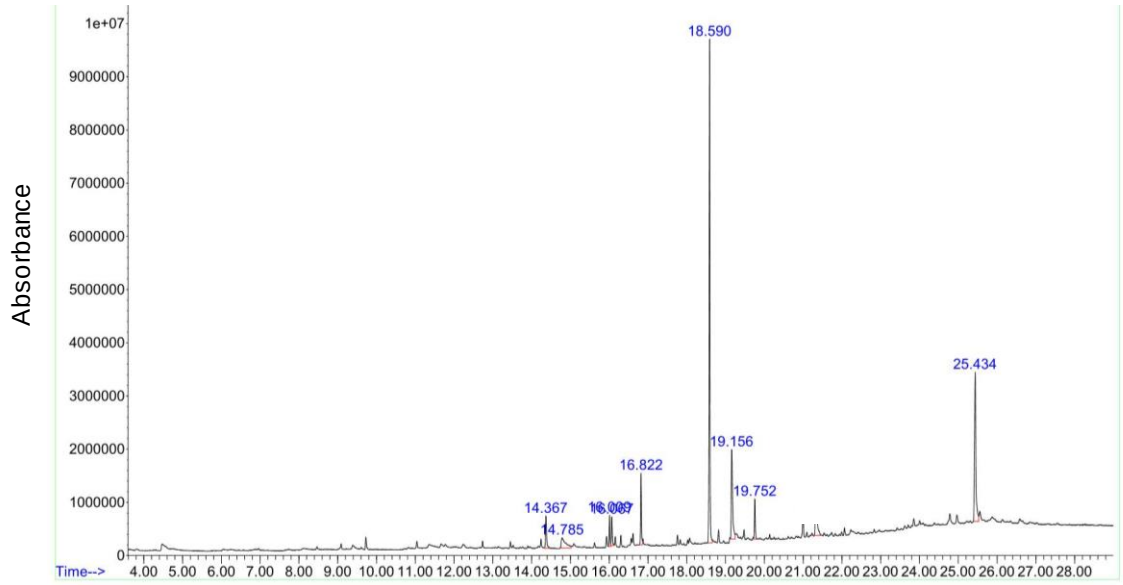

Fig. 2: GC-MS chromatogram of methanolic flower extract of *Bergenia ciliata.*

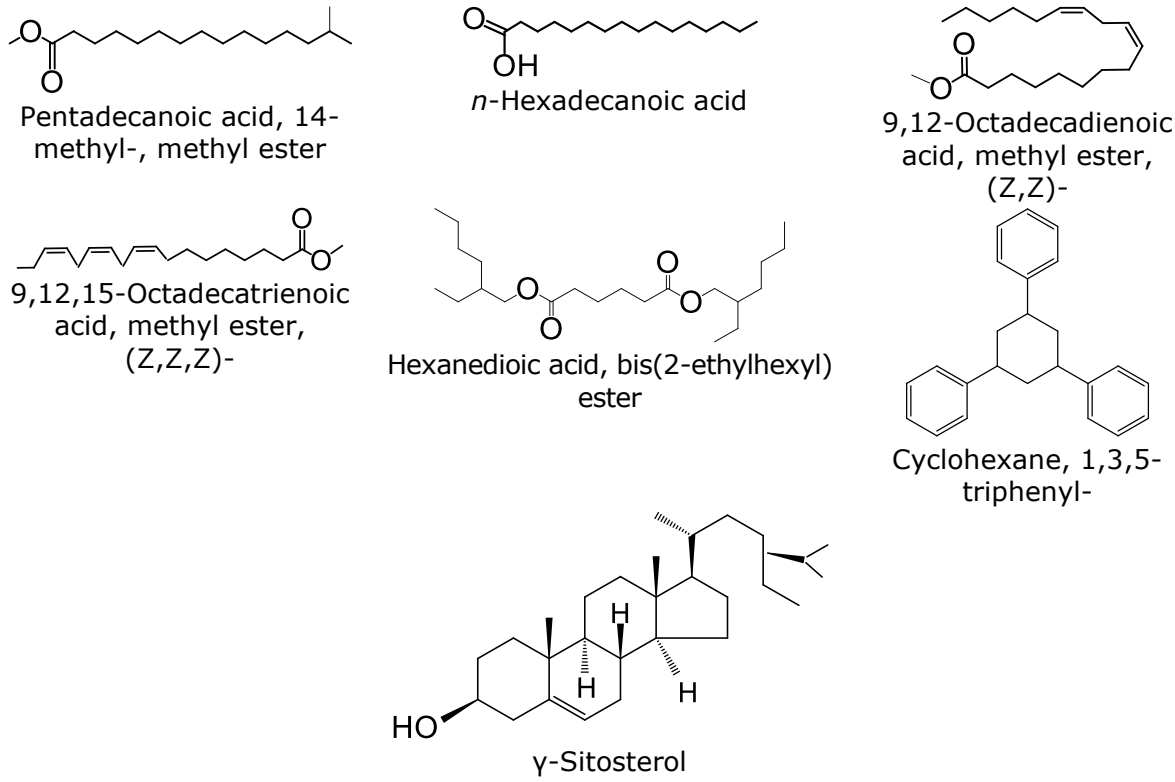

Fig. 3: Structures of compounds present in flower extract of *Bergenia ciliata.*

The second most abundant compound was γ-sitosterol. This naturally occurring steroid has been reported in many plant species including *Chenopodium quinoa* (Khan and Javaid, 2020b), *Calotropis procera* (Ferdosi *et al.,* 2021) and *Carthamus oxycantha* (Rafiq *et al.,* 2021). It has also been reported in metabolites of fungal species such as *Trichoderma pseudokoningii* (Khan ad Javaid, 2020c). Antidiabetic property of *Lippia nodiflora* is due to presence of γ-sitosterol. In addition, this compound also showed antihyperlipidemic activity by decreasing serum total cholesterol, triglycerides in treated rats (Balamurugan *et al.,* 2011). γ-Sitosterol isolated from *Acacia nilotica* showed marked anticancer activity by arresting cell cycle and the apoptosis on cancer cells (Sundarraj *et al.,* 2012). Extracts of four species of genus *Lagerstroemia,* with γ-sitosterol as the major component (14.70–34.44%), induced pronounced DNA damage in human peripheral blood mononuclear cells (Sirikhansaeng *et al.,* 2017).

n-Hexadecanoic acid or palmitic acid has many biological activities. Its occurrence has been reported in various plant species namely *Chenopodium mural*, *Coronopus didymus* and *Chenopodium quinoa* (Javaid *et al.,* 2018; Khan and Javaid, 2020b; Naqvi *et al.,* 2020), and fungal species such as *Trichoderma viride* (Khan *et al.,* 2021). It acts as an anti-inflammatory agent by designing of phospholipase A(2) inhibitors (Aparna *et al.,* 2012). It is also reported to have antimicrobial (Abubakar and Majinda, 2016), mosquito larvicidal (Rahuman *et al.,* 2000), hypocholesterolemic, antioxidant, 5-Alpha reductase inhibitor, nematicidal, pesticidal properties (Kumar *et al.,* 2010).

Pentadecanoic acid, 14-methyl-, methyl ester; 9,12-octadecadienoic acid, methyl ester, (Z,Z)- and 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)- are fatty acid methyl esters. These have been found in many plant species including *Ageratum conyzoides, Cannabis sativa* and *Coronopus didymus* (Banaras *et al.,* 2021; Javaid *et al.,* 2018, 2021). Methyl esters isolated from Euphorbia kansui showed anticancer activity by initiating growth inhibition and inducing apoptosis in tumor cells (Yu *et al.,* 2005). In additions, previous studies have also shown that fatty acid methyl ester extracts of *Arthrocnemum indicum*, *Suaeda monoica*, *Sesuvium portulacastrum, Salicornia brachiata*, *Excoecaria agallocha* and *Suaeda maritima* possess antifungal and antibacterial activities (Agoramoorthy *et al.,* 2007; Chandrasekaran *et al.,* 2008, 2011).

Conclusion

This study concludes that flowers of *B. ciliata* contain many biologically important compounds especially *n*-hexadecanoic acid, γ-sitosterol and fatty acid methyl esters.

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