PHYTOCHEMICAL PROFILE OF *BEAUMONTIA GRANDIFLORA* WALL. FLOWERS AND IDENTIFICATION OF POTENTIAL BIOACTIVE CONSTITUENTS

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ABSTRACT

Easter lily vine (*Beaumontia grandiflora* Wall.) of family Apocynaceae is a commonly cultivated ornamental plant. In the present study, its flowers were shade dried followed by grinding and soaking in pure methanol for one week. The extract was filtered and the filtrate was subjected to GC-MS analysis that showed the occurrence of 36 compounds. γ-Sitosterol (13.02%) was the principal compound followed by lupeol (10.50%), 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)- (9.18%), octadecane (7.37%), 9-tricosene, (Z)- (6.62%), hexadecanoic acid, methyl ester (5.78%), campesterol (4.16%), pentacos-1-ene (3.07%), olean-18-en-28-oic acid, 3-oxo-, methyl ester (2.71%), 2-methoxy-4-vinylphenol (2.64%), squalene (2.29%), 9,19-cyclolanost-24-en-3-ol, (3β)- (2.22%), pentacosane (2.08%) docosane (2.05%), ergosta-8,24(28)-dien-3-ol, 14-methyl-, (3β,5α)- (1.88%), (Z)-18-Octadec-9-enolide (1.61%), β-amyrin (1.46%), 26,26-dimethyl-5,24(28)-ergostadien-3β-ol (1.20%) and tetracosane (1.14%). The remaining compounds were less abundant with their peak area less than 1%. Literature survey revealed that the compounds present in *B. grandiflora* flowers possess antimicrobial, antihyperglycemic, antidiabetic, antihistaminic, anti-leukemia, anticancer, anti-inflammatory and/or nematicidal properties.

Keywords: Antimicrobial, *Beaumontia grandiflora*, Flower extract, Phytoconstituents.

INTRODUCTION

Plant derived products are a major source of therapeutic substances which are necessary for new drugs development (Atanasov *et al.,* 2021). These compounds contain unmatched chemical diversity; therefore, have received special attention since ancient times (Thomford *et al.,* 2018). These have certain biological activities in the field of pharmacology, medicine, cosmetics, agrochemicals, nanotechnology, and the food industry (Khan and Javaid, 2019, 2020; Ahmadu and Ahmad, 2021). Chemically synthesized drugs with increased microbial resistance have adverse effects on humans, turning scientist's attention towards ethnopharmacology (Süntar, 2020). Plant-based phytochemicals are safe, less adverse, and broadly effective alternatives (Naqvi *et al.,* 2020; Ferdosi *et al.,* 2021a). Bioactive compounds from plants can be used as antimicrobial, anticancer, antidiarrheal, antioxidant, wound healing and analgesic (Javaid *et al.,* 2021, 2022; Khan *et al.,* 2020, 2021).

Extraction followed by characterization of biologically active compounds are the premier steps to utilize pharmacological active compounds (Patra *et al*., 2018). Hence, GC-MS has gained widespread acceptance for the direct identification of compounds (Špánik and Machyňáková, 2018; Ferdosi *et al.,* 2021b). It is the most sensitive and selective method used widely for quantitative, qualitative, and molecular analysis of plant extracts (Abo-Altemen *et al.,* 2019). *Beaumontia grandiflora* is an evergreen plant indigenous to Asian tropical regions (Kulkarni, 2020). It is a woody climber cultivated for its elegant flowers in many parts of the world (Aslam and Afridi, 2018). It is generally cultivated in tropical regions of Asia including Pakistan, Nepal, Bangladesh, India, China, Myanmar, Laos, Cambodia, Thailand, and Vietnam (Flora of Pakistan, 1972). It also has medicinal values particularly possess strong antioxidant potential (Abdelshafeek *et al.,* 2010). It also has abortifacient, steroidogenic, and anti-implantation potential (Kulkarni, 2020). Very rare information is available about biological activities and chemical constituents of this species cultivated in Pakistan. Thus, the present study was carried out to evaluate the phytochemical profile of methanolic extracts of *B. grandiflora* flowers through GC-MS analysis and the identification of potential bioactive constituents.

MATERIALS AND METHODS

Collections of plant material

Flowers of *B. grandiflora* were collected from Lahore, Pakistan. The flowers were kept under shade for 10-days and thereafter, placed in a dry heat oven at 35 ºC for 2-days for complete removal of moisture contents.

Preparation of methanolic extract

The flowers were dried and grinded by using a pestle and mortar. The material was weighed and 50 g of it was soaked into high-grade methanol (100 mL) and placed at room temperature for two weeks. The mixture was filtered and the extracted solvent was collected into a glass vial.

GC-MS analysis and literature survey

The analysis was carried out according to Javaid *et al.* (2021) set standards for the identification of possible bioactive constituents from methanolic flower extract of *B. grandiflora*. The gas chromatography machine (model 7890B), Agilent Technologies, USA was selected to perform analysis. Helium was used as a carrier gas. The column (model DB 5MS) was used having 30 m × 0.25 µm × 0.25 µm dimensions with 1 µL of injection volume. The oven initial ramping temperature was 80 ºC which raised up to 300 ºC with an interval of 10 ºC per minute while inlet temperature was 280 ºC.

The sample was run for 28 min with a solvent delay time of 3 min in a mass spectroscopy machine (model 5977A), Agilent Technologies, USA. The Quadrupole and the source temperatures were 150 ºC and 230 ºC, respectively, with the scan range of 50-500 m/z.

A comprehensive literature survey was performed to figure out the bioactivities of identified compounds on the basis of findings of previous studies. Structures of the bioactive phytoconstituents were drawn by ChemDraw Pro 8.0 software.

RESULTS AND DISCUSSION

There were 36 compounds in the extract of *B. grandiflora* flowers as revealed through GC-MS analysis (Table 1; Fig. 1). γ-Sitosterol was the principal compound in the extract with 13.02% peak area. This compound has been reported as the frequently occurring compound in many other plant species including *Chenopodium quinoa* stem (Khan and Javaid, 2022a), *Lippia nodiflora* (Balamurugan *et al.,* 2011) and *Calotropis procera* (Ferdosi *et al.,* 2021c). It possesses antidiabetic (Balamurugan *et al.,* 2011) and anticancer activities (Sundarraj *et al.,* 2012). It is also among the major compounds of *Lagerstroemia* spp. with antihyperglycemic property (Sirikhansaeng *et al.,* 2017). Other frequently occurring compounds included lupeol (10.50%), 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)- (9.18%), octadecane (7.37%), 9-tricosene, (Z)- (6.62%), hexadecanoic acid, methyl ester (5.78%), campesterol (4.16%) and pentacos-1-ene (3.07%). Previously, lupeol was reported from *Monotheca buxifolia* as an antifungal compound against *Macrophomina phaseolina* (Javed *et al.,* 2021). It caused up to 90% inhibition in the growth of *Penicillium notatum* (Manzano *et al.,* 2013). It also showed antifungal activity against *Rhizoctoia phaseoli*, *Fusarium solani* and *Aspergillus niger* that was equivalent to the activity of fungicide miconazole (Singh and Singh, 2003). In addition, this compound has also been reported in grapes, mangoes, strawberry and olive with preventive effects against many disorders by acting as anticancer, anti-leukemia, hepatoprotective, cardioprotective and anti-inflammatory agent (Wal *et al.,* 2015). Likewise, 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)- was previously reported in quinoa leaves (Khan and Javaid, 2022b) and in many other plants, and is known for its nematicide, anticancer, antihistaminic, antiandrogenic, antieczemic, anti-inflammatory, hypocholesterolemia and anti-coronary properties (Devi and Muthu, 2014). Similarly, campesterol, a sterol, is present in some plant species such as *Vinca major*, *Cannabis sativa* and *Chrysanthemum coronarium* and has the potential to lower cholesterol and also has anticarcinogenic function (Choi *et al.,* 2007; Javaid *et al.,* 2021b, c).

Moderately occurring compounds included olean-18-en-28-oic acid, 3-oxo-, methyl ester (2.71%), 2-methoxy-4-vinylphenol (2.64%), squalene (2.29%), 9,19-cyclolanost-24-en-3-ol, (3β)- (2.22%), pentacosane (2.08%) docosane (2.05%), ergosta-8,24(28)-dien-3-ol, 14-methyl-, (3β,5α)- (1.88%), (Z)-18-octadec-9-enolide (1.61%), β-amyrin (1.46%), 26,26-dimethyl-5,24(28)-ergostadien-3β-ol (1.20%) and tetracosane (1.14%). Some of these compounds are known to have various biological activates. β-Amyrin was found inhibitory to various clinically important fungi such as *Candida stellatoidea*, *Trichophyton rubrum* and *Candida krusei* (Ogwuche *et al.,* 2014). Squalene belongs to triterpenes and mostly present in adequate quantity in amaranth, olive and palm oils, having antioxidant and antitumor properties (Huang *et al*., 2009).

The remaining compounds were less abundant with their peak area less than 1%. These included stigmasterol (0.99%), lup-20(29)-en-3-ol, acetate, (3β)- (0.94%), henicos-1-ene (0.84%), 1,7-hexadecadiene (0.82%), DL-α-tocophero (0.72%), 9,12-octadecadienoic acid (Z,Z)- (0.70%), 3-(α-hydroxyethyl)-aniline (0.68%), 9-nonadecene

(0.65%), eicosanoic acid, methyl ester (64%), pyridine, 1,2,3,6-tetrahydro-1-(phenylmethyl)- (0.52%), phenol, 2-methoxy- (0.51%), benzoic acid, methyl ester (0.48%), docosanoic acid, methyl ester (0.46%) and cholesterol (0.43%). Among these, 3-(α-hydroxyethyl)-aniline possesses antifungal potential against various fungal species (Haile and Dekebo, 2012). Stigmasterol is an unsaturated sterol that has been reported in many plant species with anti-diabetic and anti-inflammatory activities. In addition, its different derivatives namely fucosterol and spinasterol, possess many pharmacological properties (Kaur *et al.*, 2011; Wang *et al.*, 2017). 9,12-Octadecadienoic acid (Z,Z)-, methyl ester possesses many bioactive properties such as urine acidifier and uric acid inhibitor (Duke, 1992). Structures of bioactive compounds are presented in Fig. 2.

Table 1. Compounds identified in methanolic extract of *Beaumontia grandiflora* flowers through GC-MS analysis.

Sr. No.	Names of compounds	Molecular formula	Molecular weight	Retention time (min)	Peak area (%)
1	Phenol, 2-methoxy-	$C_7H_8O_2$	124.13	5.357	0.51
2	Benzoic acid, methyl ester	$C_8H_8O_2$	136.14	5.454	0.48
3	3-(α-Hydroxyethyl)-aniline	$C_8H_{11}NO$	137.17	5.603	0.68
4	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150.17	9.642	2.64
5	Pyridine, 1,2,3,6-tetrahydro-1-(phenylmethyl)-			19.751	0.52
6	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45	20.521	5.78
7	Henicos-1-ene	$C_{21}H_{42}$	294.6	22.789	0.87
8	9-Nonadecene	$C_{19}H_{38}$	266.5	22.880	0.65
9	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294.47	23.115	4.63
10	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292.45	23.227	9.81
11	Methyl stearate	$C_{19}H_{38}O_2$	298.5	23.597	1.29
12	(Z)-18-Octadec-9-enolide	$C_{18}H_{32}O$	280.45	23.901	1.61
13	Docosane	$C_{22}H_{46}$	310.6	24.688	2.05
14	4,11,11-Trimethyl-8-methylene-bicyclo[7.2.0]undec-4-ene	$C_{15}H_{24}$	204.35	24.870	0.42
15	1,7-Hexadecadiene	$C_{16}H_{30}$	222.40	25.645	0.82
16	9-Tricosene, (Z)-	$C_{23}H_{46}$	322.6	25.757	6.62
17	Octadecane	$C_{18}H_{38}$	254.5	26.142	7.37
18	Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$	326.55	26.490	0.64
19	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.44	27.378	0.70
20	Pentacos-1-ene	$C_{25}H_{50}$	350.66	28.795	3.07
21	Pentacosane	$C_{25}H_{52}$	352.68	29.229	2.08
22	Docosanoic acid, methyl ester	$C_{23}H_{46}O_2$	354.61	29.721	0.46
23	Tetracosane	$C_{24}H_{50}$	338.65	33.074	1.14
24	Squalene	$C_{30}H_{50}$	410.7	35.471	2.29
25	Lup-20(29)-en-3-ol, acetate, (3β)-	$C_{32}H_{52}O_2$	468.75	36.070	0.94
26	Olean-18-en-28-oic acid, 3-oxo-, methyl ester	$C_{31}H_{48}O_3$	468.71	37.032	2.71
27	Cholesterol	$C_{27}H_{46}O$	386.65	42.312	0.43
28	DL-α-Tocophero	$C_{29}H_{50}O_2$	430.71	42.953	0.72
29	26,26-Dimethyl-5,24(28)-ergostadien-3β-ol	$C_{30}H_{50}O$	426.7	44.826	1.20
30	Campesterol	$C_{28}H_{48}O$	400.7	45.093	4.16
31	Ergosta-8,24(28)-dien-3-ol, 14-methyl-, (3β,5α)-	$C_{29}H_{48}O$	412.7	45.328	1.88
32	Stigmasterol	$C_{29}H_{48}O$	412.7	45.724	0.99
33	9,19-Cyclolanost-24-en-3-ol, (3β)-	$C_{30}H_{50}O$	426.7	46.783	2.22
34	γ-Sitosterol	$C_{29}H_{50}O$	430.71	47.462	13.02
35	β-Amyrin	$C_{30}H_{50}O$	426.7	48.115	1.46
36	Lupeol	$C_{30}H_{50}O$	426.7	49.436	10.50

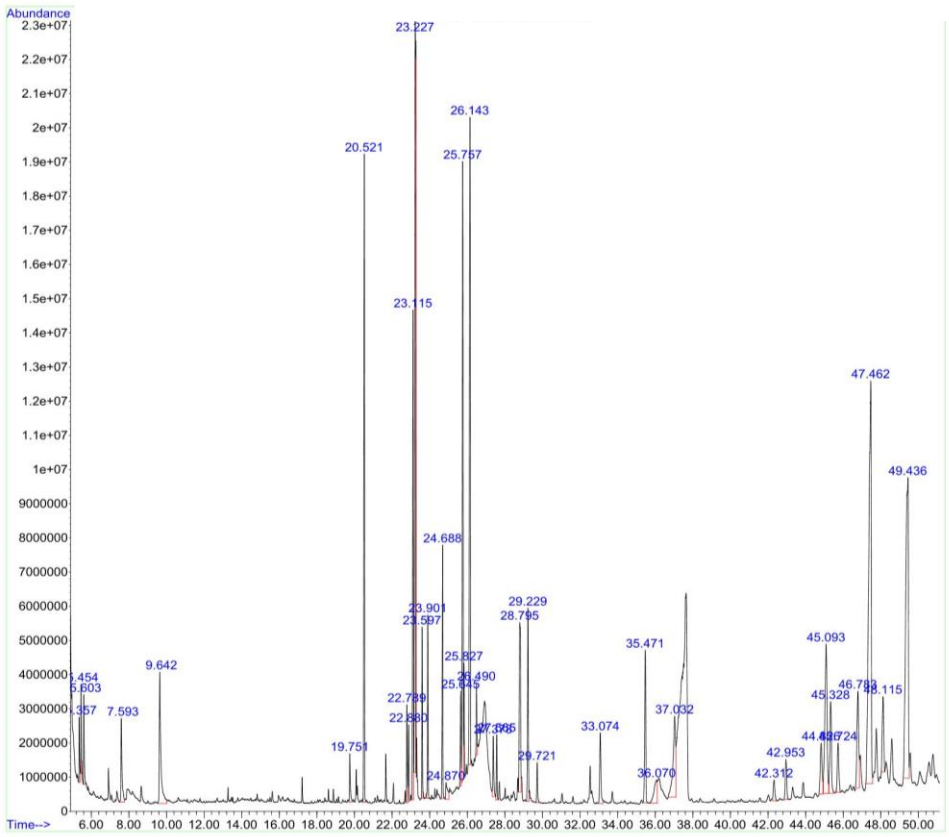

Fig. 1. GC-MS chromatogram of methanolic extract of *Beaumontia grandiflora* flowers.

γ-Sitosterol	Lupeol	Campesterol
β-Amyrin	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	Stigmasterol
Squalene	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	

Fig. 2. Structures of bioactive compounds in methanolic extract of *Beaumontia grandiflora* flowers.

Conclusion

GC-MS analysis showed the presence of 36 compounds in methanolic flower extract of *Beaumontia grandiflora*. Major compounds included the γ-sitosterol; lupeol; 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)-; octadecane and 9-tricosene, (Z)-. These compounds are known for various biological activities such as antimicrobial, antidiabetic, anticancer, antihyperglycemic, anti-leukemia, antihistaminic, anti-inflammatory and nematicidal.

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