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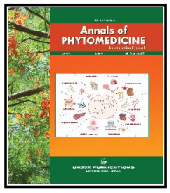

Original Article : Open Access

Biochemical and phytochemical analysis of Gypsophila (*Gypsophila paniculata* L.) using gas chromatography and mass spectroscopy (GC-MS)

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Abstract

Gypsophila (*Gypsophila paniculata* L.) necessitates the evaluation of its constituents in various fields. To detail its quality, this research was conducted to assess the biochemical and phytochemical constituents of Gypsophila at the Department of Postharvest Technology, Horticultural College and Research Institute, Periyakulam from 2022 to 2024. The study was planned in a completely randomized design with three replications. It included boric acid, sucrose, and calcium chloride at different concentrations of 0.1 to 4%, alongside a control with distilled water. The results indicate that the combination of 2% boric acid, 2% sucrose and 0.2% calcium chloride (T_2) was recorded in high levels of sugar content (9.3%), reducing sugar content (7.6%), protein content (18.1%), phenols content (3.9 mg/g), and carbohydrate content (7.2 mg/g). Major bioactive compounds identified in the Gypsophila including myo-inositol,4-C-methyl- (49.44%), stigmasterol (2.41%), hentriacontane (2.38%), 9-hexadecenoic acid (2.12%), benzoic acid, 4-ethoxy-, ethyl ester (1.70%), butyl dimethyl (12 methyleicosyloxy), silane(1.58%), 3-methyl-4-phenyl-1H-pyrrole (0.89%),vitamin E(1.33%), 2,4,7-tetra methyl-3,6,9-trioxa-2-siladecane (0.67%), t-tetracosanoic acid (0.95 %), phytol(0.95%), ethanone,1-(2-hydroxy-5-methyl phenyl)-(0.87%),1-azabicyclo hexane (0.80%), a-linolenic acid (0.59%), phenol,3,5bis (1,1dimethylethyl)-(0.69%), 21,3-propanedithiol (0.52%), 3-hydroxy-2,3-dihydromaltol (0.72%), and (3E,12Z)-nonadeca-1,3,12-triene-5,14-diol (1.51%).

1. Introduction

Gypsophila (*Gypsophila paniculata* L.) commonly known as baby's breath, is traditionally recognized for its ornamental value, but recent research highlights its potential medicinal properties. This plant, particularly its roots, contains saponins bioactive compounds known for their therapeutic benefits. Studies suggest that the saponins in Gypsophila exhibit antifungal, antibacterial, and antiviral properties, making the plant a potential agent in combating infections. For example, saponins enhance the permeability of cellular membranes, which can contribute to their antifungal efficacy by disrupting the cell walls of fungi (Jadimurthy *et al.*, 2023).

Additionally, some research points to the anti-inflammatory and immunomodulatory effects of these compounds, which could have broader applications in the treatment of diseases that involve inflammation and immune dysregulation (Shukla *et al.*, 2022). Furthermore, the cytotoxic properties of Gypsophila-derived saponins have been explored for potential cancer therapies, as they have been shown to induce apoptosis in certain cancer cell lines (Choudhary *et al.*, 2023). Despite these promising findings, more clinical research is needed to understand the scope of Gypsophila uses fully. Various pharmacological investigations have assessed the therapeutic properties of Gypsophila species.

Studies have revealed that extracts from *G. paniculata* and other species contain bioactive components like saponins and flavonoids, showcasing anti-inflammatory, antimicrobial, and antioxidant effects. Additionally, these extracts have demonstrated potential cytotoxic effects against cancer cells, suggesting possible applications in cancer therapy. As a result, Gypsophila continues to be a valuable subject in the field of pharmacological research (Dnan, 2006).

Herbal medicines have a long-standing history of being used to address various human infections and ailments, drawing from diverse biological sources such as roots, flowers, bark, stems, and leaves (Dash *et al.*, 2023). The most effective treatments for various diseases are produced from natural ingredients. These medications are sourced from nature's abundant resources and have proven beneficial in treating numerous health conditions (Salar *et al.*, 2022). The overall quality of herbal medicine can be influenced by several factors related to quality control, safety, and regulatory aspects. Factors influencing this include seasonal fluctuations, timing of harvest, cultivation areas, postharvest processing technique, the presence of adulterants or substitutes in raw materials, as well as extraction and preparation methods (Amrutanand *et al.*, 2021). Based on estimates from the World Health Organization (WHO), approximately 88% of people across 198

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countries depend on traditional medicines (Kommu *et al.*, 2023). In recent years, interest in natural products as potential sources for new medicines has grown considerably. Many of the drugs currently prescribed are derived from plant sources. Furthermore, natural products obtained from plants, animals, and minerals can be used in the treatment of various human diseases. This growing focus highlights the importance of exploring natural remedies in modern medicine. With this in mind, the present study was designed to assess the constituents of Gypsophila treated with specific vase solutions, utilizing gas chromatography-mass spectrometry (GC-MS).

2. Materials and Methods

The experiment was conducted at Postharvest Technology Laboratory, Department of Postharvest Technology, Horticultural College and Research Institute, Periyakulam, Theni District, which lies at the foothills of the Western Ghats of India, 10°12 of North latitude and 77°58 of East longitude. The experiment design used CRD and three replications and 10 treatment combinations are represented in Table 1.

The plant specimen was botanically identified and authenticated by Dr. R.Ramasubbu, Department of Biology (GUD Herbarium), Gandhigram Rural Institute, Gandhigram, Dindigul District, Tamil Nadu. Also, herbarium was submitted to Department of Biology (GUD Herbarium), Gandhigram Rural Institute, Gandhigram, Dindigul District, with Specimen Collection No. 317 for reference.

Table 1: Treatment details

T_1	1% boric acid, 1% sucrose, and 0.1% calcium chloride
T_2	2% boric acid, 2% sucrose, and 0.2% calcium chloride
T_3	2% boric acid, 3% sucrose, and 0.3% calcium chloride
T_4	3% boric acid, 1% sucrose, and 0.1% calcium chloride
T_5	3% boric acid, 2% sucrose, and 0.2% calcium chloride
T_6	3% boric acid, 3% sucrose, and 0.3% calcium chloride
T_7	4% boric acid, 1% sucrose, and 0.1% calcium chloride
T_8	4% boric acid, 2% sucrose, and 0.2% calcium chloride
T_9	4% boric acid, 3% sucrose, and 0.3% calcium chloride
T_{10}	Control (distilled water)

As post-harvest treatments boric acid, sucrose, and calcium chloride were used as vase solutions.

Figure 1: ***Gypsophila paniculata*** **L.**

2.1 Biochemical analysis

The biochemical components of Gypsophila flowers were analyzed by different methods, *viz.*, the total sugar content was measured phenol sulphuric acid method suggested by Dubois *et al.* (1956), reducing sugar content was measured by the Nelson-Somogyi method, total protein was measured by Lowry's method, total phenols content was measured by using the folin-ciocalteau method, carbohydrates was measured by anthrone method (Thimmaiah, 1999).

2.2 Extraction process

Fresh floral samples were collected and rinsed with tap water. They are dried in a shaded area for three to five days, depending on their moisture content. Once thoroughly dried, the flowers were finely ground using a blender. The resulting powder was transferred to conical flasks and directly extracted with methanol at a weight-to-volume ratio of 1:8. This mixture was stirred at 155 rpm for 24 h using an orbital shaker. Following extraction, the mixture was filtered through Whatman filter paper No. 40 (120 mm), was utilized to separate the solvent layer, resulting in a filtrate that was subsequently prepared for further analysis.

2.3 Gas chromatography-mass spectrometry (GC-MS) analysis

To commence the extraction process, a specified amount of Gypsophila floral powder was placed in a sealed flask, and methanol was added. The mixture was allowed to steep for 24 h before being filtered and evaporated using a vacuum distillation apparatus. The resulting residue was subsequently analyzed with a GC-MS system. The analysis employed a Thermo GC Ultra Clarus 550 system, which combines a gas chromatograph with a mass spectrometer (GC-MS) and utilizes an elite-I fused RMS 6 silica capillary column made of dimethylpolysiloxane. Detection was performed with an electron ionization source set at an ionization energy of 60 eV. A 1 µl sample was injected with a split ratio of 12:1, using helium (99.9%) as the carrier gas at a constant flow rate of 2 ml/min. The ion source and injector temperatures were maintained at 230°C and 240°C, respectively. The oven temperature was programmed to start at 90°C, increasing at a rate of 5°C per minute until reaching 240°C, after which, it remained isothermal for 3 min. Mass spectra were recorded for fragments within the range of 50 to 650 Da, with a scanning interval of 0.5 sec. The mass spectra and chromatograms were analyzed using Turbo Mass software, allowing for the determination of the percentage composition of each component by comparing the average peak area of each component to the total peak area (Damale *et al.*, 2023).

Methanol is commonly used as a solvent in the extraction of bioactive compounds from plant materials because it effectively dissolves a broad range of polar and non-polar compounds. Its low boiling point allows for easier evaporation and concentration of extracts. Additionally, methanol is relatively less toxic compared to other organic solvents, making it a safer option for extraction processes.

2.4 Identification of bioactive components

The mass spectra generated from the GC-MS analysis were analyzed using the National Institute of Standards and Technology (NIST) database 2008, which includes retention values for over 95,000 compounds. Spectra from both the NIST and Wiley libraries were employed to match unknown components with known substances. This approach enabled the identification, molecular weight determination, and compositional analysis of the test materials.

2.5 Biological activity of identified substances

The biological effects of the identified compounds were predicted using PASS (prediction of activity spectra for biologically active substances) based on their structural formulas. This method, as outlined by Filimonov *et al.* (2014), through the PASS online database, enabled forecasting of potential pharmacological activities, toxicities, and mechanisms of action associated with the compounds.

3. Results

3.1 Biochemical analysis

The findings from the biochemical analysis of Gypsophila are summarized in Table 2.

3.1.1 Total sugar (%)

From Table 2 and Figure 2, it was observed that the total sugar content ranged from 6.2 to 9.3 per cent. The highest sugar content of 9.3 per cent was recorded by T_2. It was followed by T_1 (8.8). However, the T_{10} registered the lowest total sugar content of 6.2 per cent.

3.1.2 Reducing sugar (%)

From Table 2 and Figure 2, it was observed that the reducing sugar content varied between 5.4 and 7.6 per cent, with the highest value observed at 7.6 per cent recorded by T_2. It was followed by T_1 (7.3). However, the T_{10} registered the lowest reducing sugar content of 5.4 per cent. Treatment T_2 (2% boric acid, 2% sucrose, and 0.2% calcium chloride) resulted in higher levels of both sugar types compared to the control and other treatments. Data represent the means of three replicates ± SE, with significant differences observed at $p < 0.05$, respectively.

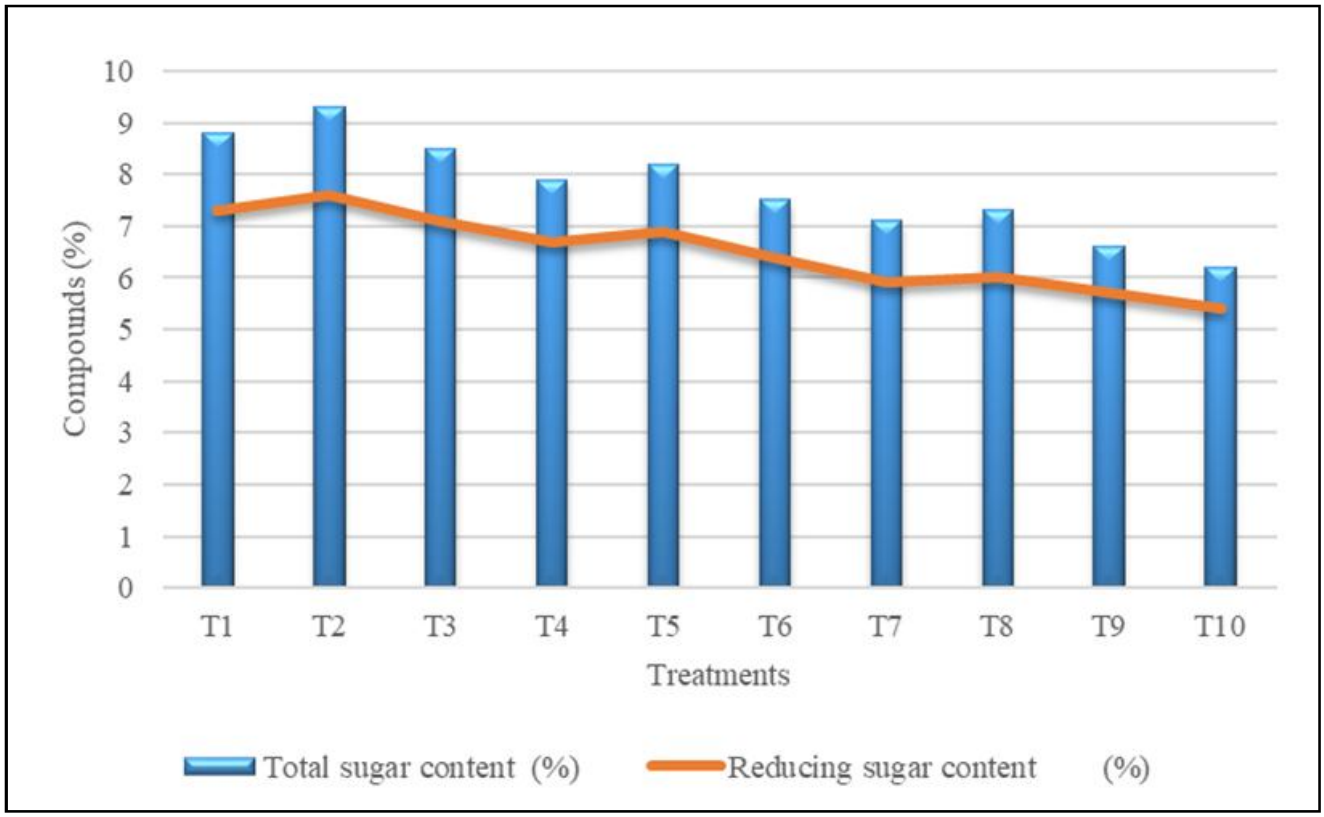

Figure 2: Total sugar content and reducing sugar content of the treated Gypsophila.

3.1.3 Phenol (mg/g)

From Table 2 and Figure 3, it was observed that the phenol content ranged from 1.9 to 3.9 per cent. The highest phenols of 3.9 per cent were recorded by T_2. It was followed by T_1 (3.7). However, the T_{10} registered the lowest phenol content of 1.9 per cent.

3.1.4 Protein content (mg/g)

From Table 2 and Figure 3, it was observed that the protein content ranged from 14.2 to 18.1 per cent. The highest protein content of 18.1 per cent was recorded by T_2. It was followed by T_1 (17.8). However, the T_{10} registered the lowest protein content of 14.2 per cent

3.1.5 Carbohydrate (mg/g)

From Table 2 and Figure 3, it was observed that the carbohydrate content ranged from 4.5 to 7.2 per cent. The highest carbohydrate content of 7.2 per cent was recorded by T_2. It was followed by T1 (7.6). However, the T_{10} registered the lowest carbohydrate content of 4.5 per cent. Treatment T_2 (2% boric acid, 2% sucrose, and 0.2% calcium chloride) led to a notable increase in all measured components when compared to the control and other treatments. The values are presented as the mean of three replicates ± SE, showing statistically significant differences at $p < 0.05$, respectively.

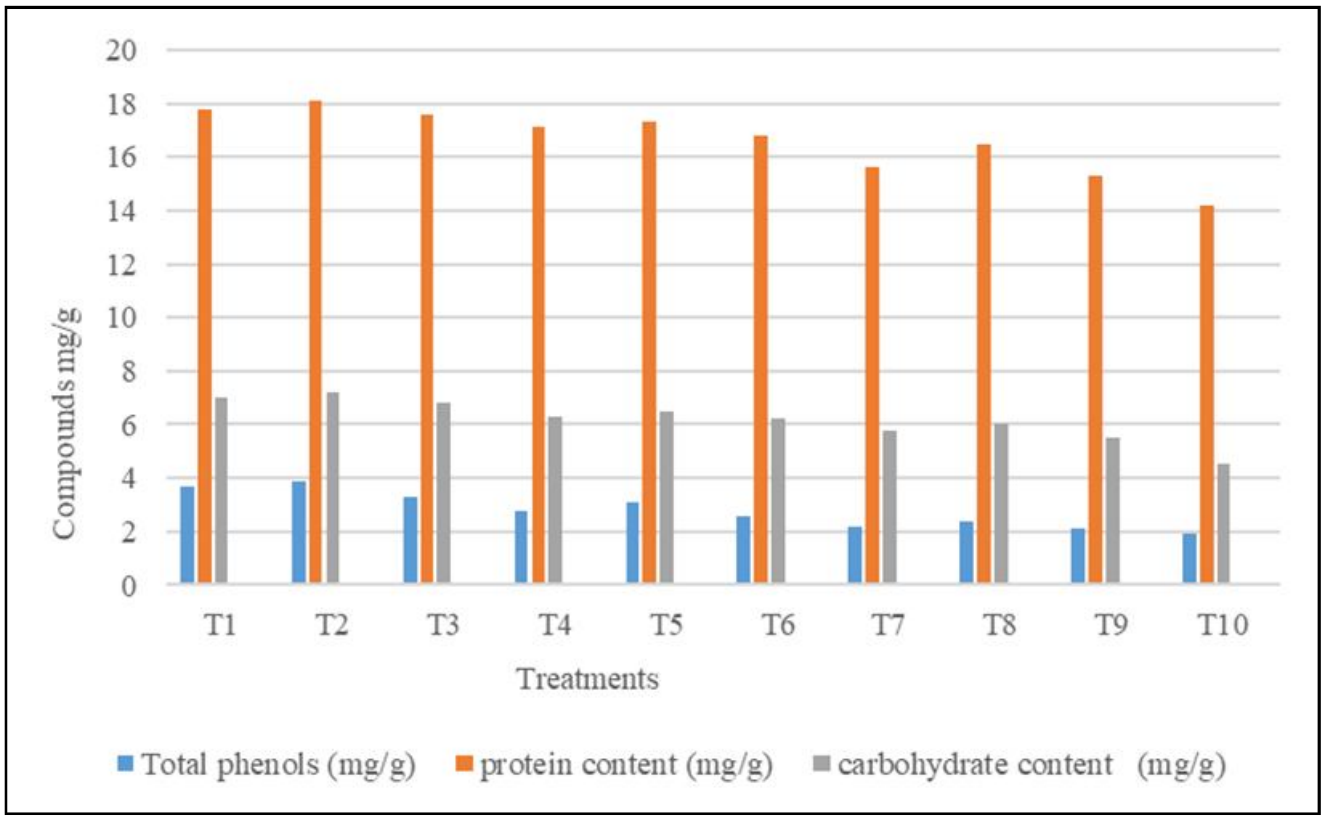

Figure 3: Total phenolic content, protein and carbohydrate levels of Gypsophila under various treatments.

Table 2: Effect of different postharvest holding solutions on total sugar (%), reducing sugar (%), total phenols (mg/g), protein content (mg/g), and carbohydrate content (mg/g) in Gypsophila

Treatments	Total sugar content (%)	Reducing sugar content (%)	Total phenols (mg/g)	Protein content (mg/g)	Carbohydrate content (mg/g)
T_1	8.8	7.3	3.7	17.8	7.0
T_2	9.3	7.6	3.9	18.1	7.2
T_3	8.5	7.1	3.3	17.6	6.8
T_4	7.9	6.7	2.8	17.1	6.3
T_5	8.2	6.9	3.1	17.3	6.5
T_6	7.5	6.4	2.6	16.8	6.2
T_7	7.1	5.9	2.2	15.6	5.8
T_8	7.3	6.0	2.4	16.5	6.0
T_9	6.6	5.7	2.1	15.3	5.5
T_{10}	6.2	5.4	1.9	14.2	4.5
Mean	7.7	6.5	2.7	16.6	6.1
SE (d)	0.181	0.140	0.053	0.357	0.114
CD (p = 0.05)	0.3787	0.2927	0.112	0.746	0.239
CV%	2.87	2.64	2.35	2.64	2.28

3.2 Metabolite profiling using GC-MS analysis

Amongst the treatments, T_2 was selected for metabolite profiling through GC-MS analysis. The chromatogram and mass spectrum (Figures 4 to 7) demonstrate that Gypsophila contains a variety of compounds. Table 3 presents the identified compounds including their RT (retention time), percentage peak area. A total of 81 compounds were detected in Gypsophila, with notable compounds including myo-inositol,4-c-methyl (49.44%), 9,12,15-octadecatrie-

noic acid, (Z, Z, Z)-(0.59%), stigmasterol (2.4%), hentriacontane (2.38%), 9-hexadecenoic acid (2.12%), benzoic acid, 4-ethoxy-, ethyl ester (1.70%), vitamin E (1.33%), t-tetracosanoic acid (0.95%), phytol (0.95%), 3-me-4-ph-pyrrole (0.89%), 1-hydroxy-2-acetyl-4-methylbenzene (0.87%), 1-azabicyclo[3.1.0] hexane (0.80%), 3-hydroxy-2,3-dihydromaltol (0.72%), phenol, 3,5-bis(1,1-dimethylethyl)- (0.69%), E,E, Z-1,3,12-nonadecatriene-5,14-diol (1.51%),1,3-propanedithiol(0.52%), TMS derivative (0.67%) and butyldimethyl-(12-methyleicosyloxy) silane (1.58%) were identified.

Table 3: Different bioactive compounds present in the Gypsophila with their IUPAC name, molecular weight, peak area, and retention time

S.No.	Compound name	IUPAC name	Molecular weight (g/ mol)	Peak area (%)	Retention time (min)
1.	2,5-furandione, 3-methyl-	2-,ethylmaleic anhydride	112.08	0.15	4.2874
2.	Pyrrolidine, N-(3-methyl-3-butenyl)-	1-(3-methyl but-3-enyl) pyrrolidine	139.24	0.24	4.3985
3.	Methane, (methylsulfinyl) (methylthio)-	Methylsulfanyl (methylsulfinyl) methane	124.23	0.15	4.5762
4.	Cyclohexane, azido-	Azidocyclo hexane	125.17	0.66	4.8762
5.	2-pentanamine, N-ethyl-4-methyl-	Octan-1-amine	129.24	0.38	4.9428
6.	3-quinuclidinyl acetate	Quinuclidin-3-ylacetate	169.22	0.17	5.1650
7.	Piperazine, 1,4-dimethyl-	1,4-dimethyl piperazine	114.19	0.14	5.2539
8.	Isopentylidene isopentyl amine	3-methyl-n-(3-methylbutylidene)-1-butanamine	155.28	0.13	5.3539
9.	Benzeneacetaldehyde	2-phenylacetaldehyde	120.15	0.47	5.4206
10.	Piperidine, 4-methyl-	4-methyl piperidine	99.17	0.18	5.5317
11.	7-deoxy-7(S)-chlorolincomycin	7-chlorolincomycin	424.98	0.06	5.6428
12.	azabicyclo[3.1.0] hexane	Azabicyclo[3.1.0] hexane	83.13	0.80	5.8316
13.	1,3-propan edithiol	Propane-1,3-dithiol	108.23	0.52	5.9316
14.	2,3-dihydro-3,5-dihydroxy-6-methyl-4-pyrone	3-hydroxy-2,3-dihydromaltol	144.12	0.72	6.4427
15.	Benzenemethanol, .alpha.-ethyl-	1-phenylpropan-1-ol	136.19	0.12	6.5316
16.	Benzoic acid, 2-ethylbutyl ester	2-ethyl butyl benzoate	206.28	0.19	6.7204
17.	1,1,3,3-tetramethyl-1,3-disilacycobutane	1,3-disilacyclobutane, 1,1,3,3-tetramethyl-	144.36	0.27	6.9982
18.	1,4-bis[(methyl)nitrosoamino] benzene	N1,N4-Dimethyl-N1,N4-dinitroso-1,4-benzenediamine;	150.18	0.28	7.1426
19.	3-hydroxy lauric acid	3-oH lauric acid	216.32	0.08	7.9203
20.	o-Acetyl-p-cresol	2-hydroxy-5-methyl acetophenone	150.17	0.87	8.1092
21.	Phenylthio(trimethylsilyl)methane	Trimethyl (phenylthiomethyl) silane	206.32	0.69	9.7756
22.	4-ethoxy ethylbenzoate	p-ethoxyethyl benzoate	194.23	1.70	9.9534
23.	1-(2-Methoxy-1-methylethoxy)-2-propanol	PPG-2 methyl ether	220.38	0.67	10.1089
24.	3-me-4-Ph-pyrrole	3-methyl-4-phenyl-1H-pyrrole	157.21	0.89	10.8866
25.	1-methylcyclohexane-1,2,3,4,5,6-hexol	1-methyl-1,2,3,4,5,6-cyclohexanehexol	194.18	49.44	1.6199
26.	9-cis-Hexadecenoic acid	Palmitolinoleic acid	254.41	0.66	12.9864
27.	(E,R,R)-PHYTOL	(E,7R,11R)-3,7,11,15-tetramethy lhexadec-2-en-1-ol	296.5	0.95	14.0974
28.	a-Linolenic acid	alpha linolenic acid	278.4	0.59	14.2640
29.	Octadecanoic acid	octadecanoic acid	284.5	0.68	14.3973
30.	9-hexadecenoic acid	(E)-hexadec-9-enoic acid	254.41	2.12	13.1086
31.	Glycerol 1-palmitate	2,3-dihydroxy propyl hexadecanoate	330.5	0.92	16.4082
32.	Di(2-ethylhexyl) phthalate	Palatinol AH	390.6	0.42	16.5082

33.	E,E,Z-1,3,12-nonadecatriene-5,14-diol	(3E,12Z)-nonadeca-1,3,12-triene-5,14-diol	294.5	1.51	17.3525
34.	Heneicosyl heptafluorobutyrate	Henicosyl 2,2,3,3,4,4,4-heptafluorobutanoate	508.6	0.30	18.5524
35.	Hentriacontane	Hentriacontane	436.8	2.38	19.7745
36.	Vitamin E	Ascorbutina	176.12	1.33	20.4966
37.	t-Butyldimethyl-(12-methyleicosyloxy) silane	Tert-butyl-dimethyl-(12-methylicosoxy)silane	426.8	1.58	20.5633
38.	Tetracosanoic acid	Tetracosanoic acid	368.6	0.95	20.9410
39.	Stigmasterin	Delta5-Stigmasterol	412.7	2.41	22.7630
40.	Trichlorpyrphos	Danusban	350.6	0.49	13.2975

Table 4: Gypsophila compounds and their pharmaceutical activity identified by gas chromatography and mass spectrometry (GC-MS)

Compound name	Pharmaceutical activity
2,5-furandione, 3-methyl-	Antimicrobial properties and the ability to inhibit biofilm formation.
Pyrrolidine, N-(3-methyl-3-butenyl)-	Pharmaceutical research and drug development strategies.
Methane, (methylsulfinyl)(methylthio)-	Fungal inhibition and mycotoxin suppression activity.
Cyclohexane, azido-	Significantly boosting antioxidant activity.
2-pentanamine, N-ethyl-4-methyl-	A foundation employed for amphoteric surfactants, corrosion inhibitors, and emulsifying agents.
Aceclidinum	Mitigation of psychotic disorders and cognitive impairments.
Piperazine, 1,4-dimethyl-	Nematode-targeting agents.
1-butanamine, 3-methyl-N-(3-methylbutylidene)-	Applications in pharmaceuticals and agrochemicals.
Benzeneacetaldehyde	Utilized as a flavoring agent or additive.
Piperidine, 4-methyl-	Produce bioactive compounds.
Clindamycin	It is used to treat a range of bacterial infections, such as those involving the skin, respiratory system, and soft tissues.
1-azabicyclo[3.1.0]hexane	Antimicrobial, antifungal, and anticancer properties.
1,3-propanedithiol	Bacterial inhibition properties.
Methyl fumarate	Applications in managing osteoporosis, diabetes, and cardiovascular diseases.
Benzenemethanol, .alpha.-ethyl-	Employed as a solvent, antioxidant, fragrance, and metabolic byproduct.
Benzoic acid, 2-ethyl butyl ester	Repellent for ticks, chiggers, and mosquitoes, as well as a pediculicide and scarified in dogs.
1,1,3,3-tetramethyl-1,3-disiletane	The development of novel pharmaceuticals and therapeutic agents.
p-toluidine, N-methyl-N-nitroso-	Dyes and the manufacture of organic chemicals.
Dodecanoic acid, 3-hydroxy-	Disorders related to fatty acid metabolism.
Ethanone, 1-(2-hydroxy-5-methylphenyl)-	It also acts as a flavoring agent and can be evaluated through RP-HPLC for impurity isolation and pharmacokinetic studies.
3,5-di-tert-butyl phenol	It demonstrates antifungal and antibiofilm properties and is categorized as a volatile organic compound, while also enhancing the accumulation of reactive oxygen species (ROS).
1-(2-Methoxy-1-methylethoxy)-2-propanol,	A stabilizing and solubilizing agent for diverse pharmaceutical formulations.
3-methyl-4-phenyl-1H-pyrrole	Aminopyrine, antipyrine, dipyrone, propyphenazone, and ramifenazone are all analgesic and antipyretic agents.
Myo-Inositol, 4-C-methyl-	This compound is utilized to treat insulin resistance, gestational diabetes, polycystic ovary syndrome (PCOS), and depression-related disorders.
cis-9-Hexadecenoic acid	It acts as a biomarker for triglyceridemia, abdominal fat accumulation, and lipogenesis, while also stimulating insulin in muscle tissues as a lipid hormone.
Phytol	It is involved in the synthesis of vitamins E and K1 modulates transcription through PPAR-alpha and RXR pathways, and also displays antibacterial and anticancer properties.
(9,12,15)-linolenic acid	A micronutrient nutraceutical that has the potential to reduce the risk of cardiovascular disease.

Octadecanoic acid	An emulsifying agent used in pharmaceutical tablets and capsules to improve their stability and bioavailability.
9-hexadecenoic acid	Typically present in cheeses made from goat and sheep milk, as well as in partially hydrogenated vegetable oils.
Glycerol 1-palmitate	Biochemical studies aimed at distinguishing enzymes that hydrolyze or transfer monoacylglycerols, serving as a biomarker.
Fleximel	Commonly used in coatings and adhesives and as a solvent in various applications. Also used plasticizer in polyvinyl chloride (PVC)
(3E,12Z)-1,3,12-Nonadecatriene-5,14-diol	Shows promising medicinal potential as both an antimicrobial and anti-inflammatory agent.
Heneicosyl heptafluorobutyrate	Improving the solubility and stability of active ingredients in agricultural formulations.
Hentriacontane	Frequently present in natural sources and utilized across a range of industrial applications.
Vitamin E	Vitamin E is a fat-soluble antioxidant that promotes skin health by reducing signs of aging, addressing hyperpigmentation, aiding in healing, and providing moisture to dry skin.
t-Butyldimethyl-(12-methyleicosyloxy)silane	A protective group in organic synthesis and a reagent for synthesizing various compounds.
Tetracosanoic acid	A potential therapeutic option for neurological disorders and an excipient in pharmaceutical formulations.
Stigmasterol	It has the potential to lower cholesterol and reduce inflammation.

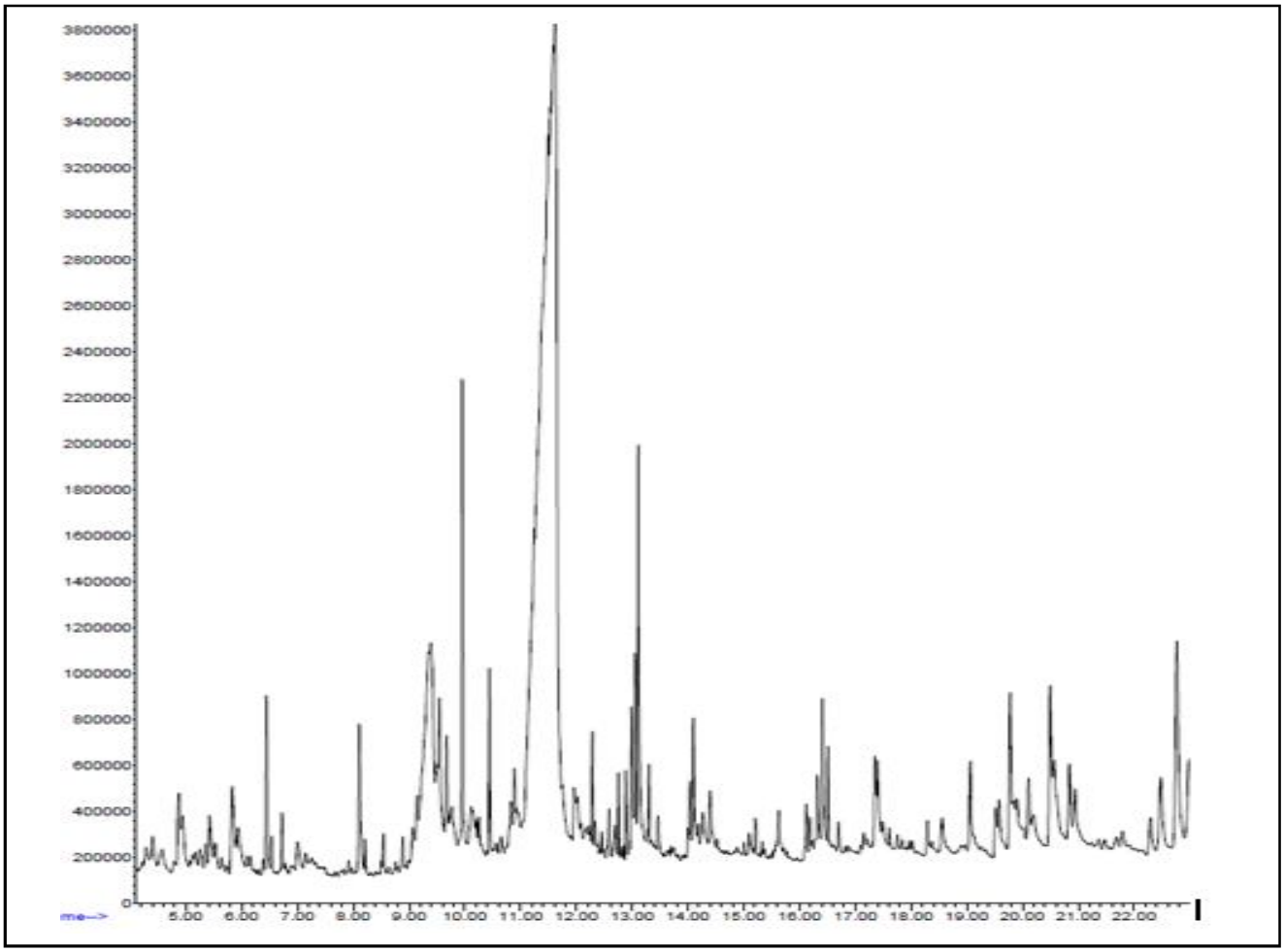

Figure 4: Chromatogram of methanol extract of Gypsophila by GC-MS.

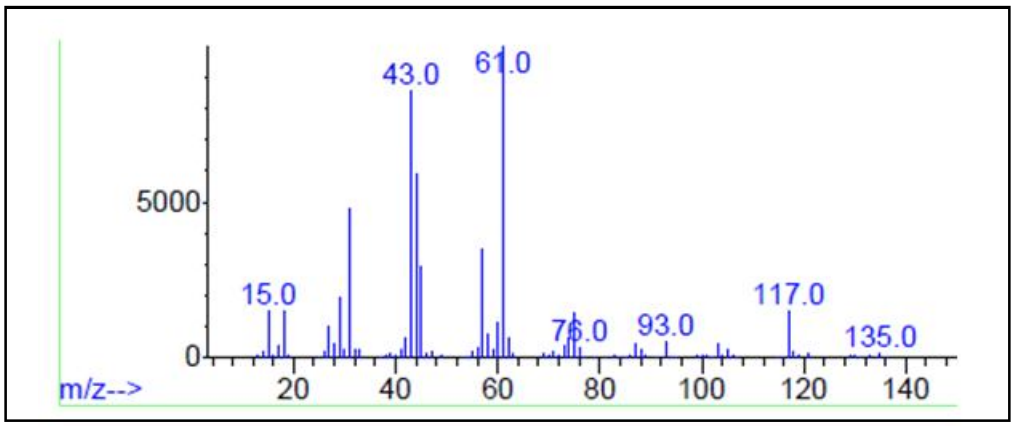

Figure 5: Mass spectrum of diglycerol (0.48%).

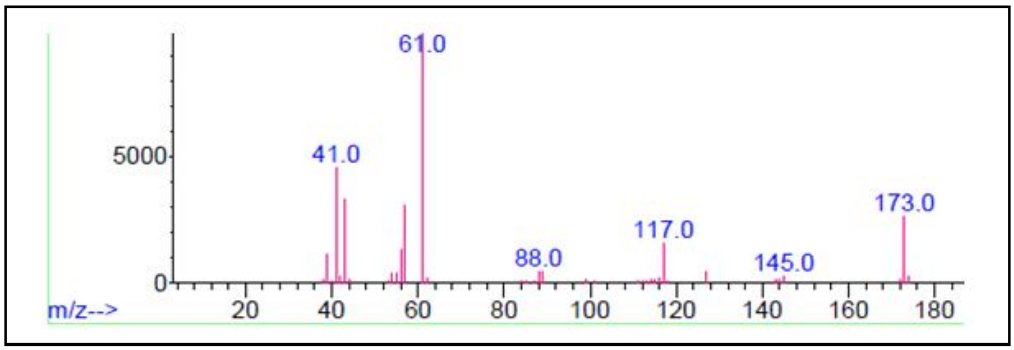

Figure 6: Mass spectrum of bis (butoxy) propionic acid (0.42%).

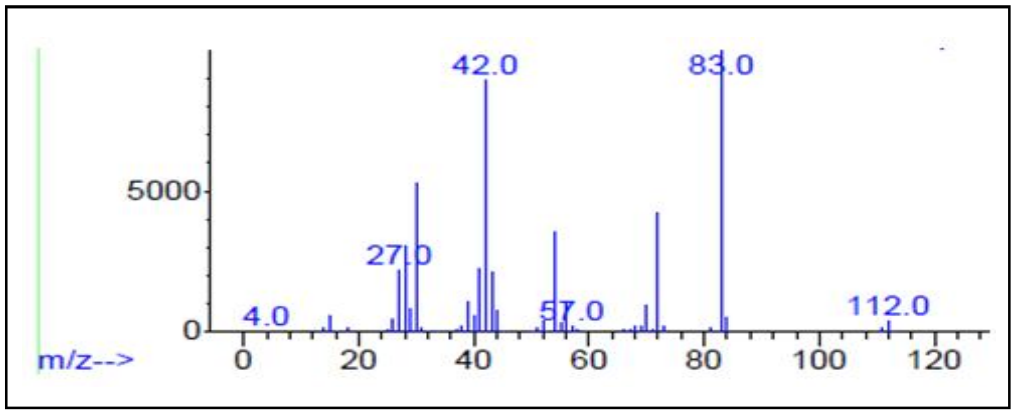

Figure 7: Mass spectrum of propanenitrile, 3-(propylamino) (0.93%).

4. Discussion

Gypsophila species, known for their diverse biological activities, have been traditionally used in folk medicine and the food industry. The findings from this study revealed that Gypsophila extracts are rich in phenolic and flavonoids, exhibiting strong antioxidant and antiproliferative effects against various cancer cell lines. Additionally, the extracts demonstrated significant antidiabetic and anticholinergic properties, making gypsophila a potential source for developing natural anticancer, antidiabetic, and anticholinergic drugs (Altay *et al.*, 2019).

Treatment T_2 led to increased levels of biochemical and phytochemical properties, which are crucial for the metabolic processes and freshness of the flowers during their vase life. In contrast, the control group exhibited lower levels of reducing sugars. Boric acid is known for its antiseptic, antifungal, and antiviral properties and is classified as a weakly acidic hydrate of boric oxide. It is utilized in treating yeast infections and herpes, although the precise mechanism of action remains unclear (anonymous)

Calcium chloride helps to enhance the level of bioactive compounds during the storage of Gypsophila by stabilizing cell membranes and reducing oxidative stress. Calcium role in cell wall reinforcement and delayed senescence helps preserve the integrity of bioactive compounds such as phenolics, flavonoids, and antioxidants. This not only maintains flower quality but also supports higher retention of these key compounds throughout the storage period (Ehsanimehr *et al.*, 2024).

Elevated levels of bioactive compounds with higher doses of sucrose are primarily due to its role as an energy source, which supports metabolic processes. Sucrose promotes increased respiration, activates antioxidant pathways, and helps in synthesizing secondary metabolites such as phenolics and flavonoids. By maintaining cell turgor and delaying senescence, sucrose also aids in reducing oxidative damage, leading to higher retention and accumulation of bioactive compounds during the postharvest period (Sim *et al.*, 2020).

A significant relationship was observed between reducing and total sugar content, showing the highest levels of strong antioxidant activity. These may provide valuable health and pharmaceutical benefits. Stefaniak and Grzeszczuk, (2019) reported that Hemerocallis × hybrida exhibited the highest levels of reducing sugars among the species studied. This finding indicates its potential as a rich source of easily metabolizable sugars, making it particularly useful for applications in food products that benefit from natural sweeteners or high-energy ingredients.

Phenolic compounds offer therapeutic benefits in treating inflammation, obesity, immune disorders, and various diseases, including cancer, diabetes, and kidney conditions. Rop *et al.*(2012) recorded a total phenolic content of 3.49 g GA kg{ ¹ FW in *Antirrhinum majus* L. flowers, which is consistent with the results obtained for this species. This suggests a reliable phenolic profile for reinforcing its potential as a source of bioactive compounds.

Carbohydrates from medicinal plants provide energy and play a role in wound healing, while certain polysaccharides exhibit antiviral and antimicrobial properties. These compounds support therapeutic applications in various health conditions (Takahashi *et al.*, 2020). Reported that flowers typically exhibit high carbohydrate content, with Centaurea petals containing 88.39% (dry matter), surpassing the carbohydrate levels found in rose petals. Despite this, rose petals are known to impart a sweeter taste to foods, indicating that factors beyond carbohydrate content influence flavor profiles.

Enzymes derived from Gypsophila proteins have pharmacological relevance, particularly in the field of detoxification and immune modulation (Kamali *et al.*, 2024). Found that proteolytic enzymes from Gypsophila could break down harmful proteins and peptides in human cells, offering potential treatments for conditions involving protein aggregation, such as Alzheimer's disease. Additionally, these enzymes have been shown to stimulate immune responses, making them promising candidates for immunomodulatory therapies.

Gas chromatography and mass spectrometry (GC-MS) serve as an effective tool for biological analysis, as also observed by (Shenbagavalli *et al.*, 2024). The various phytochemicals found in Gypsophila, listed in Table 3 with the chromatogram shown in Figure 4, revealed a total of 81 bioactive compounds. These compounds are responsible for its nutraceutical benefits. Additionally, the phytochemicals in Gypsophila possess unique physiological effects that can be harnessed for medicinal purposes. A similar result was observed by Nazaruk and Galicka (2014) and isolated ten flavonoids from *Cirsium palustre* leaves using multistep chromatographic separation and identified through spectroscopic methods. Except for luteolin 7-O-glucoside, all compounds were new. Four flavonoids were tested for their effects on collagen expression in human dermal fibroblasts.

5. Conclusion

The study showed that Gypsophila contains a wide variety of bioactive compounds, including important metabolites with potential health benefits. Notable compounds like phytosterols, fatty acids, and vitamins indicate its potential therapeutic applications. This chemical diversity enhances its relevance for applications in medicine, cosmetics, and other fields. The findings offer valuable insight into the plant's potential uses and contributions. Combined use of boric acid (2%) + sucrose (2%) + calcium chloride (0.2%) performed well among the ten treatment combinations tested. Boric acid (2%) aids in maintaining cell wall integrity and boosting biochemical stability. Calcium chloride (0.2%) fortifies cells, slowing senescence and improving quality. Sucrose (2%) provides energy, enhancing water uptake, maintaining turgidity, and increasing biochemical compounds. Together, these treatments extend vase life while significantly enhancing flower quality. The methanolic extraction of gypsophila identified over 81 compounds, providing scientific evidence for their pharmacological potential. The compounds identified through GC-MS exhibit a range of biological activities including hepatoprotective, antioxidant, anticarcinogenic, antibacterial, antifungal, and anti-inflammatory properties and anticancer effects, making them valuable for pharmaceutical applications. The pharmacological and phytochemical properties of Gypsophila species, which include around 150 flowering plants known for their ornamental and medicinal applications, these species contain a diverse array of volatile and non-volatile compounds, such as phenolics, flavonoids, triterpenoids, saponins, and alkaloids. The genus is particularly rich in fatty acids, monoterpenes, sesquiterpenes, sterols, and cyclopeptides. Hence, the potential medicinal properties of Gypsophila may be commercially exploited in the pharmaceutical industries.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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