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Phytochemical Profiling of Methanolic and Ethanolic Extracts of *Pemphis acidula* (J.R.Forst. & G.Forst.) Leaf using LC–MS/MS and GC–MS

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ABSTRACT

Pemphis acidula (J.R.Forst. & G.Forst.) is a mangrove species of the Lythraceae family widely distributed along the Indonesian coasts. Traditionally used for medicinal and cosmetic purposes, information on its phytochemical composition remains limited. Comprehensive chemical profiling is essential to elucidate its bioactive potential and development. This study aimed to identify, characterize, and compare the phytochemical and bioactive profiles of methanolic and ethanolic extracts of *Pemphis acidula* using LC–MS/MS and GC–MS to determine which solvent yields richer bioactive composition. Leaf samples were collected and authenticated at Herbarium Bogoriense (BRIN). The dried powders were extracted with methanol or ethanol by triple maceration for 24 hours, and filtrates were evaporated under reduced pressure. Crude extracts were analyzed using LC–MS/MS (UPLC-QTOF, ESI ±) and GC–MS; compound identification employed UNIFI software referencing the Waters Traditional Medicine Library and Wiley 275 database. The results showed that LC–MS/MS identified 25 compounds in methanolic and 49 in ethanolic extracts, while GC–MS detected 13 and 19 compounds, respectively, with quality matches ≥90%. These compounds comprised alkaloids, flavonoids, phenols, polyphenols, and terpenoids with antioxidant, anti-inflammatory, antibacterial, antiviral, and anticancer activities. Eleven non-volatile compounds were identified in both extracts, namely kaempferol-3-glucuronide, desmanthin, quercetin-3-O-α-D-glucuronide, quercetin-3-O-glucuronide 6″-methylester, ellagic acid, Z-ligustilide, melazolide A, cimicifugic acid B, vellerdiol, 5,6,7,7α-tetrahydro-4,4,7α-trimethyl-2(4H)-benzofuranone, and farnesyl acetate and seven volatile compounds were identified in both extracts, i.e. supraene, hexadecanoic acid methyl ester, loliolide, 9,12-octadecadienoic acid (Z,Z)-methyl ester, phytol, 9-octadecenoic acid, and tocopherol. In conclusion, *Pemphis acidula* extracts contain bioactive compounds with medicinal potential, and ethanol extract yielded a broader phytochemical profile than methanol's.

***Keywords*:** *Pemphis acidula* leaf; LC–MS/MS; GC–MS; Phytochemical profiling; Bioactive compounds

INTRODUCTION

Exploration of natural sources for medicinal bioactive compounds has received considerable attention in pharmaceutical and biomedical research nowadays. Recent metabolomic studies employing advanced analytical platforms

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such as Liquid Chromatography–Mass Spectrometry (LC–MS) and Gas Chromatography–Mass Spectrometry (GC–MS) have successfully identified and profiled secondary metabolites from several medicinal plants, demonstrating their potential pharmacological activities.[1-3]

Indonesia's ethnopharmacological heritage comprises thousands of medicinal plant species, as documented in national inventories such as Ristoja, highlighting the country's extensive biodiversity and long-standing tradition of medicinal plant utilization.[1] Indonesia is renowned for its rich biodiversity, and among the medicinal plants that flourish and are commonly found along the country's coastal areas is *Pemphis acidula*.[2–6]

Pemphis acidula, widely known by local names such as bantigue, mentigi, setigi, or sentigi, is a mangrove species in the family Lythraceae[7] characterized as a dense, long-lived shrub or small tree that grows in rocky or sandy coastal regions and mangrove habitats across the tropical Indo-Pacific. Field observations in Gili Sulat identify *Pemphis acidula* as an indicator species for sandy coastal habitats, ecotone regions, and areas in a climax successional stage.[8] Recent molecular analysis revealed that *Pemphis acidula* populations from the southern coast of Gorontalo, Sulawesi Island, exhibit a high level of genetic variation, with a polymorphism rate of 88.92% and a mean polymorphic information content (PIC) value of 0.49, indicating strong adaptability to diverse coastal environments.[9]

The leaves of *Pemphis acidula* are green in color, simple, thick, succulent, narrowly elliptic to lanceolate, measuring 1–3 cm in length and width of 5–15 mm, and arranged oppositely, sessile or with very short petioles alternately. Flowers are small, white, and crumpled, usually solitary or in pairs, whereas its fruit takes the form of a dry capsule containing several tiny seeds.[2,6]

The Togian people of Central Sulawesi utilize the leaves as a traditional remedy to reduce fever, whereas the inhabitants of Pari Island, Indonesia, use them to treat mouth ulcers. On Talaud Island, the leaves and bark are used in the preparation of traditional medicines and cosmetics. In Vanuatu, an infusion of *Pemphis acidula* sap combined with a handful of bark has been used as an abortifacient.[10]

Previous studies reported that the ethanol extract of *Pemphis acidula* exhibited antioxidant activity, anti-inflammatory,[11] antibacterial,[12,13] and topoisomerase-I activity.[13] Recent findings further corroborate this by demonstrating that the ethanolic extract of *Pemphis acidula* leaves possesses strong antioxidant and α-amylase inhibitory activities, along with high levels of phenolic (5390 mg GAE/100 g) and flavonoid (581.66 mg QE/100 g) compounds, indicating its potential medicinal properties.[14] These studies suggest that *Pemphis acidula* has considerable medicinal potential, highlighting the need for an in-depth investigation of its chemical constituents.

The most abundant and easiest part of the plant to use as a raw material for medicinal purposes is the leaves. To measure the potential of *Pemphis acidula* leaves as a raw material for medicines, it is necessary to determine the chemical composition of the leaf extract.

Information about the chemical content of *Pemphis acidula* leaf is very limited. Phytochemical analysis of whole plant extracts has shown that both methanol and ethanol extracts of *Pemphis acidula* contain alkaloids, flavonoids, phenols, quinones, saponins, steroids, tannins, terpenoids, glycosides, sugars, and xanthoproteins.[11] The bark is reported to have a tannin content ranging from 19–43%. Matsuda et al.[15] also reported that methanolic extracts of *Pemphis acidula* leaves contain four types of galloyl flavonol glycosides, which are believed to contribute to its antioxidant properties.

Comparing the chemical content of ethanolic and methanolic extracts of *Pemphis acidula* leaves is important for optimizing its medicinal and pharmacological applications, as solvent choice significantly influences extraction efficiency, bioactive compound profiles, and biological activity. Ethanol and methanol differ in polarity, affecting their ability to extract specific compounds.

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Several studies have reported differences in phytochemical composition and biological activity between ethanolic and methanolic plant extracts. Idoko et al.[16] reported the difference in phytochemical content of ethanolic and methanolic extracts of *Flacourtia indica* leaf. Proteins and steroids were not detectable in ethanolic extract of the leaf, while they were present in moderately high amounts in the methanolic leaf extract. The quantitative screening showed that the ethanolic leaf extract had lower levels of phenols and higher levels of glycosides, flavonoids, alkaloids, and tannins than the methanolic extract.

Moonmun et al.[17] also found that the phytochemicals present in the methanolic extract of *Heliconia rostrata* rhizome were likewise detected in the ethanolic extract, except for glycosides, which were identified only in the methanolic extract. The ethanolic extract showed higher levels of total phenolics and flavonoids compared to the methanolic extract, while the methanolic extract had a higher total tannin content. Both ethanolic and methanolic extracts of *Heliconia rostrata* rhizome showed antibacterial activity, however, the antibacterial activity of the ethanolic extract was significantly higher compared to the methanolic extract.

Recent findings have also reported that different solvents used to extract cumin seeds produce extracts with varying contents, both qualitatively and quantitatively.[18] In a different study, Nadeem et al.[19] reported that the ethanolic extracts of *Thymus linearis* stem showed higher antioxidant activity compared to methanolic stem extracts. Likewise, the cytotoxicity of polar, semipolar, and nonpolar extracts of *Andrographis paniculata* leaves on cancer cells showed different strength. The ethyl acetate and ethanol fractions of the leaves had stronger cytotoxic activity against HepG2 cells compared to the hexane extract.[20]

Based on the results of the above studies, which indicate that the phytochemical content and bioactivity of the extract can be significantly influenced by the solvent used, this study was conducted to compare the chemical content of ethanolic and methanolic extracts of *Pemphis acidula* leaf. To obtain more comprehensive results, this study was conducted by LC–MS/MS and GC–MS analysis.

MATERIALS AND METHODS

Sample collection and identification

Fresh leaves of *Pemphis acidula* were collected from Pulau Air, Seribu Islands, Jakarta. A herbarium specimen comprising the plant's roots, stems, leaves, flowers, and fruits was prepared for species identification. The specimen was authenticated at the Herbarium Bogoriense, Center for Biological Research, National Research and Innovation Agency (BRIN), Indonesia.

Materials and Equipment

The materials used in this study included *Pemphis acidula* leaves, methanol (technical grade), and ethanol (technical grade) as extraction solvents. Analytical-grade methanol was used for LC–MS/MS and GC–MS analyses to ensure solvent purity. All solvents and reagents were of analytical or chromatography grade and were used without further purification.

The instruments used included an LC–MS/MS QTOF system (Waters Corporation, Milford, MA, USA) operated at PT Saraswanti Indo Genetech (SIG), Bogor, Indonesia, and a GC–MS system (Agilent 7890B GC coupled with 5977A MSD; Agilent Technologies, USA) operated at the Advanced Characterization Laboratory, Serpong – BRIN.

Preparation of extract

Fresh leaves were separated from other plant parts, soil, and debris, and washed twice with distilled water. After removing excess water, the leaves were dried in an oven at 40 °C for 24 hours. The dried leaves were then ground and sieved through a 40-mesh sieve to obtain a fine powder. The leaf powder was macerated with either ethanol or methanol on a shaker for 24 hours, and the process was repeated three times. All filtrates were combined and evaporated under reduced

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pressure. The dried extract was stored in a refrigerator until further analysis.

LC–MS/MS Analysis

LC–MS/MS analysis was performed following previously reported procedure [21–23] with slight modifications. An amount of 0.5 g of extract was dissolved in 10 mL methanol, sonicated for 30 minutes, further diluted with methanol to the desired concentration, homogenized, and filtered through a 0.22 µm GHP/PTFE syringe filter.

The LC–MS system was equipped with an HSS T3 column, an auto-sampler, column manager, and an adjustable MS detector. The mobile phase consisted of 0.1% formic acid in bidistilled water (solvent A) and 0.1% formic acid in acetonitrile (solvent B), at a constant flow rate of 0.6 mL/min. The total chromatographic run time was 2.0 minutes. The column and auto-sampler temperatures were maintained at 40 °C and 15 °C, respectively. The injection volume was 10 µL.

Mass spectrometric detection employed an electrospray ionization (ESI) source operated in both positive and negative ion modes. MS data were collected over an m/z range of 50–1200 using MSE TOF mode. Compound identification was performed using UNIFI data processing software, referencing the Waters Traditional Medicine Scientific Library for UPLC/QTOF MSE data with automated compound identification.

GC–MS Analysis

GC–MS analysis was conducted following previously reported methods[1–3] with slight modifications. The analysis employed an Agilent 7890 GC coupled with a 5977 MSD and an autosampler. An HP-5MS UI (5%) capillary column (30 m × 250 µm × 0.25 µm) was used. Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min in splitless mode. The oven temperature was initially set at 80 °C, increased at 3 °C/min to 150 °C (held for 1 minute), and then ramped at 20 °C/min to 300 °C. The ion source and interface temperatures were maintained at 230 °C and 250 °C, respectively.

Electron Impact (EI) ionization was applied at 70 eV. The structures of the detected compounds were determined by comparing their fragmentation patterns with reference spectra, using a split ratio of 1:8. Chromatograms were examined to calculate the relative abundance of each compound. Identification was performed by matching the mass spectra with the Wiley 275 library. The relative composition of each compound was estimated from the total ion chromatogram (TIC) peak areas, expressed as percentages of total abundance.

RESULTS AND DISCUSSION

Plant Identification

The plant specimen used in this study was taxonomically identified and authenticated at the Herbarium Bogoriense, Directorate of Scientific Collection Management, National Research and Innovation Agency (BRIN), Cibinong, Bogor, Indonesia. The identification was conducted by Dr. Ratih Damayanti, S.Hut., M.Si., confirming the species as *Pemphis acidula* J.R.Forst. & G.Forst., belonging to the family Lythraceae. A voucher specimen was deposited at the Herbarium Bogoriense under Collection No. B-1671/II.6.2/IR.01.02/7/2023 for future reference.

LC–MS/MS Analysis of Methanolic and Ethanolic Extracts of *Pemphis acidula* Leaves

The methanolic and ethanolic extracts of *Pemphis acidula* were analyzed using LC–MS/MS, a tandem mass spectrometry technique in which the sample passes through two mass analyzers. This method provides greater selectivity and sensitivity, enabling detailed structural elucidation and quantification of compounds in complex matrices. The LC–MS/MS chromatograms of the methanolic extract of *Pemphis acidula* leaves analyzed in both positive and negative ionization modes are presented in Figure 1, whereas those of the ethanolic extract are shown in Figure 2.

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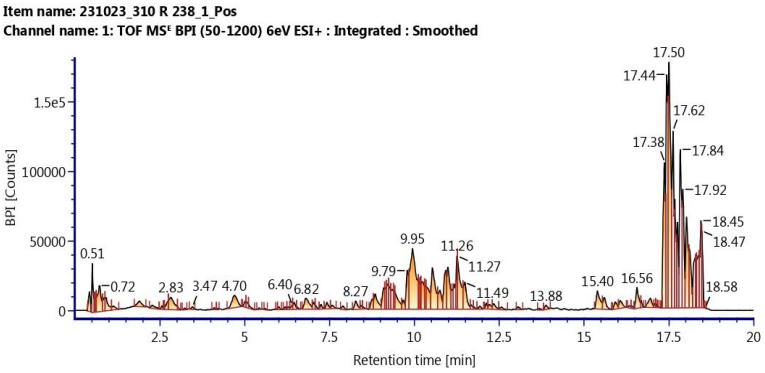
a) TOF MS^E BPI (50-1200) 6eV ESI+



b) TOF MS^E BPI (50-1200) 6eV ESI-

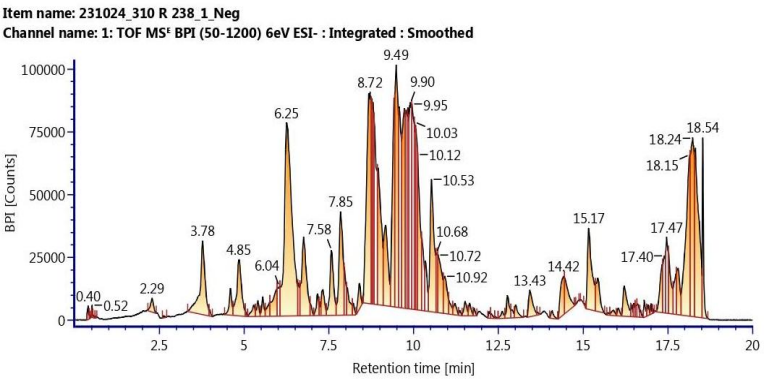
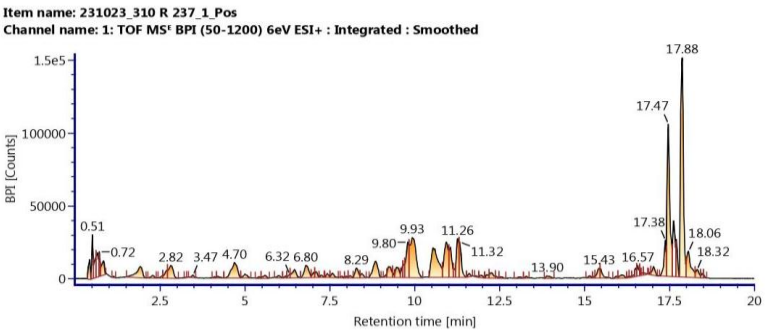


Figure 1. LC–MS/MS chromatogram of the methanolic extract of *Pemphis acidula* leaf in a) ESI+ and b) ESI- mode

a) TOF MS^E BPI (50-1200) 6eV ESI+



b) TOF MS^E BPI (50-1200) 6eV ESI-

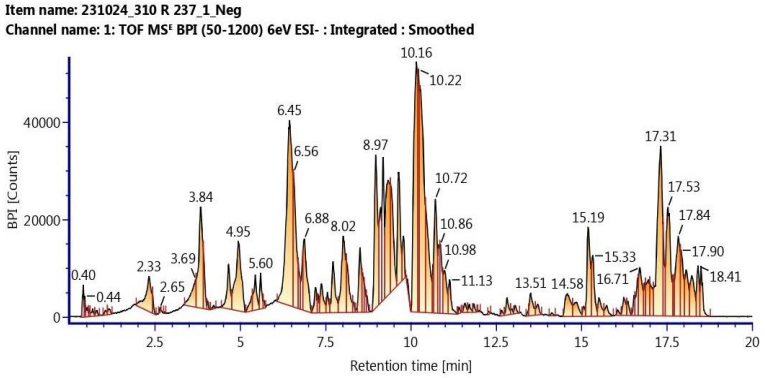


Figure 2. LC–MS/MS chromatogram of the ethanolic extract of *Pemphis acidula* leaf in a) ESI+ and b) ESI- mode

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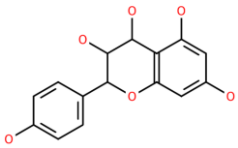
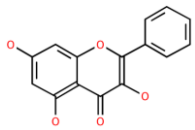
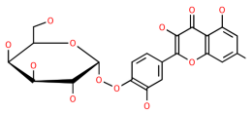
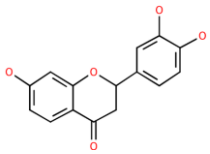
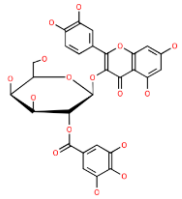
From the chromatograms, 25 compounds belonging to alkaloids, flavonoids, phenols, polyphenols, and terpenoids were identified in the methanolic extract, whereas 49 compounds were identified in the ethanolic extract (Tables 1 and 2). No steroidal compounds were detected in either extract, and other compound classes besides alkaloids, flavonoids, phenols, polyphenols, terpenoids, and steroids were not analyzed. The 25 compounds identified in the methanolic extract are summarized in Table 1 along with their retention times, responses, molecular formulas, and chemical structures. These compounds comprise 11 flavonoids, 3 phenols, 1 polyphenol, and 10 terpenoids. On the other hand, the ethanolic extract contained 49 identified compounds, including 2 alkaloids, 24 flavonoids, 6 phenols, 2 polyphenols, and 15 terpenoids (Table 2). All compounds were identified based on their MS/MS fragmentation patterns and by cross-referencing the literature. According to Tables 1–3, flavonoids represent a major proportion of the compounds detected in both extracts: 11 in the methanolic extract and 24 in the ethanolic extract. Only the same four flavonoids were found in both extracts—kaempferol-3-glucuronide, desmanthin, quercetin-3-O-α-D-glucuronide, and quercetin-3-O-glucuronide 6″-methyl ester (Table 3).

Table 1. Phytochemicals identified in methanolic extract of *Pemphis acidula* Leaf by LC–MS/MS analysis

No.	ESI Mode	RT (min)	Response	Compounds	Molecular Formula	Chemical Structure
Alkaloids:						
	-	-	-	-	-	-
Flavonoids:						
	(-)	-	-	-	-	-
1	(+)	6.29	13499	Leucopelargonidin	$C_{15}H_{14}O_6$	
2	(+)	7.66	1663	Galangin (Norizalpinin)	$C_{15}H_{10}O_5$	
3	(+)	8.86	18860	Isohyperoside	$C_{21}H_{20}O_{13}$	
4	(+)	9.18	1367	3′,4′,7-Trihydroxy-flavanone	$C_{15}H_{12}O_5$	
5	(+)	9.24	66237	2″-O-Galloylhyperin	$C_{28}H_{24}O_{16}$	

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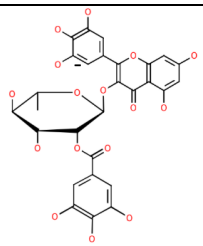
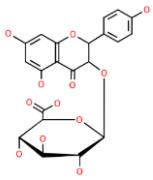
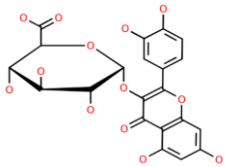
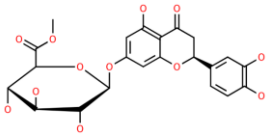
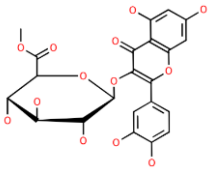
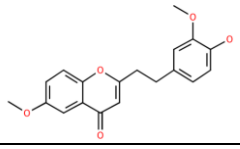
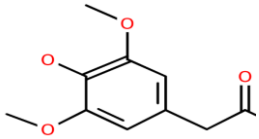
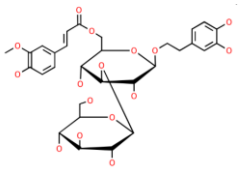
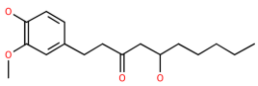
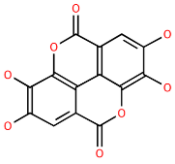
No.	ESI Mode	RT (min)	Response	Compounds	Molecular Formula	Chemical Structure
6	(+)	9.46	79223	Desmanthin-1	$C_{28}H_{24}O_{16}$	
7	(+)	9.80	35941	Kaempferol-3-glucuronide	$C_{21}H_{20}O_{12}$	
8	(+)	9.90	78496	Quercetin-3-O-α- D-glucuronide	$C_{21}H_{18}O_{13}$	
9	(+)	11.10	14935	Eriodictyol-7-O-β-D-methyl glucurono-pyranoside	$C_{22}H_{22}O_{12}$	
10	(+)	11.30	23942	Quercetin-3-O-glucuronide 6″-methylester	$C_{22}H_{20}O_{13}$	
11	(+)	13.29	722	6-Methoxy-2-[2-3'-methoxy-4'-hydroxy-phenyl) ethyl] chromone	$C_{19}H_{18}O_5$	
Phenols:						
	(-)	-	-	-	-	-
12	(+)	2.03	2040	3-Hydroxy-1-(4-hydroxy-3,5 dimethoxyphenyl)-2-propanone	$C_{11}H_{14}O_5$	
13	(+)	4.91	5705	Scroside E	$C_{30}H_{38}O_{16}$	
14	(+)	12.66	512	6-Gingerol	$C_{17}H_{26}O_4$	
Polyphenol:						
	(-)	-	-	-	-	-
15	(+)	5.53	655	Ellagic acid	$C_{14}H_6O_8$	

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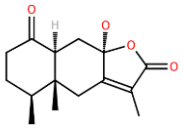
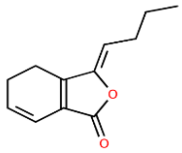
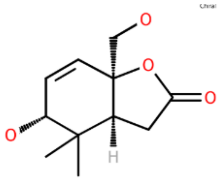
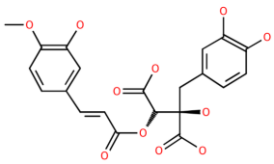
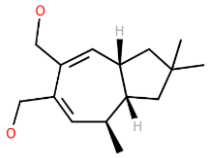
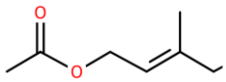
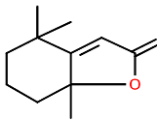
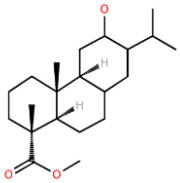
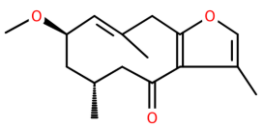
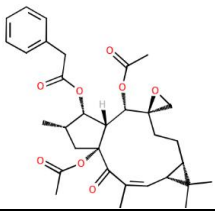
No.	ESI Mode	RT (min)	Response	Compounds	Molecular Formula	Chemical Structure
Terpenoids:						
	(-)	-	-	-	-	- -
16	(+)	5.41	1989	(-)-Istanbulin A	$C_{15}H_{20}O_4$	
17	(+)	6.35	1147	Z-Ligustilide	$C_{12}H_{14}O_2$	
18	(+)	7.24	3193	Melazolide A	$C_{11}H_{16}O_4$	
19	(+)	10.55	22142	Cimicifugic acid B	$C_{21}H_{20}O_{11}$	
20	(+)	12.23	1610	Vellerdiol	$C_{15}H_{24}O_2$	
21	(+)	15.41	1149	Farnesyl acetate	$C_{17}H_{28}O_2$	
22	(+)	16.08	30299	5,6,7,7α-Tetrahydro-4,4,7α-trimethyl-2(4H)-benzofuranone	$C_{11}H_{16}O_2$	
23	(+)	16.28	831	12-Hydroxyhydromethyl abietate	$C_{21}H_{36}O_3$	
24	(+)	16.67	2856	Rel-2R-methoxy-4R-furanogermacr-1 (10)E-en-6-one	$C_{16}H_{22}O_3$	
25	(+)	17.94	15199	6,17-Epoxylathyrol-5,15-diacetate-3-phenylacetate	$C_{32}H_{40}O_8$	

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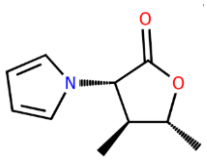
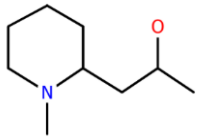
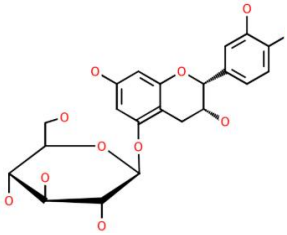
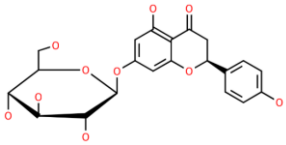
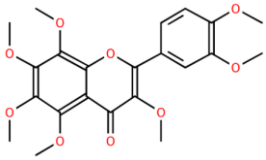
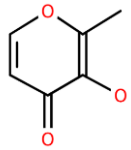
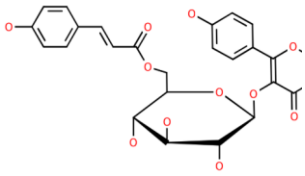
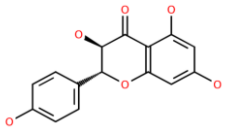
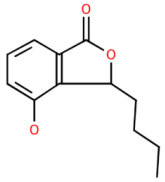
Table 2. Phytochemicals identified in Ethanolic Extract of *Pemphis acidula* Leaf by LC–MS/MS Analysis

No.	ESI Mode	RT (min)	Response	Compounds	Molecular Formula	Chemical Structure
Alkaloids:						
	(-)	-	-	-	-	-
1	(+)	1.83	750	(3α,4β,5α)-4,5-Dihydro-4,5-dimethyl-3-(1-pyrrolyl)-furan-2(3H)-one	$C_{10}H_{13}NO_2$	
2	(+)	15.71	2837	Dihydro-N-methylisopelletierine	$C_9H_{19}NO$	
Flavonoids:						
3	(-)	9.12	364	Epicatechin 5-O-β-D-glucopyranoside	$C_{21}H_{24}O_{11}$	
4	(-)	10.26	6993	Prunin	$C_{21}H_{22}O_{10}$	
5	(-)	11.85	7605	3,5,6,7,8,3',4'-Heptemethoxyflavone	$C_{22}H_{24}O_9$	
6	(+)	3.24	2748	Maltol	$C_6H_6O_3$	
7	(+)	4.08	5047	Tiliroside	$C_{30}H_{26}O_{13}$	
8	(+)	4.28	940	Dihydrokaempferol	$C_{15}H_{12}O_6$	
9	(+)	5.62	1748	4-Hydroxy-3-butylphthalide	$C_{12}H_{14}O_3$	

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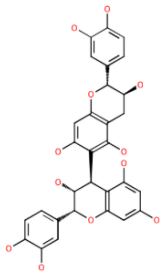
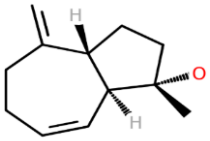
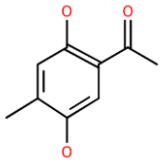
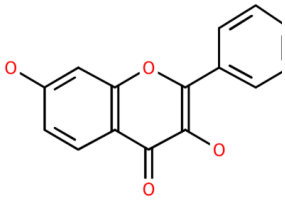
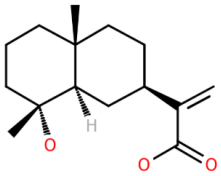
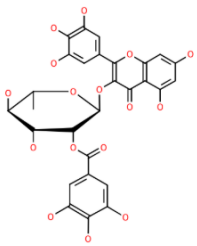
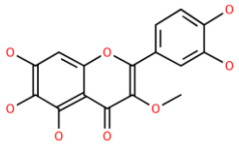
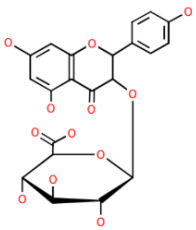
No.	ESI Mode	RT (min)	Response	Compounds	Molecular Formula	Chemical Structure
10	(+)	6.02	4256	Procyanidin B7	$C_{30}H_{26}O_{12}$	
11	(+)	6.82	2220	Dictamnol	$C_{12}H_{18}O$	
12	(+)	7.24	3402	2,5-Dihydroxy-4-methyl-hypnone	$C_9H_{10}O_3$	
13	(+)	7.65	2151	Resokaempferol	$C_{15}H_{10}O_5$	
14	(+)	8.62	859	Ilicic acid	$C_{15}H_{24}O_3$	
15	(+)	9.26	82365	Desmanthin-1	$C_{28}H_{24}O_{16}$	
16	(+)	9.41	1012	5,6,7,3',4'-Pentahydroxy-3-methoxyflavone	$C_{16}H_{12}O_8$	
17	(+)	9.82	41309	Kaempferol-3-glucuronide	$C_{21}H_{20}O_{12}$	

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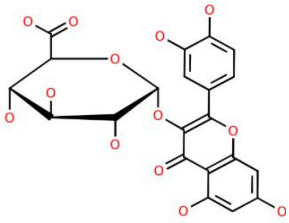
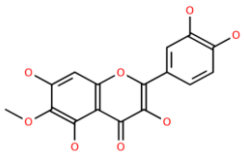
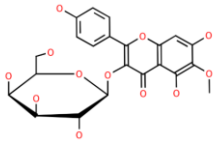
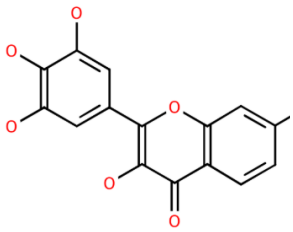
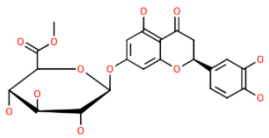
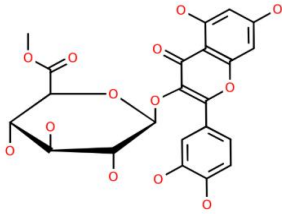
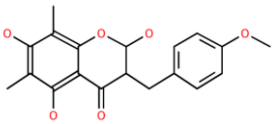
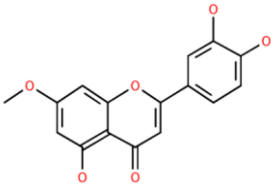
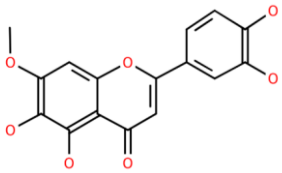
No.	ESI Mode	RT (min)	Response	Compounds	Molecular Formula	Chemical Structure
18	(+)	9.95	90137	Quercetin-3-O-α-D-glucuronide	$C_{21}H_{18}O_{13}$	
19	(+)	10.12	9560	Patuletin	$C_{16}H_{12}O_8$	
20	(+)	10.91	7253	6-Methoxykaempferol-3-O-β-D-galactopyranoside	$C_{22}H_{22}O_{12}$	
21	(+)	11.03	106463	Robinetin	$C_{15}H_{10}O_7$	
22	(+)	11.13	11889	Eriodictyol-7-O-β-D-methyl-glucuronopyranoside	$C_{22}H_{22}O_{12}$	
23	(+)	11.31	25518	Quercetin-3-O-glucuronide 6″-methylester	$C_{22}H_{20}O_{13}$	
24	(+)	13.30	1009	2,5,7-Trihydroxy-6,8-dimethyl-3-(4'-methoxybenzyl) chroman-4-one	$C_{19}H_{20}O_6$	
25	(+)	15.97	601	Hydroxygenkwanin	$C_{16}H_{12}O_6$	
26	(+)	16.22	2984	Pedalitin	$C_{16}H_{12}O_7$	

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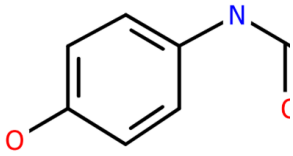
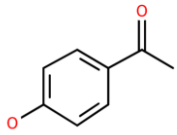
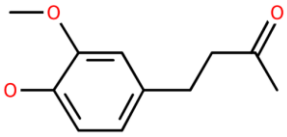
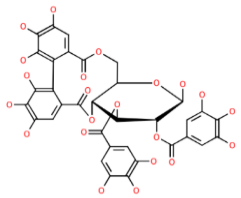
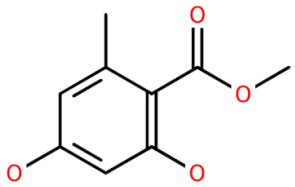
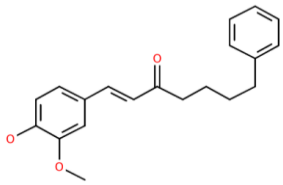
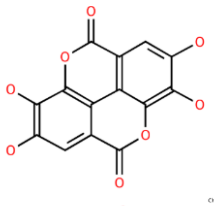
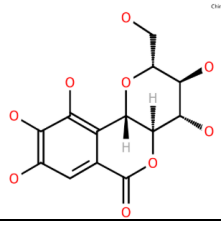
Phenols:						
	(-)	-	-	-	-	-
27	(+)	1.10	2886	p-Hydroxyacetanilide	$C_8H_9NO_2$	
28	(+)	3.56	3679	4-Hydroxyacetophenone	$C_8H_8O_2$	
29	(+)	7.25	1586	Gingerone	$C_{11}H_{14}O_3$	
30	(+)	7.34	1726	Collinin	$C_{34}H_{26}O_{22}$	
31	(+)	9.17	10082	Methyl-β-orsellinate	$C_9H_{10}O_4$	
32	(+)	17.09	1668	Yakuchinone B	$C_{20}H_{22}O_3$	
Polyphenols:						
	(-)	-	-	-	-	-
33	(+)	1.99	690	Ellagic acid	$C_{14}H_6O_8$	
34	(+)	8.29	4200	Norbergenin	$C_{13}H_{14}O_9$	
Terpenoids:						
	(-)	-	-	-	-	-

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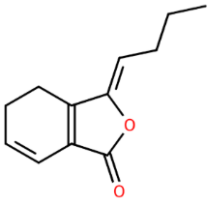
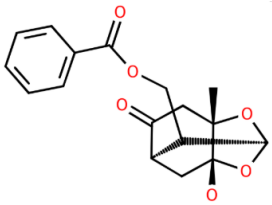
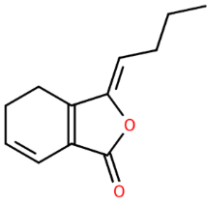
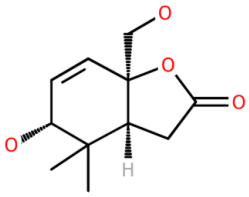
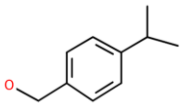
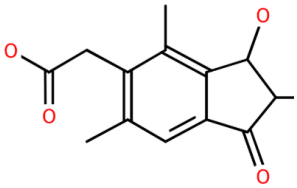
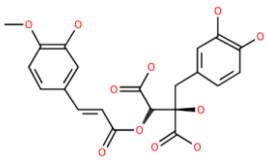
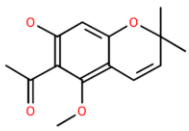
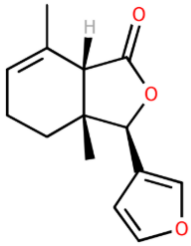
No.	ESI Mode	RT (min)	Response	Compounds	Molecular Formula	Chemical Structure
35	(+)	6.35	1158	Z-Ligustilide	$C_{12}H_{14}O_2$	
36	(+)	6.69	1359	Paeoniflorigenone	$C_{17}H_{18}O_6$	
37	(+)	7.03	3119	Neoligustilide	$C_{12}H_{14}O_2$	
38	(+)	7.24	2377	Melazolide A	$C_{11}H_{16}O_4$	
39	(+)	7.40	1414	Cuminic alcohol	$C_{10}H_{14}O$	
40	(+)	8.00	8307	Isohistiopterosin A	$C20H_{30}O_3$	
41	(+)	8.45	23820	Cimicifugic acid B	$C_{21}H_{20}O_{11}$	
42	(+)	8.68	1862	Evodionol	$C_{15}H_{16}O_4$	
43	(+)	9.34	1327	Isofraxinellone	$C_{14}H_{16}O_3$	

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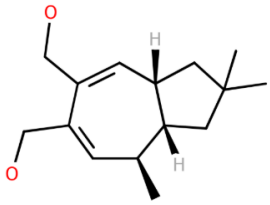
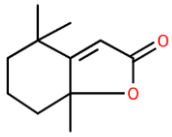
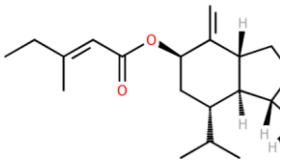
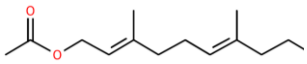
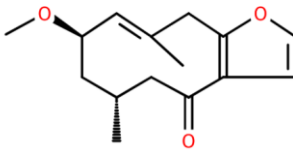
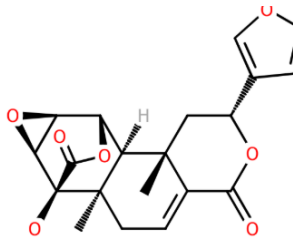
No.	ESI Mode	RT (min)	Response	Compounds	Molecular Formula	Chemical Structure
44	(+)	12.26	997	Vellerdiol	$C_{15}H_{24}O_2$	
45	(+)	12.73	648	5,6,7,7α-Tetrahydro-4,4,7α-trimethyl-2(4H)-benzofuranone	$C_{11}H_{16}O_2$	
46	(+)	14.45	955	7β-(3-Ethyl-cis-crotonoyloxy)-14-hydroxynotonipetranone	$C_{21}H_{32}O_4$	
47	(+)	15.45	626	Farnesyl acetate	$C_{17}H_{28}O_2$	
48	(+)	16.63	1297	Rel-2R-methoxy-4R-furanogermacr-1(10)E-en-6-one	$C_{16}H_{22}O_3$	
49	(+)	16.72	914	Fibraurin	$C_{20}H_{20}O_7$	

Table 3. Comparison of phytochemical content in methanolic and ethanolic extracts of *Pemphis acidula* leaf analyzed by LC–MS/MS

No.	ESI Mode	RT (min)	Compounds	Methanolic Extract	Ethanolic Extract
Alkaloids:					
1	(+)	1.83	(3α,4β,5α)-4,5-Dihydro-4,5-dimethyl-3-(1-pyrrolyl)-furan-2(3H)-one	-	+
2	(+)	15.71	Dihydro-N-methylisopelletierine	-	+
Flavonoids:					
3	(+)	6.29	Leucopelargonidin	+	-
4	(+)	7.66	Galangin (Norizalpinin)	+	-
5	(+)	8.86	Isohyperoside	+	-

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No.	ESI Mode	RT (min)	Compounds	Methanolic Extract	Ethanolic Extract
6	(+)	9.18	3′,4′,7-Trihydroxy-flavanone	+	-
7	(+)	9.24	2″-O-Galloylhyperin	+	-
8	(+)	9.80	Kaempferol-3- glucuronide	+	+
9	(+)	9.90	Quercetin-3-O-α- D-glucuronide	+	+
10	(+)	11.10	Eriodictyol-7-O-β-D-methyl glucurono- pyranoside	+	-
11	(+)	13.29	6-Methoxy-2-[2-3'-methoxy-4'-hydroxy-phenyl) ethyl] chromone	+	-
12	(-)	9.12	Epicatechin 5-O-β-D-glucopyranoside	-	+
13	(-)	10.26	Prunin	-	+
14	(-)	11.85	3,5,6,7,8,3',4'-Heptemethoxyflavone	-	+
15	(+)	3.24	Maltol	-	+
16	(+)	4.08	Tiliroside	-	+
17	(+)	4.28	Dihydrokaempferol	-	+
18	(+)	5.62	4-Hydroxy-3-butylphthalide	-	+
19	(+)	6.02	Procyanidin B7	-	+
20	(+)	6.82	Dictamnol	-	+
21	(+)	7.24	2,5-Dihydroxy-4-methyl-hypnone	-	+
22	(+)	7.65	Resokaempferol	-	+
23	(+)	8.62	Ilicic acid	-	+
24	(+)	9.26	Desmanthin-1	+	+
25	(+)	9.41	5,6,7,3',4'-Pentahydroxy-3-methoxyflavone	-	+
26	(+)	10.12	Patuletin	-	+
27	(+)	10.91	6-Methoxykaempferol-3-O-β-D-galactopyranoside	-	+
28	(+)	11.03	Robinetin	-	+
29	(+)	11.13	Eriodictyol-7-O-β-D-methyl-glucuronopyranoside	-	+
30	(+)	11.31	Quercetin-3-O-glucuronide 6″-methylester	+	+
31	(+)	13.30	2,5,7-Trihydroxy-6,8-dimethyl-3-(4'-methoxybenzyl) chroman-4-one	-	+
32	(+)	15.97	Hydroxygenkwanin	-	+
33	(+)	16.22	Pedalitin	-	+
Phenols:					
34	(+)	2.03	3-Hydroxy-1-(4-hydroxy-3,5 dimethoxyphenyl)-2-propanone	+	-
35	(+)	4.91	Scroside E	+	-
36	(+)	12.66	6-Gingerol	+	-

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No.	ESI Mode	RT (min)	Compounds	Methanolic Extract	Ethanolic Extract
37	(+)	1.10	p-Hydroxyacetanilide	-	+
38	(+)	3.56	4-Hydroxyacetophenone	-	+
39	(+)	7.25	Gingerone	-	+
40	(+)	7.34	Collinin	-	+
41	(+)	9.17	Methyl-β-orsellinate	-	+
42	(+)	17.09	Yakuchinone B	-	+
Polyphenols:					
43	(+)	1.99	Ellagic acid	+	+
44	(+)	8.29	Norbergenin	-	+
Terpenoids:					
45	(+)	5.41	(-)-Istanbulin A	+	-
46	(+)	16.28	12-Hydroxyhydromethyl abietate	+	-
47	(+)	17.94	6,17-Epoxylathyrol-5,15-diacetate-3-phenylacetate	+	-
48	(+)	6.35	Z-Ligustilide	+	+
49	(+)	6.69	Paeoniflorigenone	-	+
50	(+)	7.03	Neoligustilide	-	+
51	(+)	7.24	Melazolide A	+	+
52	(+)	7.40	Cuminic alcohol	-	+
53	(+)	8.00	Isohistiopterosin A	-	+
54	(+)	8.45	Cimicifugic acid B	+	+
55	(+)	8.68	Evodionol	-	+
56	(+)	9.34	Isofraxinellone	-	+
57	(+)	12.26	Vellerdiol	+	+
58	(+)	12.73	5,6,7,7α-Tetrahydro-4,4,7α-trimethyl-2(4H)-benzofuranone	+	+
59	(+)	14.45	7β-(3-Ethyl-cis-crotonoyloxy)-14-hydroxynotonipetranone	-	+
60	(+)	15.45	Farnesyl acetate	+	+
61	(+)	16.63	Rel-2R-methoxy-4R-furanogermacr-1(10)E-en-6-one	-	+
62	(+)	16.72	Fibraurin	-	+

According to the LC–MS/MS analysis, the compounds identified in the methanolic extract of *Pemphis acidula* leaves were distinct from those in the ethanolic extract. Ethanol was able to extract a wider diversity of compounds compared to methanol. Table 3 summarizes the differences between the methanolic and ethanolic extracts of *Pemphis acidula* leaves. Overall, a total of 62 compounds were identified, with eleven found in both extracts: Kaempferol-3-glucuronide, Desmanthin, Quercetin-3-O-α-D-glucuronide, Quercetin-3-O-glucuronide 6″-methylester (flavonoid), Ellagic acid (polyphenol), Z-Ligustilide, Melazolide A, Cimicifugic acid B, Vellerdiol, 5,6,7,7α-Tetrahydro-4,4,7α-trimethyl-2(4H)-benzofuranone, and Farnesyl acetate (terpenoids).

Among the 62 compounds identified in the methanolic and ethanolic extracts of *Pemphis acidula* leaves, 31 are flavonoids. Flavonoids are compounds, many of which possess antioxidant and anti-inflammatory activities—important biological functions

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that contribute to their medicinal benefits in preventing and managing various diseases and health disorders.

Numerous epidemiological studies have shown a correlation between high intake of dietary flavonoids and a reduced risk of degenerative diseases, such as cardiovascular disease, type 2 diabetes, atherosclerosis, and various forms of cancer. Flavonoids provide diverse health benefits, including lowering the risk of chronic diseases, enhancing immune response, and demonstrating antibacterial, antimalarial, anticancer, antiviral, antiangiogenic, and neuroprotective activities. Recent findings also emphasize the anti-aging properties of flavonoids, suggesting their potential in supporting longer and healthier lifespans. [24–26]

Leucopelargonidin, a type of leucoanthocyanidin, is a flavonoid recognized for its antioxidant and anti-inflammatory potential. Present in various plants, this compound has been investigated for its ability to reduce oxidative stress, protect nerve cells—particularly in the context of neurodegenerative diseases—and contribute to cardiovascular health. An *in silico* analysis also suggested leucopelargonidin as a promising compound for managing PCOS (Polycystic Ovary Syndrome) with hyperandrogenism and estrogen dominance.[27,28]

Galangin, a flavonoid primarily found in *Alpinia officinarum*, exhibits various biological activities, such as neuroprotective, antitumor, and anti-inflammatory effects. Recent studies have shown that galangin provides notable neuroprotective benefits through several mechanism.[29,30] Huang et al.[29] reported that galangin inhibits Beclin-1-dependent autophagy and activates the PI3K/AKT signaling pathway, thereby reducing neuroinflammatory damage and enhancing motor coordination in MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydro-pyridine)-induced Parkinson's mice.

Kaempferol-3-glucuronide is a glycoside form of kaempferol, a flavonoid widely distributed among plant species. Numerous studies have demonstrated its anti-inflammatory activity, including improving barrier function, reducing gastric inflammation, enhancing antioxidant capacity, and aiding in the treatment of obesity and colorectal cancer. Clinical trials also reported that diabetic patients consuming kaempferol-rich diets experienced significant reductions in TNF-α, IL-6, and CRP levels.[31–33]

Quercetin-3-O-α-D-glucuronide is a ubiquitous flavonoid that exhibits strong antioxidant, anti-inflammatory, antimicrobial, antihyperglycemic, and antihypertensive activities.[34,35] Desmanthin, a flavonoid C-glycoside commonly found in several legumes, shows significant inhibitory activity against aldose reductase—an enzyme implicated in diabetic complications such as cataracts and neuropathy—making it a promising candidate as an antidiabetic agent.[15] Robinetin, a naturally occurring polyhydroxylated flavonol, has been reported to exhibit broad biological activities, including antioxidant, anti-inflammatory, antiviral, and hepatoprotective effects[36.] Hayat et al.[37] also reported its palliative role in preventing polystyrene microplastic-induced lung toxicity in rats.

GC–MS Analysis of Methanolic and Ethanolic Extracts of *Pemphis acidula* Leaves

GC–MS analysis of the methanolic extract of *Pemphis acidula* leaves identified 13 chemical compounds with match qualities of 90% or higher, whereas the ethanolic extract contained 19 compounds meeting the same criteria (Tables 4 and 5). Match quality is a parameter indicating the degree of similarity between the library spectrum and the unknown spectrum; a higher match value reflects a greater likelihood of correct identification. The chromatograms are presented in Figures 3 and 4, respectively, and the relative abundance of each compound was calculated from its peak area percentage.

The major compounds identified in the methanolic extract, based on relative abundance, were Neophytadiene (24.55%),

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Supraene (20.10%), and Hexadecanoic acid, methyl ester (7.10%), while the major compounds in the ethanolic extract of *Pemphis acidula* were Supraene (13.86%), Heptadecane (13.48%), Bicyclo[3.1.1] heptane, 2,6,6-trimethyl-, [1R-(1α,2β,5α)]- (11.49%), and Hexadecanoic acid, methyl ester (10.38%). Supraene was detected in substantial amounts in both extracts; however, its relative concentration in the methanolic extract (20.10%) was higher than that in the ethanolic extract (13.86%) (Tables 4 and 5). Hexadecanoic acid, methyl ester was also present in both extracts, with a lower concentration in the methanolic extract (7.10%) compared to the ethanolic extract (10.38%)

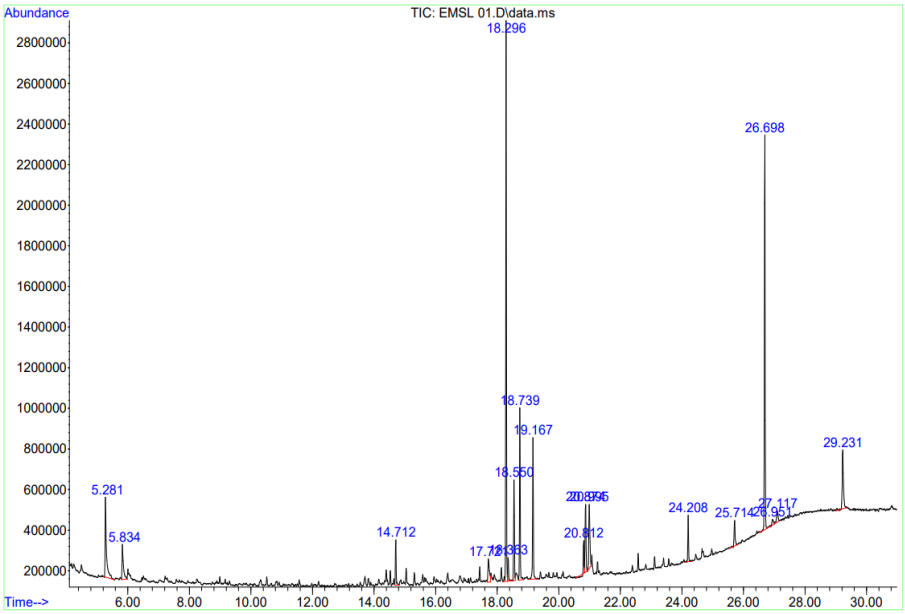

Figure 3. GC–MS Chromatogram of Methanolic Extract of *Pemphis acidula* Leaf

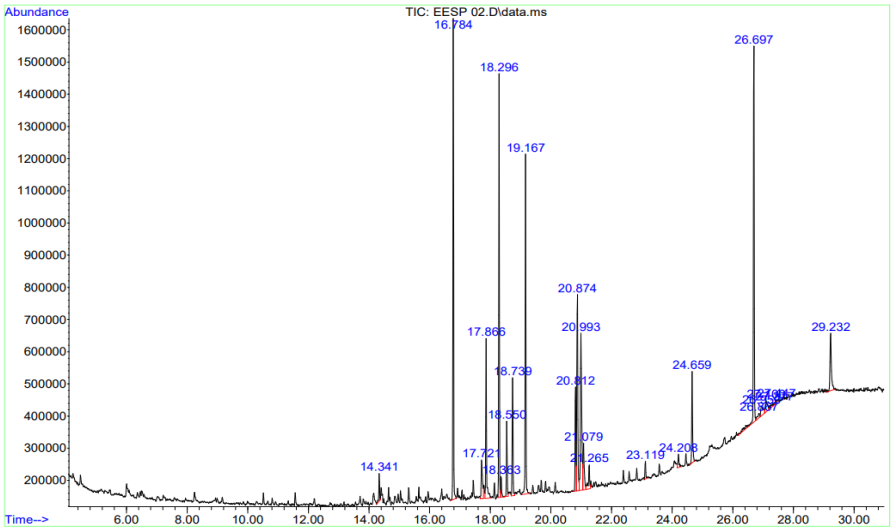

Figure 4. GC–MS Chromatogram of Ethanolic Extract of *Pemphis acidula* Leaf

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Table 4. Chemical Composition of Methanolic Extract of *Pemphis acidula* Leaf Identified by GC–MS Analysis

No.	RT	Compound Name	Mol. Formula	Mol.Wt (g/mol)	Area %	Quality
1	5.284	1-Methoxy-2-propyl acetate	$C_6H_{12}O_3$	132.16	6.77	72
2	5.839	Ethanol, 2-butoxy-	$C_6H_{14}O_2$	118.176	2.95	70
3	14.711	Butylated Hydroxytoluene	$C_{15}H_{24}O$	220.35	2.33	98
4	17.724	Loliolide	$C_{11}H_{16}O_3$	196.24	2.13	98
5	18.291	Neophytadiene	$C_{20}H_{38}$	278.5157	24.55	94
6	18.366	5-Eicosene, (E)-	$C_{20}H_{40}$	280.532	1.32	58
7	18.555	Neophytadiene	$C_{20}H_{38}$	278.5157	4.66	70
8	18.744	Neophytadiene	$C_{20}H_{38}$	278.5157	7.85	76
9	19.173	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45	7.10	98
10	20.811	9,12-Octadecadienoic acid (Z,Z)-methyl ester	$C_{19}H_{33}O_2$	294.4721	1.09	99
11	20.874	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292.4562	4.29	99
12	21.000	Phytol	$C_{20}H_{40}O$	296.53	4.63	91
13	24.202	Tritetracontane	$C_{44}H_90$	651.2	2.17	91
14	25.714	Eicosane	$C_{20}H_{42}$	282.5475	1.71	95
15	26.697	Supraene	$C_{30}H_{50}$	410.73	20.10	99
16	26.949	9-Octadecenoic acid	$C_{18}H_{34}O_2$	282.4614	0.58	95
17	27.113	9-Octadecenoic acid	$C_{18}H_{34}O_2$	282.4614	1.03	95
18	29.230	Vitamin E	$C_{29}H_{50}O_2$	430.71	4.75	99

Table 5. Chemical Composition of Ethanolic Extract of *Pemphis acidula* Leaf Identified by GC–MS Analysis

No.	RT	Compound Name	Mol. Formula	Mol.Wt (g/mol)	Area %	Quality
1	14.346	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	$C_{15}H_{22}$	202.36	0.91	98
2	16.778	Heptadecane	$C_{17}H_{36}$	240.47	13.48	98
3	17.724	Loliolide	$C_{11}H_{16}O_3$	196.24	2.02	99
4	17.862	Octadecane	$C_{18}H_{38}$	254.49	5.43	97
5	18.291	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, [1R (1.alpha.,2.beta.,5.alpha.)]-	$C_{10}H_{18}$	138.25	11.49	93
6	18.366	Cyclododecanol, 1-ethenyl-	$C_{14}H_{28}O$	212.37	0.80	42
7	18.555	Neophytadiene	$C_{20}H_{38}$	278.52	2.19	70
8	18.744	2-n-Octylfuran	$C_{12}H_{20}O$	180.29	3.67	72
9	19.173	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45	10.38	98
10	20.811	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294.47	3.38	99
11	20.874	7,10,13-Hexadecatrienoic acid, methyl ester	$C_{17}H_{28}O_2$	264.40	8.58	96
12	20.988	Phytol	$C_{20}H_{40}O$	296.53	8.68	99
13	21.076	Methyl stearate	$C_{19}H_{38}O$	298.50	2.26	98
14	21.265	Heptadecyl heptafluorobutyrate	$C_{21}H_{35}F_7O_2$	452.49	1.32	55

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No.	RT	Compound Name	Mol. Formula	Mol.Wt (g/mol)	Area %	Quality
15	23.118	cis-Vaccenic acid	$C_{18}H_{34}O$	282.5	0.63	90
16	24.214	6-Octadecenoic acid	$C_{18}H_{34}O_2$	282.46	0.85	95
17	24.454	9-Octadecenoic acid	$C_{18}H_{34}O_2$	282.5	0.70	84
18	24.655	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.55	3.18	91
19	26.697	Supraene	$C_{30}H_{50}$	410.72	13.86	98
20	26.861	9-Octadecenoic acid	$C_{18}H_{34}O_2$	282.5	0.43	95
21	26.949	4-Trifluoroacetoxytetradecane	$C_{16}H_{29}F_3O_2$	310.39	0.90	38
22	27.100	6-Octadecenoic acid, (Z)-	$C_{18}H_{34}O_2$	282.46	0.98	94
23	27.377	9-Octadecenoic acid	$C_{18}H_{34}O_2$	282.5	0.36	93
24	27.440	9-Octadecenoic acid	$C_{18}H_{34}O_2$	282.5	0.36	95
25	29.230	dl-.alpha.-Tocopherol	$C_{29}H_{50}O_2$	416.68	3.38	99

Supraene, a natural triterpenoid, is a stereoisomer of squalene known as trans-squalene (Fig. 5). Supraene has been reported to possess several important biological activities, including antioxidant, anticancer, and detoxifying effects. It also exhibits antifungal activity against fungi and oomycetes, as well as hypoglycaemic activity through the inhibition of α-glucosidase and α-amylase.[38]

Squalene, a naturally occurring unsaturated triterpenoid hydrocarbon, is abundant in shark liver oil and various plant seeds such as amaranth, palm, wheat germ, and olive. It demonstrates diverse biological activities, including antioxidant, anti-inflammatory, immune-enhancing, anti-aging, lipid-lowering, antitumor, antibacterial, and detoxifying effects.[39] Moreover, squalene is widely utilized in pharmaceuticals and dietary supplements due to its ability to protect cells from oxidative stress and support detoxification processes, as well as for its well-known skin-protective properties.[40]

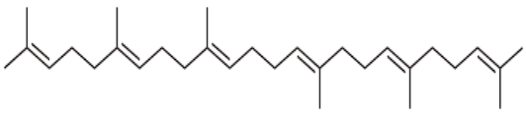

Figure 5. Supraene, a natural triterpenoid identified in methanolic and ethanolic extracts of *Pemphis acidula* leaves, is a stereoisomer of squalene (NIST Chemistry WebBook, SRD 69)

Neophytadiene is a sesquiterpenoid compound (Fig. 6) that has been demonstrated to possess microbicidal, anti-inflammatory, antioxidant, anticancer, analgesic, antipyretic, and neuropharmacological activities. It also exhibits carminative, gastric inhibitory, antiulcerative, histamine-release inhibitory, antiprotozoal (against Leishmania), and antiparasitic properties. Therefore, neophytadiene is considered a potential therapeutic agent.[41]

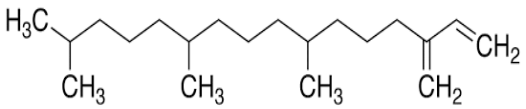

Figure 6. Neophytadiene

Heptadecane (Fig. 7) is a straight-chain alkane with the chemical formula $C_{17}H_{36}$, known for its strong antioxidant and anti-inflammatory properties. Kim et al.[42] reported that it reduces COX-2 and iNOS expression by suppressing NF-κB signaling and modulating MAPK pathways, thereby alleviating oxidative stress and inflammation. Recent studies further support its biological activity; heptadecane detected in *Gracilaria salicornia* and various ethanolic plant extracts, exhibited strong antioxidant and antibacterial potential. [43,44] Its presence in methanolic extracts of *Brassica oleracea* var. italica was also linked to antioxidant capacity and cytotoxicity against A549 cells,[45] while its derivatives such as E-15-heptadecenal from *Rhynchoglossum notonianum* exhibited antibacterial and anti-inflammatory activity.[46] These findings indicate that heptadecane is a multifunctional compound with significant antioxidant,

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antimicrobial, and cytoprotective properties.

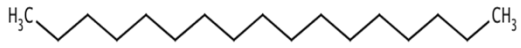

Figure 7. Heptadecane

Bicyclo[3.1.1] heptane, 2,6,6-trimethyl-, [1R(1α,2β,5α)] (Fig. 8) is a terpenoid derivative with demonstrated antioxidant and antiproliferative activities. Toha et al.[47] suggest that this compound has the potential to inhibit cell proliferation and reduce oxidative stress, making it a promising candidate for use in anticancer therapy.

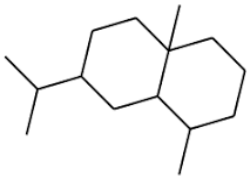

Figure 8. Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, [1R (1.alpha.,2.beta.,5.alpha.)]

Hexadecanoic acid methyl ester (Fig. 9), also known as methyl palmitate, is a fatty acid ester that is widely distributed in plants. It has been reported to exhibit multiple biological activities, including antioxidant, anti-inflammatory, antimicrobial, antihypercholesterolemic, hepatoprotective, anti-arthritic, and cancer-preventive properties.[48,49]

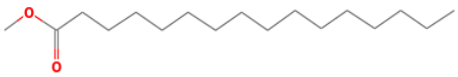

Figure 9. Hexadecanoic acid, methyl ester

Other compounds identified in both extracts, in addition to the major ones were Loliolide; 9,12-Octadecadienoic acid (Z,Z)-, methyl ester; Phytol; 9-Octadecenoic acid; and Tocopherol or Vitamin E (Table 6).

Loliolide (Fig. 10) is a naturally occurring and widely distributed monoterpenoid lactone that has been isolated from various plants, including *Vicia tenuifolia* Roth, white mulberry (*Morus alba*), tobacco (*Nicotiana tabacum*), and *Pemphis acidula*. Loliolide is also found in marine organisms, such as *Sargassum horneri*[50] and *Codium tomentosum*.[51] Liolide, a carotenoid metabolite, exhibits numerous pharmacological activities, including anti-Parkinson, antioxidant, anticholinesterase, antidepressant, antitumor, and antimicrobial properties.[50,52]

Table 6. Comparison of phytochemical content which showed a match quality of 90% or higher in methanolic and ethanolic extracts of *Pemphis acidula* leaf analyzed by GC–MS

No.	RT (min)	Compound	Methanolic Extract	Ethanolic Extract
1	14.346	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	-	+
2	14.711	Butylated Hydroxytoluene	+	-
3	16.778	Heptadecane	-	+
4	17.724	Loliolide	+	+
5	17.862	Octadecane	-	+
6	18.291	Neophytadiene	+	-
7	18.291	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, [1R (1.alpha.,2.beta.,5.alpha.)]-	-	+
8	18.366	Cyclododecanol, 1-ethenyl-	-	+
9	18.555	Neophytadiene	+	-

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No.	RT (min)	Compound	Methanolic Extract	Ethanolic Extract
10	19.173	Hexadecanoic acid, methyl ester	+	+
11	20.811	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	+	+
12	20.874	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	+	-
13	20.874	7,10,13-Hexadecatrienoic acid, methyl ester	-	+
14	20.988/21.000	Phytol	+	+
15	21.076	Methyl stearate	-	+
16	23.118	cis-Vaccenic acid	-	+
17	24.202	Tritetracontane	+	-
18	24.214	6-Octadecenoic acid	-	+
19	24.454/26.861/ 26.949	9-Octadecenoic acid	+	-
20	24.655	Bis(2-ethylhexyl) phthalate	-	+
21	25.714	Eicosane	+	-
22	26.697	Supraene	+	+
23	26.949	4-Trifluoroacetoxytetradecane	-	+
	26.949	9-Octadecenoic acid	+	+
24	27.100	6-Octadecenoic acid, (Z)-	-	+
25	29.230	dl-alpha-Tocopherol/ Vitamin E	+	+

In a recent study, Loliolide isolated from *Sargassum horneri* was reported to alleviate oxidative stress and inflammatory responses by activating the Nrf2/HO-1 pathway, suggesting its potential as a natural cytoprotective compound.[53] Recent studies have demonstrated that Loliolide interacts with CXCR4/7 and MnSOD, effectively suppressing cancer cell invasion, migration, and metastasis, as well as inhibiting the epithelial–mesenchymal transition (EMT) process. These findings suggest that Loliolide is a promising EMT inhibitor in colon and breast cancer.[54]

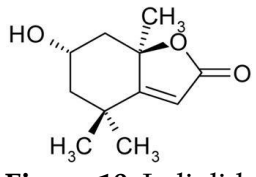

Figure 10. Loliolide

Phytol is an acyclic diterpene alcohol, a diterpenoid, and a long-chain primary fatty alcohol (Fig. 11). It is a constituent of chlorophyll. Phytol has been reported to occur in *Camellia sinensis*, *Desmos chinensis*, *Artemisia princeps*, and other organisms. Phytol has several industrial applications. It is primarily known for its fragrance and flavoring properties and is widely used in perfumes, cosmetics, and food products. Additionally, phytol acts as a starting material in the production of synthetic vitamins E and K_1.

Besides its industrial significance, phytol exhibits diverse pharmacological properties, including antioxidant, anti-inflammatory, antimicrobial, and antiallergic effects.[55] Recent research has revealed that phytol and its metabolite, phytanic acid, can modulate cellular

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transcription by activating peroxisome proliferator-activated receptors (PPAR-α, PPAR-γ) and retinoid X receptors (RXR), indicating their involvement in lipid metabolism and anti-inflammatory responses. Recent reports state that phytol exhibits anticancer activity by inhibiting the growth of human non-small cell lung cancer (A549) cells.

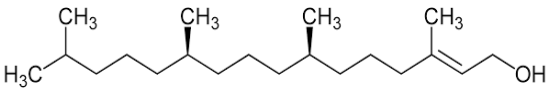

Figure 11. Phytol

Although it is not the main compound in the extracts, tocopherol (vitamin E) has been confirmed to be present in both methanolic and ethanolic extracts of *Pemphis acidula* leaves. Vitamin E, or α-tocopherol (Fig. 12), is a fat-soluble vitamin that has long been recognized as a potent natural antioxidant and anti-inflammatory agent, notable for its role in promoting health and preventing diseases associated with oxidative stress and free radical damage. It plays an essential role in maintaining cellular integrity and supporting immune function.[56,57]

Furthermore, the biological activities of vitamin E independent of its antioxidant function have also been reported. These include potential roles in immune function, cell signaling, gene expression regulation, and interactions with long-chain fatty acids and other vitamins.[58] This highlights the importance of vitamin E in natural resources that could be developed as raw materials for medicinal products.

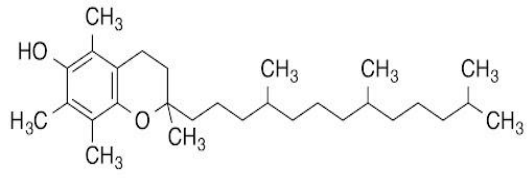

Figure 12. α-tocopherol

CONCLUSIONS

Results of the study revealed a diversity of bioactive compounds with medicinal potential found in both methanolic and ethanolic extracts of *Pemphis acidula* leaf. Ethanol demonstrated superior extraction capacity, yielding a broader diversity of phytochemical constituents compared to methanol. In the methanolic extract, 25 compounds were identified by LC–MS/MS and 13 volatile compounds by GC–MS analysis, whereas in the ethanolic extract, 49 compounds were identified by LC–MS/MS and 19 volatile compounds by GC–MS analysis. These findings confirm that solvent polarity significantly influences the chemical profiles of *Pemphis acidula* leaf extracts and highlight their potential as promising sources of bioactive compounds for pharmaceutical development.

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