

# Papaver Decaisnei: GC-MS Alkaloids Profiling, in Vitro Antioxidant, and Anticancer Activity

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## Research Article

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# *Papaver Decaisnei*: GC-MS Alkaloids Profiling, in Vitro Antioxidant, and Anticancer Activity

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# Abstract

The *Papaver L.* plant have been well known as a source of pharmaceutically valuable alkaloids (noscapine, thebaine, codeine, roemerine, papaverine and morphine). The current study investigates the phytochemical, in-vitro antioxidant, and anticancer activities of *papaver decaisnei*, an endemic plant species to the flora of Kurdistan-Iraq. The chemical analysis of the methanolic (MeOH) extracts of flowers, leaves, and roots of *papaver decaisnei* were made by using gas chromatography-mass spectrophotometry (GC-MS), and the antioxidant activity evaluation done by radical scavenging [on 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2]-azino-bis (3- ethylbenzothiazoline-6-sulfonic acid) (ABTS)], and reducing power [cupric reducing antioxidant capacity (CUPRAC), and ferric reducing antioxidant power (FRAP)] assays. The anticancer actions were presented as IC<sub>50</sub> (inhibitory concentration at 50%) on human colorectal adenocarcinoma (Caco-2), mammary cancer cells (MCF-7), and human cervical carcinoma (HeLa) cells. The results of the phytochemical analysis showed 17, 19, and 22 chemical compounds for flowers, leaves, and roots of *P. decaisnei*, respectively. The prevalent organic compounds of *P. decaisnei* were alkaloids, phenolics, fatty acids, esters, and phytosterols, namely **Roemerine (70.44%)**, Decarbomethoxytabersonine, 9,12,15-Octadecatrien-1-ol, Hexadecanoic acid, 6,8-Dioxa-3-thiabicyclo(3,2,1)octane 3,3-dioxide, and  $\gamma$ -Sitosterol. The antioxidant activity of plant organ extracts was within 39.1-143.5  $\mu\text{g/ml}$  for DPPH and 123.12-276.4  $\mu\text{g/ml}$  for ABTS assays, while, the FRAP and CUPRAC values ranged within 12.4- 34.3 and 42.6-75.8  $\mu\text{g/ml}$ , respectively. The anticancer action of *P.decaisnei* organ extracts was found against all tested human cell lines (Caco-2, MCF-7, HeLa) with inhibitory concentrations (IC<sub>50</sub>) values between 125.3-388.4  $\mu\text{g/ml}$ . The presented data on alkaloid contents and biological activity of *P. decaisnei* can serve a ground knowledge for the future biomedical synthesis and cancer research projects.

## Introduction

The poppy (*Papaver L.*) belongs to the family Papaveraceae with about 820 species belonging to 43 genera [1]. In the flora of Iraq, the genus *Papaver* comprises of 15 annual and perennial species and most of them occurred in Kurdistan (80% in Rwanduz district), one of which species are endemic namely: *papaver decaisnei* Hochst. [2]. The *Papaver* species share different characteristics with releasing approximately 170 different alkaloids [3]. The alkaloid chemicals (thebaine, morphine, and codeine) of *Papaver* plant have been used as pharmaceutical and curative agents [4]. Thebaine is commonly used for the production of pentacyclic-morphinanbased remedies. Morphine, codeine, and noscapine have shown great analgesic as well as anti-proliferative efficacy [5]. Over the recent years, the global trends show increased usage of *Papaver* alkaloids and their derivatives in pharmaceutical industries [6].

Natural antioxidants have gained more popularity in the recent years and numerous *Papaver* species have been reported to possess this activity [7],[8]. This biological activity by *Papaver* species has been linked with its moderate hydrophilic secondary metabolites such as phenolic acids, flavonoids, and its glycosides, as well as compounds soluble in lipid fractions like sterols, tocopherols, tocotrienols, carotenoids and zeaxanthins, which may act as antioxidant agents in diseases related with oxidative stress [9],[10]. Alkaloid chemicals (macranthine, berberine, roemerin, and Noscapine) of *Papaver* species acquired more attention as anticancer agents in recent years [11],[12],[13]. Enlightened by the above passage, identifying the

biologically active chemicals in the *Papaver* species and *Papaver* organs aids breeders to select desired poppy genotypes for the purpose of harvesting and breeding process, which could help in developing functional foods with better health benefits for the consumers.

Therefore, the current study aimed to determine the GC-MS phytochemical profile, the antioxidant and antiproliferative activity of roots, flowers, and leaves of *P. decaisnei*.

## Materials And Methods

### Plant collection

The roots, leaves parts, and flowers of *Papaver decaisnei* (figure 1) were collected at different growing stages during spring of 2021 from Erbil, Iraq (Altitude: 36.609153, Latitude: 44.526220). The plant was identified and the voucher specimen was deposited from the Salahaddin University Herbarium-Education College (ESUH). (voucher no. 6548).

### Plant extract preparation

The dried roots, flowers, and leaves (100 mg each) of *P. decaisnei* were macerated with 1 L of Methanol (99.9 % absolute methanol) extracting solvent by aluminum foil and ultrasonic incubation at room temperature for 2 hours. The solvent drainage done by rotary evaporator in water bath 40 °C to synthesis the crude extract, then the extracts freeze-dried to remove the solvent completely. The obtained MeOH extract was 21.4, 25.2, 19.6 % (w/w) for flowers, leaves, and roots, respectively. The extracts were stored at +4°C for further investigation[14].

### Phytochemical Profiling

The *Papaver* organ extracts were screened qualitatively for their alkaloid contents using the GC/GC-MS technique. The methanol extract examined by Shimadzu Model QP-2010 GC coupled with MS. GC equipped with HP-5 MS (5% phenylmethyl siloxane), capillary column (30 m × 0.25 mm i.d., film thickness 0.25µm) with temperature 60°C (2') to 250 °C for 10 minutes at a rate of 20 °C /min, helium flow rate 1.61ml/minute. The sustenance ion source was at 250 °C and 70 eV electron energy. The extracts were mixed with methanol before injecting 1µl into the column. The Wiley GC/MS Library and Adams Library, and Mass Finder Library were used to determine the exact name and molecular weight of the unknown component by comparing their mass spectrum with the reference spectrum[15],[16].

### Antioxidant activity

The free radical scavenging activity of *Papaver* extracts was evaluated by [1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)] assays, and by reducing power [cupric reducing antioxidant capacity (CUPRAC) and ferric reducing antioxidant power (FRAP)] assays as explained by [17]. The antioxidant activity was presented as milligrams of Trolox equivalents (TE) per liter.

### Anticancer activity

The Papaver extracts of flowers, leaves, and roots were tested for anti-proliferative effectivity by evaluating their minimal inhibitory concentration (IC<sub>50</sub>) on Caco-2 (human colorectal adenocarcinoma), MCF-7 (human breast adenocarcinoma), and HeLa (human cervical cancer) cell lines using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide) assay as previously explained [18]. The counting of viable cells made at 580nm by ELISA plate reader as equal value to the intensity in light absorbance.

### **Statistical Analysis.**

Multivariate statistical analysis of GC-MS experimental data was performed by GC Shimadzu software. All experiments were carried out in triplicate. The IC<sub>50</sub> values were determined as drug concentrations leading to a 50% reduction in the viability or inhibition of the biological activity. The IC<sub>50</sub> values were calculated using a four-parametric logistic curve (Sigma Plot 11.0). The biological activity data interpretation made by student t-test ( $\alpha = 0.05$ ) using the SPSS v. 14.0 program. Finally, the data measurement was done by one-way analysis (ANOVA) of SPSS statistical software package, version 24.0 for Windows. The significance value is considered as  $p < 0.05$ .

## **3. Results And Discussion**

### **3.1 GC-MS profile**

The chromatogramatical study of MeOH extracts of flowers, leave, and roots of *P.decaisnei* showed different alkaloid contents (Table 1). The tested plant organs namely flowers, leaves, and roots contained 17, 19, and 22 chemical compounds, respectively, accounting for 100% of their volatile compounds (fig. 2, 3, 4).

**Table 1.** Shows phytochemical contents of roots, leaves, and flowers of *P.decaisnei*.

NO.	RT	Components Name	Similarity	Peak area percentage %		
				PF	PL	PR
1	7.55	Glycerin	9	6.41	-	-
2	8.666	Uracil, 1-N-Methyl-	59	-	1.76	-
3	8.686	Thymine	72	-	-	0.96
4	9.003	Guaiacol	94	1.85	-	0.99
5	10.259	4H-Pyran-4-One, 2,3-Dihydro-3,5-Dihydroxy-6-Methyl-	72	-	-	1.19
6	10.58	Silane, Triethylmethoxy-	72	1.99	-	-
7	11.021	2-Methyl[1,3,4]Oxadiazole	59	-	-	0.72
8	11.659	1,2-Benzenediol	81	-	-	0.54
9	12.038	2,3-Dihydro-Benzofuran	90	-	2.68	0.77
10	13.029	2H-Pyran-2-One, Tetrahydro-4-Hydroxy-4-Methyl-	91	4.85	-	-
11	13.642	Trans-Anethole	98	-	-	2.55
12	14.269	2-Methoxy-4-Vinylphenol	96	2.18	4.14	1.63
13	15.079	2,6-Dimethoxyphenol	95	-	-	0.61
14	15.592	DL-Proline, 5-Oxo-, Methyl Ester	78	2.80	-	0.44
15	16.127	Vanillin	96	-	-	0.52
16	17.201	6,8-Dioxa-3-Thiabicyclo(3,2,1)Octane 3,3-Dioxide	47	-	8.07	2.49
17	17.958	1,6-Anhydro-.Beta.-D-Glucopyranose (Levoglucozan)	47	2.67	-	-
18	18.457	2,4-Di-Tert-Butylphenol	96	-	2.80	-
19	18.462	Phenol, 2,4-Bis(1,1-Dimethylethyl)-	96	0.60	-	0.84
20	20.376	.Alpha.-Cedrol	99	-	-	1.40
21	21.907	3a-Hydroxy-1,2,3,3a,8,8a-Hexahydropyrrole(2,3b)Indole	90	-	-	0.45
22	22.763	4-Methoxy-6-Methyl-2-Propylpyridine	50	-	-	1.27
23	23.297	Benzyl Benzoate	98	3.59	2.52	-
24	24.496	Neophytadiene	96	-	4.79	-

25	25.944	Hexadecanoic Acid, Methyl Ester	99	-	1.70	-
26	26.52	Hexadecanoic Acid	99	16.40	14.66	2.66
27	28.725	Methyl 9,12,15-Octadecatrienoate	99	-	2.19	-
28	28.901	Phytol	91	-	3.10	-
29	29.187	9,12-Octadecadienoic Acid (Z,Z)-	96	3.47	-	1.45
30	29.192	Linoleic Acid	97	-	2.18	-
31	29.29	9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-	94	12.00	25.45	-
32	29.607	Octadecanoic Acid	98	-	2.98	-
33	35.32	8.Beta.,13:8.Alpha.,14	86	-	8.81	-
34	35.325	Decarbomethoxytabersonine	56	24.49	-	-
35	35.382	Roemerine	58	-	-	<b>70.44</b>
36	36.145	3-(3-Methoxyphenyl)Propenenitrile, 2-(Diethoxyphosphinyl)-	95	-	-	<b>1.23</b>
37	36.98	Butriptyline	72	-	2.25	-
38	36.98	Amitriptylinoxide	59	3.60	-	-
39	39.279	4-Methoxybenzene, 1-(2-Hydroxynaphthylmethyleneamino)-	95	-	2.08	-
40	39.284	3'-Methyl-1'-Phenylspiro(Indoline-2,4'-(2)Pyrazoline)-5'-One	72	3.18	-	-
41	39.289	3'-Methyl-1'-Phenylspiro(Indoline-2,4'-(2)Pyrazoline)-5'-One	72	-	-	5.16
42	43.139	3,4-Dihydro-6,7-Dimethoxyisoquinoline 2-Oxide	43	5.62	-	-
43	43.238	Gibberellin A3	58	-	2.55	-
44	44.695	$\gamma$ -Sitosterol	95	4.31	5.31	1.69

**Table 1. (continue)**

	<b>Compound type (Total number in 3 plant organs)</b>	<b>% and (number) in PF</b>	<b>% and (number) in PL</b>	<b>% and (number) in PR</b>
	Alkaloids (12)	15.78 (3)	23.52 (4)	22.72 (5)
	Phenolics (11)	10.52 (2)	17.64(3)	27.27 (6)
	Fatty acids (8)	15.78 (3)	17.64(3)	9.09 (2)

Esters (6)	15.78 (3)	11.76 (2)	4.54 (1)
Terpenoids (5)	21.05 (4)	0 (0)	4.54 (1)
Phytosterol (3)	5.26 (1)	5.88 (1)	4.54 (1)
Coumaranes (2)	5.26 (1)	0 (0)	4.54 (1)
Organosulfur (2)	5.26 (1)	0 (0)	4.54 (1)
Alcohols (2)	5.26 (1)	5.88 (1)	0 (0)
Oxapanes (1)	0 (0)	5.88(1)	0 (0)
Aromatics (1)	0 (0)	0 (0)	4.54 (1)
Others (5)	0 (0)	11.76 (2)	13.63 (3)
Total	100%	100%	100%

a: Retention time (tR [min]) on a Restek Rtx-5 column. Peak area percentage calculated from the GC-FID chromatogram. PF: Papaver flower, PL: Papaver leaves, PR: Papaver roots.

The chemical profiling of flower extracts showed 17 phytochemicals (shown in Table 1 and Figure 2), includes majorly Decarbomethoxytabersonine (24.49%), Hexadecanoic acid (16.40%), 9,12,15-Octadecatrien-1-ol, (Z,Z,Z)- (12%), Glycerin(6.41%), 3,4-dihydro-6,7 dimethoxyisoquinoline 2-oxide(5.62%), 2H-Pyran-2-one, tetrahydro-4-hydroxy-4-methyl-(4.85%),  $\gamma$ -Sitosterol(4.31%), Benzyl benzoate (3.60%), and Amitriptylinoxide (3.59%). Researchers have declared that Decarbomethoxytabersonine acts as a strong alkaloid that could play important role as anticancer and reduce free radicals during oxidative stress conditions [19].

The major phytochemicals of methanolic leave extracts were found as 9,12,15-octadecatrien-1-ol (25.45%), hexadecanoic acid (14.66%), 8.beta.,13:8.alpha.,14(8.81%), 6,8-dioxa-3-thiabicyclo(3,2,1)octane 3,3-dioxide (8.07%),  $\gamma$ -sitosterol (5.31%), neophytadiene (4.79), 2-methoxy-4-vinylphenol (4.14%), and phytol (3.10%) (table 1 and figure 3). previous researches have showed different biological activities such as antioxidant and anticancer of plant extracts enriched in 9,12,15-Octadecatrien-1-ol and Hexadecanoic acids [20]. The antioxidant and anti-proliferative effect of  $\gamma$ -Sitosterol has been also reported previously by the researchers. [21]. The analysis of methanolic extract of *P.decaisnei* leaves also showed some chemicals in low amount including octadecanoic acid (2.98%), 2,4-di-tert-butylphenol (2.80%), 2,3-dihydro-benzofuran (2.68%), gibberellin a3 (2.55%), and few others, which were not discussed in this article.

The main detected chemicals of MeOH root extracts were Roemerine (70.44%), 3'-methyl-1'-phenylspiro (indoline-2,4'-(2)pyrazoline)-5'-onE (5.16%), and Hexadecanoic acid (2.66%) ( Table 1 and Figure 4). Roemerine is a naturally occurring alkaloid that was reported to facilitate in reducing symptoms of Neurodegenerative diseases [22]. Previous work also detected significant amount of Roemerine in *P. lacerum* and *P. syriacum* and labeled it as possible strong antidepressant drug [23]. The current study also detected a variety of alkaloids in the plant organs as mentioned in the table 1, which were not discussed in this report.



The organic class of major detected chemicals were found as alkaloids, phenolics, and fatty acids, esters, and terpenoids. While, the organic classes of minor detected phytochemicals in the three plant organs were organosulfur, coumaranes, fatty alcohols, and phytosterols (Table 1). Our data results are in agreement with a recent study on the chemical profiling of *P.decaisnei* by thin layer chromatography (TLC), which reported alkaloids as the main organic class content in *P.decaisnei*, namely aporphine-type roemerine and proaporphine-type mecambrine (PD2) [24]. Similar alkaloid contents (roemerine, dehydroromerine, roemerine N-oxide, rhoeagenine) have been reported from *P. glaucum* [24]. Furthermore, same alkaloid constituents were reported from *P. somniferum* [25], *P. bracteatum* [26], and *P. rhoeas* [27].

To the researcher's best knowledge, there is no previous research on the GC-MS profiling of *P. decaisnei*, Therefore, the current study considered as the first record on the identification of specific phytochemicals in flowers, leaves, and roots of *P.decaisnei*.

### 3.2 Antioxidant activity

The results of the antioxidant activity evaluation of *P.decaisnei* organs demonstrated that the species possessed profound antioxidant capacity. This may be due to the presence of alkaloid and polyphenolic chemicals. The antioxidant activity measured by radical scavenging (DPPH and ABTS) and reducing activity (FRAP and CUPRAC) assays, as presented in Table 2. The plant extraction needed to inhibit 50% of essay reagents is presented as IC<sub>50</sub>. The lower the IC<sub>50</sub> value of an extract indicates its efficiency as an antioxidant agent. In antioxidant activity measurement by DPPH and ABTS assay, flowers (39.1 and 135.4 µg /mL trolox) were superior to leaves (81.35 and 245.6 µg /mL trolox) and roots (143.5 and 276.4 µg /mL trolox), respectively. In the antioxidant evaluation by FRAP and CUPRAC assays, leaves recorded the highest values (12.4 and 42.6 µg /mL trolox) followed by roots (18.3 and 68.1 µg /mL trolox) and flowers (34.3 and 75.8 µg /mL trolox), respectively.

**Table 2.** Antioxidant activity of MeOH extracts of roots, leaves, and flowers of *P.decaisnei*<sup>1</sup>.

Plant extracts	DPPH scavenging <sup>2</sup>	ABTS scavenging <sup>2</sup>	FRAP reducing <sup>3</sup>	CUPRAC reducing <sup>3</sup>
Flowers	39.1 ± 0.53 <sup>b</sup>	135.4 ± 0.78 <sup>b</sup>	34.3 ± 0.05 <sup>b</sup>	75.8 ± 0.9 <sup>b</sup>
Leaves	81.35 ± 0.111 <sup>a</sup>	245.61 ± 0.23 <sup>a</sup>	12.4±0.08 <sup>a</sup>	42.6±0.3 <sup>a</sup>
Roots	143.5 ± 3.06 <sup>a</sup>	276.4±0.045 <sup>a</sup>	18.3±1.023 <sup>c</sup>	68.1±0.9 <sup>c</sup>
Trolox	1.4±0.02 <sup>c</sup>	2.29±0.02 <sup>c</sup>	2435.1±0.01 <sup>a</sup>	2255.2±0.02 <sup>a</sup>
EDTA <sup>4</sup>	ND <sup>5</sup>	ND	ND	ND

<sup>1</sup>The values indicated by different superscripts within the same column are different according to the Tukey's honestly significant difference post hoc test at 5% significance level. (value as mean ±standard deviation)

<sup>2</sup>IC<sub>50</sub> (µg /mL), inhibition concentration at which 50 % of the DPPH (2,2-Diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) radicals were scavenged and the ferrous ion-ferrozine complex were inhibited.

<sup>3</sup> EC<sub>50</sub> (µg /mL): Effective concentration at which the absorbance was 0.5 for CUPRAC (Cupric ion reducing antioxidant capacity) and FRAP (Ferric reducing antioxidant power) assays.

<sup>4</sup> EDTA: Ethylenediaminetetraacetic acid (disodium salt).

<sup>5</sup> nf: Not detected.

The antioxidant activity shown by different organs of *P.decaisnei* could be linked to their phytochemical contents. The alkaloid and polyphenolic of different plant species have been reported to possess antioxidant activity [28],[29],[30],[31]. The current study shows flowers as the most active antioxidant part in DPPH and ABTS assays, these could be correlated with its chemical contents, mainly terpenoids, esters, and fatty acids, namely decarbomethoxy tabersonine, hexadecanoic acid, and anthocyanins. The present outcome agrees with previous claims [29],[32],[33],[34]. Previous studies have also reported alkaloids namely, thebaine, noscapine, morphine, and codeine as antioxidant agents [10],[35].

The antioxidant estimation by FRAP and CUPRAC assays shows leaves as a superior part in antioxidant activity. These increased reducing power activity of leaves could be associated with its alkaloid, fatty acids and polyphenolic contents, mainly 9,12,15-Octadecatrien-1-ol, Hexadecanoic acid, and 8.beta.,13:8.alpha.,14. The previous investigation also showed Papaver leave extracts as a stronger radical scavenging agent [36],[37]. Previous phytochemical screening of Papaver leaves concluded flavonols, like quercetin, kaempferol, myricetin, and isorhamnetin, as effective antioxidants [38]. Furthermore, scientists have correlated increased total phenolic content in *Papaver rhoeas* L. leave extracts with its high antioxidant activity [36].

The current research study detected significant amount of Roemerine in MeOH root extracts of *P.decaisnei*, a known aporphine alkaloid, which was reportedly stated as antioxidant and anticancer drugs [39]. Furthermore, a study by D. muthna and his colleagues confirmed the antioxidant and anticancer efficacy of aporphine members like roemerine [40]. Accordingly, numerous studies reported the antioxidant capacity of alkaloids isolated from different plant species [8], [41], [42]. The data stated above could be a reliable evidence for the antioxidant activity of *P.decaisnei*.

### 3.3 Anticancer activity

The anti-proliferative activity of *P.decaisnei* organs presented as IC<sub>50</sub> value, which ranged from 165.2-388.4 µg/mL on cancer cells derived from Caco-2 (human colorectal adenocarcinoma), MCF-7 (human breast adenocarcinoma), and HeLa (human cervical cancer) (Table 4). Moreover, Doxorubicin was used as a reference against same cancer cell lines.

**Table 4.** The Anti-proliferative activity IC<sub>50</sub> (µg/mL) of extract on human cell lines after 24 hr of treatment.

Cell line	IC <sub>50</sub> values (µg/mL) <sup>1</sup>			
	<i>Flowers MeOH extract</i>	<i>Leaves MeOH extract</i>	<i>Roots MeOH extract</i>	DOX <sup>2</sup>
Caco-2	223.4±2.1	176.2±3.8	194.7±6.5	6.7±0.4
MCF-7	306.5±9.8	268.2±12.3	388.4±11.2	18.8±0.3
HeLa	228.4±3.1	165.3±2.3	125.3±4.2	14.0±0.1

Key: Caco-2 (human colorectal adenocarcinoma), MCF-7 (human breast adenocarcinoma), and HeLa (human cervical cancer). (\* value as mean±standard deviation (n = 3).)

<sup>1</sup>Mean value ±S D of IC<sub>50</sub> (µg/mL), inhibition concentration at which 50 %

<sup>2</sup> DOX: Doxorubicin.

Over the last decades, many cancer diseases like human colorectal adenocarcinoma, hepatocellular carcinoma were tried to be treated by plant-derived compounds as a useful alternative source with less downside effects than synthetic drugs. Several flowering plants including Papaver species produces enormous alkaloids and aromatic hydrocarbon compounds [43]. Furthermore, alkaloids (narcotine, morphine, codeine, narceine and thebaine) are nitrogenous waste producing compounds which have medicinal importance including antidepressant and pain relieving effects for humans and animals [44]. The anticancer activity of plant alkaloids raises scientist's hope in dealing with this deadly disease because of its less side effects than synthetic chemo-therapy. Furthermore, recent decades witness an intense race between the scientists to find specific alkaloids with the most active indole ring and hydrocarbon chains against cell lines, as these properties significantly affect the delivery of drug [45],[46]. Therefore, we investigated the potency of MeOH extracts of flowers, leaves, and roots of *P.decaisnei* against the growth of Caco-2, MCF-7, and HeLa cell lines. The results show higher anticancer activity of leaves extracts (176.2, 268.2 µg/mL) against Caco-2 and MCF-7 cell lines as compared to that activity of roots (194.7, 388.4 µg/mL), and flower extract (223.4, 306.5 µg/mL) against the same cell lines, respectively. This leave superiority as anticancer agent could be related to its higher content of alkaloids, fatty acids, and phytosterols namely 9,12,15-Octadecatrien-1-ol (25.45%), Hexadecanoic acid (14.66%), 8.beta.,13:8.alpha.,14(8.81%), and γ -Sitosterol (5.31%), which were reportedly considered as the strong anticancer agents [47], [48],[49], [50].

The methanolic extract of *P.decaisnei* roots was superior in anticancer activity against HeLa cell lines (125.3 µg/mL), which were higher than that (165.3 µg/mL) and (228.4 µg/mL) for leaves and flowers, respectively. This root extract superiority against certain cell lines could be ascribed to its increased content of alkaloids and phenols namely roemerine (70.44%) and 3'-methyl-1'-phenylspiro(indoline-2,4'-(2)pyrazoline)-5'-one (5.16%), which were already highlighted as a significant anti-proliferative agent by previous natural product studies [51],[39],[52]. The effectivity of roemerine to reduce the proliferation and migration of different human cell lines and stimulated their apoptosis in different degrees have also reported previously [11]. Similar to our findings, researches have also reported the superiority of

*P.somniferum* roots against HeLa cell lines [12]. Moreover, it could be emphasized that Papaveraceae members are rich with alkaloid contents and many studies have shown their anticancer efficacy against several human cancer cell lines [52],[53]. The systematic search showed no previous anticancer study of *P.decaisnei*, therefore, the current work considered as the first record regarding the anticancer of *P.decaisnei* plant organs.

## Conclusion

The current study reports GC-MS profiling, antioxidant, and anticancer of flowers, leaves, and root extracts of *P.decaisnei* for the first time. The major alkaloids of flowers were found as Decarbomethoxytabersonine, Hexadecanoic acid, and anthocyanin. Furthermore, the MeOH leave extract analysis showed 9,12,15-Octadecatrien-1-ol, Hexadecanoic acid, and  $\gamma$ -Sitosterol as their main alkaloid constituents. The main alkaloid compound of root extract was **roemerine** (70.44%), a known alkaloid as antidepressant and antianxiety-like remedy. The antioxidant analysis showed flower extracts as superior part in DPPH and ABTS assays and root extracts as the most effect plant organ in reducing power activities by FRAP and CAPRAC assays. The anticancer investigation of flowers, leaves, and roots of *P.decaisnei* showed significant potency of plant organs against the growth of Caco-2, MCF-7, and HeLa cell lines. Our study provides a detailed phytochemistry and biological activities of *P.decaisnei* that can be used for numerous biomedical production and cancer research however, further research is needed to explore the toxicity and down side effects of *P.decaisnei* as a possible curative remedy for various human diseases.

## References

1. Walsh NG, Kellermann Papaveraceae J, Kellermann J (eds) (2011) State Herbarium of South Australia, Adelaide
2. Almousawi UMN, Alwan AA (2016) Distribution of the genus Papaver L. in Iraq Introduction Distribution of the genus Papaver L. in Iraq. Basrah J Sci 34:9–24
3. Langlois A, Mulholland DA, Crouch NR (2004) Grace OM Aporphine alkaloid from *Papaver aculeatum* (sect. *Horrida*; Papaveraceae) of southern Africa. Biochem Syst Ecol 32:1087–1090
4. Pathan H, Williams J (2012) Basic opioid pharmacology: an update. Br J pain 6:11–16
5. Tomar V, Kukreti S, Prakash S, Madan J, Chandra R (2017) Noscapine and its Analogs as Chemotherapeutic Agent: Current updates. Curr Top Med Chem 17:174–188
6. Tisserat B, Berhow M (2009) Production of Pharmaceuticals from Papaver Cultivars in Vitro. Eng Life Sci 9:190–196
7. Hussain G, Rasul A, Anwar H, Aziz N, Razzaq A, Wei W, Ali M, Li J, Li X (2018) Role of plant derived alkaloids and their mechanism in neurodegenerative disorders. Int J Biol Sci 14:341–357
8. Krošlák E, Maliar T, Nemeček P, Viskupičová J, Maliarová M, Havrlentová M, Kraic J Antioxidant and Proteinase Inhibitory Activities of Selected Poppy (*Papaver somniferum* L.) Genotypes. Chem Biodivers. <https://doi.org/10.1002/cbdv.201700176>

9. Bozan B, Temelli F (2008) Chemical composition and oxidative stability of flax, safflower and poppy seed and seed oils. *Bioresour Technol* 99:6354–6359. DOI: 10.1016/j.biortech.2007.12.009
10. Yoshida Y, Niki E, Noguchi N (2003) Comparative study on the action of tocopherols and tocotrienols as antioxidant: chemical and physical effects. *Chem Phys Lipids* 123:63–75
11. Paul BD, Dreka C, Knight ES, Smith ML (1996) Gas chromatographic/mass spectrometric detection of narcotine, papaverine, and thebaine in seeds of *Papaver somniferum*. *Planta Med* 62:544–547. doi: 10.1055/s-2006-957966
12. Güler DA, Aydın A, Koyuncu M, Parmaksız İ, Tekin Ş (2016) Anticancer Activity of *Papaver Somniferum*. *J Turkish Chem Soc Sect A Chem* 3:349–366
13. Demirgan R, Karagöz A, Pekmez M, Önay-Uçar E, Artun FT, Gürer Ç (2016) Mat A In vitro anticancer activity and cytotoxicity of some papaver alkaloids on cancer and normal cell lines. *African J Tradit Complement Altern Med* 13:22–26. DOI: 10.4314/ajtcam.v13i3.3
14. Momchev P, Ciganović P, Jug M, Marguí E, Jablan J Zovko Končić M Comparison of Maceration and Ultrasonication for Green Extraction of Phenolic Acids from *Echinacea purpurea* Aerial Parts. *Molecules*. <https://doi.org/10.3390/molecules25215142>
15. Ab.jabbar A *Onosma mutabilis*: Phytochemical composition, antioxidant, cytotoxicity, and acute oral toxicity. *Food Sci Nutr*; 9:5755–5764 <https://doi.org/10.1002/fsn3.2544>
16. McLafferty FW, Stauffer DB, Stenhagen E, Heller SR, Fred W, McLafferty Douglas B. Stauffer. Wiley, New York
17. Nakajima J, Tanaka I, Seo S, Yamazaki M, Saito (2004) K LC/PDA/ESI-MS Profiling and Radical Scavenging Activity of Anthocyanins in Various Berries. *J Biomed Biotechnol* 2004:469084
18. Nemati F, Dehpouri AA, Eslami B, Mahdavi V, Mirzanejad S (2013) Cytotoxic properties of some medicinal plant extracts from mazandaran, iran. *Iran Red Crescent Med J* 15:e8871–e8871
19. Activities P, Uses FMA (2021) Review of *Rhazya stricta* Decne Phytochemistry, Bioactivities, Pharmacological Activities, Toxicity, and Folkloric Medicinal Uses. *Plants*;10:1–18
20. Zhou J-X, Braun M, Wetterauer P, Wetterauer B, Wink M, Antioxidant (2019) Cytotoxic, and Antimicrobial Activities of *Glycyrrhiza glabra* L., *Paeonia lactiflora* Pall., and *Eriobotrya japonica* (Thunb.) Lindl. *Extracts Medicines* 6:43
21. Sirikhansaeng P, Tanee T, Sudmoon R, Chaveerach A (2017) Major Phytochemical as  $\gamma$ -Sitosterol Disclosing and Toxicity Testing in *Lagerstroemia* Species. *Evidence-Based Complement Altern Med* 2017:7209851
22. Bayazeid O, Nemutlu E, Eylem CC, İlhan M, Küpeli-Akkol E, Karahan H, Kelicen-Uğur P, Ersoz T Yalçın FN Neuroactivity of the naturally occurring aporphine alkaloid, roemerine. *Nat Prod Res*. <https://doi.org/10.1080/14786419.2020.1830395>
23. Bayazeid O, Eylem CC, Reçber T, Yalçın FN, Kır S, Nemutlu E, An (2018) LC-ESI-MS/MS method for the simultaneous determination of pronuciferine and roemerine in some *Papaver* species. *J Chromatogr B, Anal Technol Biomed life Sci* 1096:223–227

24. Jafaar HJ, Isbilen O, Volkan E, Sariyar G (2021) Alkaloid profiling and antimicrobial activities of *Papaver glaucum* and *P. decaisnei*. *BMC Res Notes* 14:348
25. Paul BD, Dreka C, Knight ES, Smith ML (1996) Gas chromatographic/mass spectrometric detection of narcotine, papaverine, and thebaine in seeds of *Papaver somniferum*. *Planta Med* 62:544–547
26. Huo X (1999) [Determination of thebaine in various tissues of *papaver bracteatum* by gas chromatography]. *Se pu =. Chinese J Chromatogr* 17:70–72
27. Oh J-H, Ha IJ, Lee MY et al (2018) Identification and metabolite profiling of alkaloids in aerial parts of *Papaver rhoeas* by liquid chromatography coupled with quadrupole time-of-flight tandem mass spectrometry. *J Sep Sci* 41:2517–2527
28. Czapski GA, Szypuła W, Kudlik M, Wileńska B, Kania M, Danikiewicz W, Adamczyk A (2014) Original article Assessment of antioxidative activity of alkaloids from *Huperzia selago* and *Diphasiastrum complanatum* using in vitro systems. *Folia Neuropathol* 52:394–406
29. Sharopov F, Valiev A, Gulmurodov I, Sobeh M, Satyal P, Wink M (2018) Alkaloid Content, Antioxidant and Cytotoxic Activities of Various Parts of *Papaver somniferum*. *Pharm Chem J* 52:459–463
30. Li C-Y, Meng Y-H, Ying Z-M, Xu N, Hao D, Gao M-Z, Zhang W-J, Xu L, Gao Y-C (2016) Ying X-X Three Novel Alkaloids from *Portulaca oleracea* L. and Their Anti-inflammatory Effects. *J Agric Food Chem* 64:5837–5844
31. Wojdyło A, Oszmiański J (2007) Czemerys R Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chem* 105:940–949
32. Ishtiaque S, Khan N, Siddiqui MA, Siddiqi R, Naz S (2013) Antioxidant Potential of the Extracts, Fractions and Oils Derived from Oilseeds. *Antioxidants*;2:246–256
33. Chung HS, Woo (2001) WS A quinolone alkaloid with antioxidant activity from the aleurone layer of anthocyanin-pigmented rice. *J Nat Prod* 64:1579–1580
34. Hasplova K, Hudecova A, Miadokova E, Magdolenova Z, Galova E, Vaculcikova L, Gregan F (2011) Dusinska M Biological activity of plant extract isolated from *Papaver rhoeas* on human lymphoblastoid cell line. *Neoplasma* 58:386–391
35. Karajibani M, Montazerifar F, Feizabad AK Study of oxidants and antioxidants in addicts. *Int J High Risk Behav Addict*. <https://doi.org/10.5812/ijhrba.35057>
36. Selen Isbilir S, Sagiroglu A (2012) An assessment of in Vitro antioxidant activities of different extracts from *Papaver rhoeas* L. Leaves. *Int J Food Prop* 15:1300–1308
37. Katarzyna J, Karolina J, Patrycja K, Mateusz B, Izabela G (2021) Mineral Composition and Antioxidant Potential in the Common Poppy (*Papaver rhoeas* L.) Petal Infusions. *Biol Trace Elem Res* 199:371–381
38. Trichopoulou A, Vasilopoulou E, Hollman P et al (2000) Nutritional composition and flavonoid content of edible wild greens and green pies: a potential rich source of antioxidant nutrients in the Mediterranean diet. *Food Chem* 70:319–323
39. Liu C-M, Kao C-L, Wu H-M, Li W-J, Huang C-T, Li H-T, Chen C-Y (2014) Antioxidant and anticancer aporphine alkaloids from the leaves of *Nelumbo nucifera* Gaertn. cv. *Rosa-plena* *Molecules* 19:17829–17838

40. Muthna D, Cmielova J, Tomsik P, Rezacova M (2013) Boldine and related aporphines: From antioxidant to antiproliferative properties. *Nat Prod Commun* 8:1797–1800
41. Belyagoubi-Benhammou N, Belyagoubi L, Gismondi A, Di Marco G, Canini A, Atik Bekkara (2019) F GC/MS analysis, and antioxidant and antimicrobial activities of alkaloids extracted by polar and apolar solvents from the stems of *Anabasis articulata*. *Med Chem Res* 28:754–767
42. Shaghaghi A, Alirezalu A, Nazarianpour E, Sonboli A, Nejad-Ebrahimi S (2019) Opioid alkaloids profiling and antioxidant capacity of *Papaver* species from Iran. *Ind Crops Prod* 142:111870
43. Bilková A, Balážová A, Obložinský M, Bilka F (2006) Biochemical aspect of production Plants., of secondary metabolites in opium poppy. *Farm Obz* 75:152–158
44. Morimoto S, Suemori K, Moriwaki J, Taura F, Tanaka H, Aso M, Tanaka M, Suemune H, Shimohigashi Y (2001) Shoyama Y Morphine metabolism in the opium poppy and its possible physiological function. Biochemical characterization of the morphine metabolite, bismorphine. *J Biol Chem* 276:38179–38184
45. Gao P, Nie X, Zou M, Shi Y, Cheng G (2011) Recent advances in materials for extended-release antibiotic delivery system. *J Antibiot (Tokyo)* 64:625–634
46. Tan Q, Liu S, Chen X, Wu M, Wang H, Yin H, He D, Xiong H, Zhang J (2012) Design and evaluation of a novel evodiamine-phospholipid complex for improved oral bioavailability. *AAPS PharmSciTech* 13:534–547
47. Sharma N, Samarakoon KW, Gyawali R, Park Y-H, Lee S-J, Oh SJ, Lee T-H Jeong DK Evaluation of the Antioxidant, Anti-Inflammatory, and Anticancer Activities of *Euphorbia hirta* Ethanolic Extract. *Mol.* <https://doi.org/10.3390/molecules190914567>
48. Sundarraj S, Thangam R, Sreevani V, Kaveri K, Gunasekaran P, Achiraman S, Kannan S (2012)  $\gamma$ -Sitosterol from *Acacia nilotica* L. induces G2/M cell cycle arrest and apoptosis through c-Myc suppression in MCF-7 and A549 cells. *J Ethnopharmacol* 141:803–809
49. Bharath B, Perinbam K, Devanesan S, AlSalhi MS, Saravanan M (2021) Evaluation of the anticancer potential of Hexadecanoic acid from brown algae *Turbinaria ornata* on HT–29 colon cancer cells. *J Mol Struct* 1235:130229
50. Liu J, Zhou H, Song L, Yang Z, Qiu M, Wang J, Shi S (2021) Anthocyanins: Promising natural products with diverse pharmacological activities. *Molecules.*;26:1–23
51. You M, Wickramaratne DB, Silva GL, Chai H, Chagwedera TE, Farnsworth NR, Cordell GA, Kinghorn AD, Pezzuto JM (1995) (-)-Roemerine, an aporphine alkaloid from *Annona senegalensis* that reverses the multidrug-resistance phenotype with cultured cells. *J Nat Prod* 58:598–604
52. Saleh NM, El-Gazzar MG, Aly HM, Othman RA Novel Anticancer Fused Pyrazole Derivatives as EGFR and VEGFR-2 Dual TK Inhibitors. *Front Chem.* 202AD;7:917
53. Boulaaba M, Kalai FZ, Dakhlaoui S, Ezzine Y, Selmi S, Bourgou S, Smaoui A, Isoda H, Ksouri R (2019) Antioxidant, antiproliferative and anti-inflammatory effects of *Glaucium flavum* fractions enriched in phenolic compounds. *Med Chem Res* 28:1995–2001

## Figures

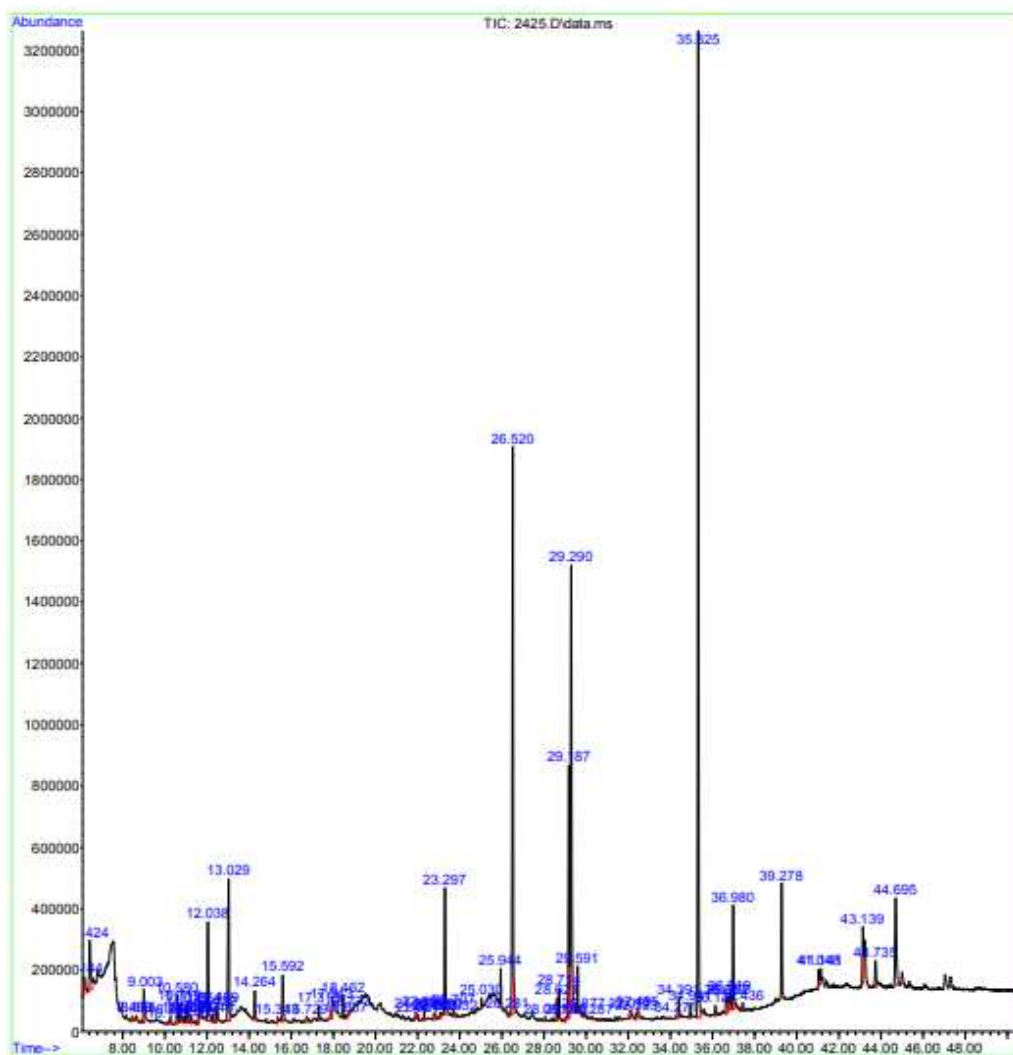




**Figure 1**

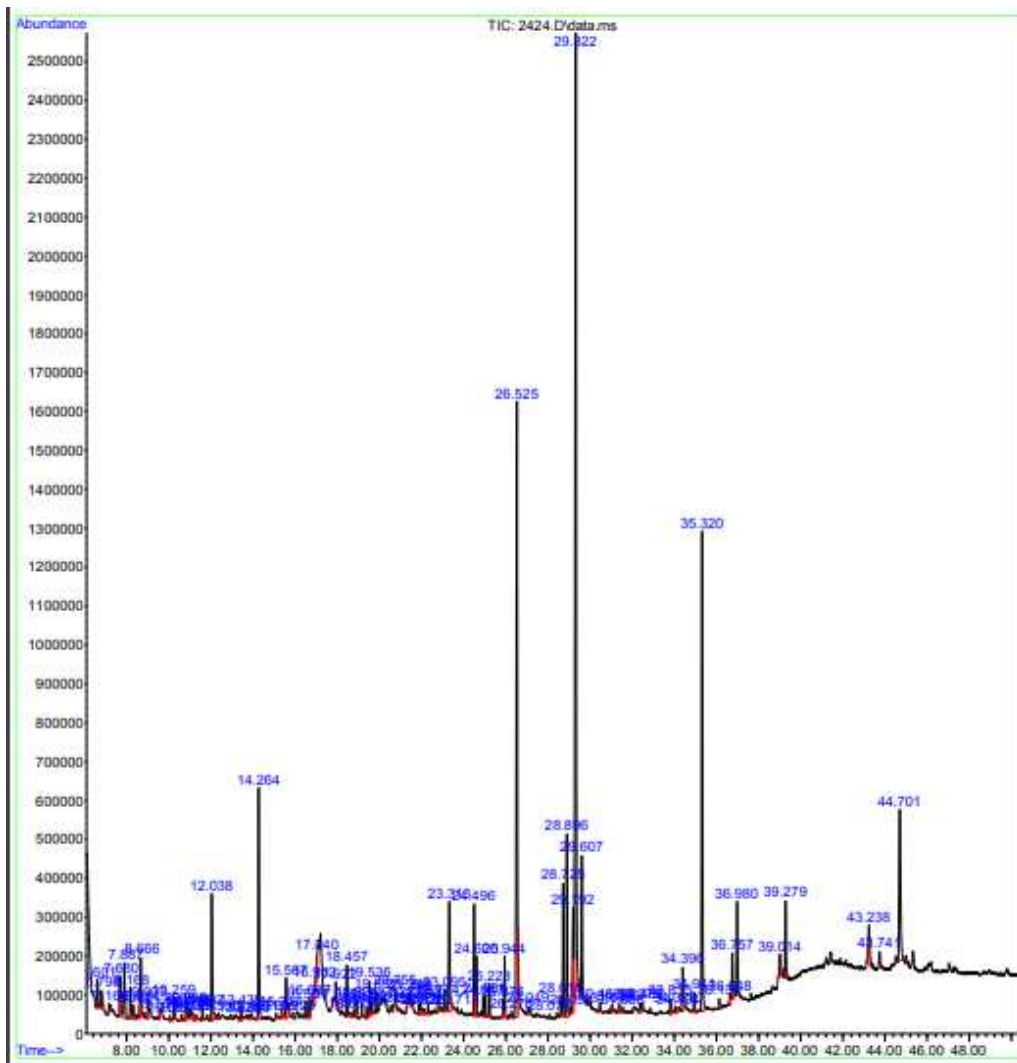
The collected parts of *P.decaisnei*





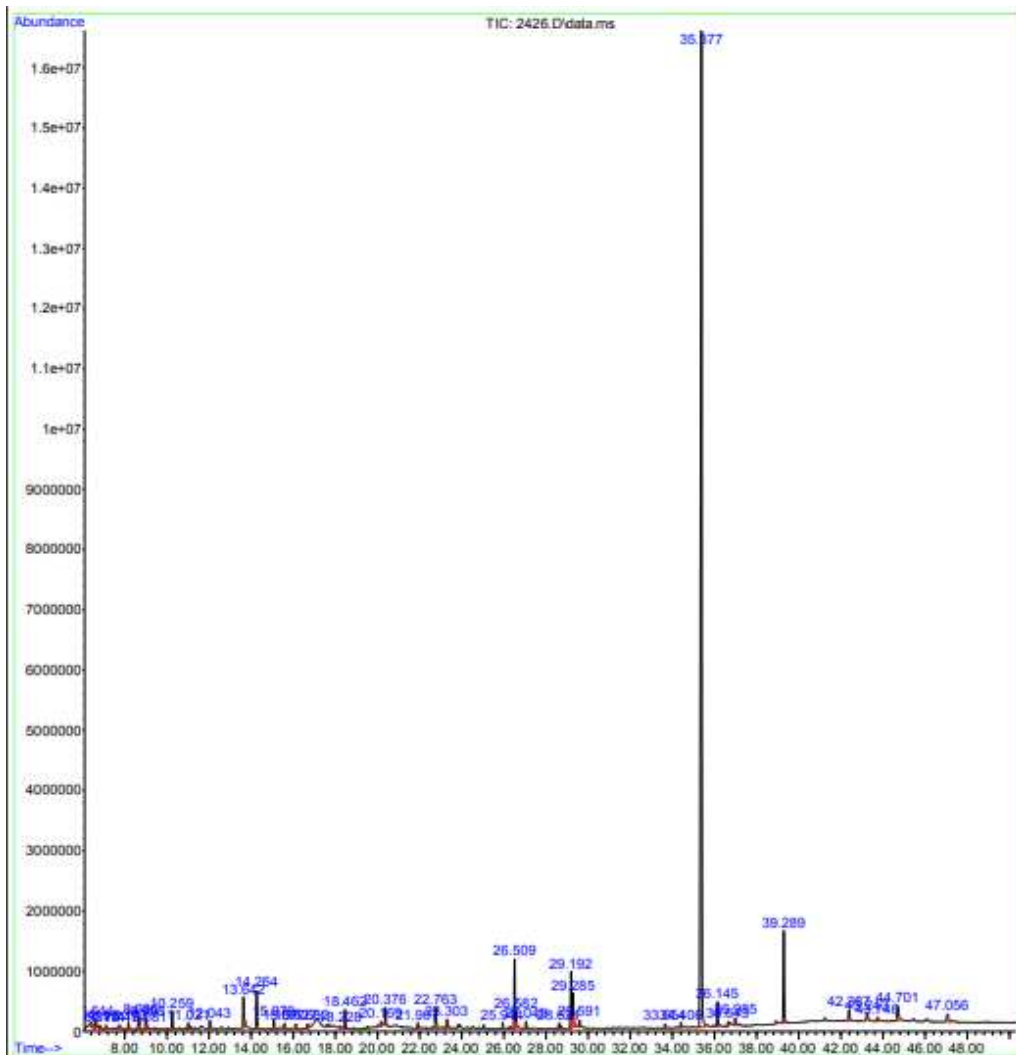
**Figure 2**

Chromatogram of MeOH flower extracts of *P. decaisnei*. *uracil*, *1-n-methyl-, 2,3-dihydro-benzofuran, 2-methoxy-4-vinylphenol*, *6,8-dioxo-3-thiabicyclo(3,2,1)octane 3,3-dioxide*, *2,4-di-tert-butylphenol*, *benzyl benzoate*, *neophytadiene*, *hexadecanoic acid, methyl ester*, *hexadecanoic acid, methyl 9,12,15-octadecatrienoate*, *phytol*, *linoleic acid*, *9,12,15-octadecatrien-1-ol*, *octadecanoic acid, 8.beta.,13:8.alpha.,14, butriptyline.*, *4-methoxybenzene, 1-(2-hydroxynaphthylmethylamino)-*, *gibberellin a3*,  $\gamma$  -*sitosterol*.



**Figure 3**

Chromatogram of MeOH leaf extracts of *P. decaisnei*. *glycerin, guaiacol, silane, triethylmethoxy-, 2h-pyran-2-one, tetrahydro-4-hydroxy-4-methyl-, 2-methoxy-4-vinylphenol, dl-proline, 5-oxo-, methyl ester, 1,6-anhydro-.beta.-d-glucofuranose (levoglucosan), phenol, 2,4-bis(1,1-dimethylethyl)-, benzyl benzoate, hexadecanoic acid, 9,12-octadecadienoic acid (z,z)-, 9,12,15-octadecatrien-1-ol, (z,z,z)-, decarbomethoxytabersonine, amitriptylinoxide, 3'-methyl-1', phenylspiro(indoline-2,4'-(2)pyrazoline)-5'-one, 3,4-dihydro-6,7-dimethoxyisoquinoline 2-oxide,  $\gamma$ -sitosterol*



**Figure 4**

Chromatogram of MeOH root extracts of *P.decaisnei*. *thymine*, *guaiacol* *4h-pyran-4-one*, *2,3-dihydro-3,5-dihydroxy-6-methyl-*, *2-methyl[1,3,4]oxadiazole*, *1,2-benzenediol*, *2,3-dihydro-benzofuran*, *trans-anethole*, *2-methoxy-4-vinylphenol*, *2,6-dimethoxyphenol*, *dl-proline*, *5-oxo-*, *methyl ester*, *vanillin*, *6,8-dioxa-3-thiabicyclo(3,2,1)octane 3,3-dioxide*, *phenol*, *2,4-bis(1,1-dimethylethyl)-,alpha.-cedrol*, *3a-hydroxy-1,2,3,3a,8,8a, hexahydropyrrole(2,3b)indole*, *4-methoxy-6-methyl-2-propylpyridine*, *hexadecanoic acid*, *9,12-octadecadienoic acid (z,z)-*, *roemerine*, *3-(3-methoxyphenyl)propenenitrile*, *2-(diethoxyphosphinyl)-*, *3'-methyl-1'-phenylspiro(indoline-2,4'-(2)pyrazoline)-5'-one*,  $\gamma$  -*sitosterol*.

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