



**Isolation of Fatty Acid, Elements Protein Contents in Flowers of *Calendula Officinalis L.*,  
from Jamshoro, Sindh- Pakistan**

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**Abstract:** This report described that *Calendula officinalis L.* flowers, extract which contain total 37 fatty acids among which 13 were saturated 24 unsaturated, 2nd 10 elements viz: arsenic, cadmium, copper, iron, sodium, manganese, magnesium, potassium, lead and zinc. accumulation of lead 0.37 mg/kg was the lowest and zinc 15.90 mg/kg in middle and highest accumulation was of potassium 71.79 mg/kg in *C.officinalis L.* Total protein in *Calendula officinalis L.* was found between 30.20 to 28.6 %.

**Keywords:** *Calendula officinalis L.* flowers, fatty acids, elements, Total protein Sindh- Pakistan

**1. INTRODUCTION**

*Calendula officinalis L.* (family Asteraceae) with its pale green leaves and golden orange flowers is commonly known as the Marigold. It was well known to the herbalists as a garden flower and for its use in cookery and medicine. The leaves and the flowers of *C. officinalis* are used for a number of remedies, e.g. headache, jaundice, red eyes, toothache and ague. It has been also used for strengthen the heart and to assuage swellings. The herbal acne ointment to treat a range of skin burn is considered control the bleeding, inflammation and soothes irritated issue. This major injuries, eczemas and cyst in children as well as for diaper rash and cradle cap is used for internal or topically. They are often used to add color to salads and *C. officinalis* extract is commonly added to chicken feed to yield darker egg yolks. The oil from its seed contains calendic acid. Many scientists have discovered other chemical compounds. The oils contain fatty acids with conjugated double bonds that display high rates of oxidation compared with oils that contain unsaturated fatty acids with the methylene-interrupted double bonds, however oil enriched in fatty acids with conjugated double bonds are used commercially as drying agents in paints and varnishes calendic acid, the primary fatty acid of *C. officinalis* seeds have also been identified with cDNAs for two highly expressed FAD2-related polypeptides (CoFADX-1 and CoFADX-2) from seed. A tissue that is enriched in calendic acid (18: 3D8trans, 10trans, 12cis), exhibit an unusual conjugated trienoic fatty acid (Formo, 1979 Sonntag, 1979, McClean, Chisholm, Edgar). Anti-inflammatory activity from the compounds obtained from *C. officinalis* such as flavonoids, saponins, triterpene, carotenoids, calendic

acid, eleostearic acid, linolenic acid are cited (Mors *et al.*, 2000; Ferreira, 1992a Lindahl 1997; Olabanji *et al.*, 1997; Singh, 1997; Chisholm, 1967). Flowers are used in the West and Asia for their anti-inflammatory properties. (Neukirch, 2004-2005). Extracts from *C. officinalis* have shown anti-viral activity, anti-HIV properties of therapeutic interest (Janiszowska, 1982) as well as anti-genotoxic properties (Chudnicka, 2005). Essential oil has high-quality potential antifungal activity and is effective against all fungal problems and also used as a food component.  $\alpha$ -cadinene is a significant essence agent in baked foodstuff, chocolate, chew gum and fragrance in cosmetic and detergents (Gazim 2008, Okoh, *et al.*, 2007).

In the present study, extract from the flowers and seeds of *C. officinalis* were studied and a number of fatty acids, proteins and elements has been isolated and identified.

**2. MATERIALS AND METHODS**

**Collection of plant material:** *C.officinalis* flowers (Asteraceae) were collected from University of Sindh, Jamshoro in November-December 2010. Reference samples were identified by (Dr. Iqbal Azhar, University of Karachi) through literature

**Extraction:** The dried *C.officinalis* flowers (300 g) were chopped into small pieces and dipped into 2 Liter ethanol (EtOH) for about one month at room temperature.

Equal quantity of 150 ml. Ethanol (EtOH) and water (H<sub>2</sub>O) (1:1, V/V) containing 10% KOH was added to above plant residue. This reaction mixture was

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saponified by refluxing at 100 °C for 6 h. The mixtures were concentrated under reduced pressure in a rotary evaporator, and thereafter water (H<sub>2</sub>O) and diethyl ether (Et<sub>2</sub>O) were added, and extraction with ether was repeated for three times. The unsaponifiable matter was partitioned and thereby separated. The aqueous alkaline fraction was acidified with 6N HCl (pH 5-6) and then extracted several times with (Et<sub>2</sub>O). The total Et<sub>2</sub>O fraction was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and on evaporation of Et<sub>2</sub>O a residue was obtained.

The column temperature was kept at 70 °C for 2 min with increased @ the rate of 4 °C per min up to 260 °C. Injection temperature 250 °C, split ratio 1:45, the carrier gas (Helium) flow rate 1.0 ml/min (Khanzada *et al.*, 2008, 2013)

**Digestion of *Calendula officinalis* L. flowers:** The plant material was initially dried under shade at room temperature and later on in an oven at 60-80 °C for 1 h. 01. g of manually crushed sample material was carefully the sample was nearly dried. When the sample became cool

**Elemental assay: Method.** All elements were determined in medicinal plants under this investigation procedure. A blank reading was also taken. (Khanzada *et al.*, 2008, 2013, 2014)

**Total protein by kjeldhal:** The samples were digested in (30 ml) H<sub>2</sub>SO<sub>4</sub> in the presence of catalysts, CuSO<sub>4</sub>, (1g) and K<sub>2</sub>SO<sub>4</sub> (10g). After digestion Sodium hydroxide (NaOH, 33%) was added followed by steam distillation, the distillate was collected in 20 ml (4%) boric acid. Then nitrogen content was determined by using titration with HCl (0.01 N). Indicators used in titration were Bromocresol, green and Methyl red. A factor of 6.25 was used to evaluate total protein concentration following the protocol of ISI-24-1-e. (Khanzada *et al.*, 2008, 2013)

### 3. RESULTS AND DISCUSSION

The physical significance of the Plant that can synthesize a large variety of chemical substances (Kretovich, 2005). A literature search was carried out to find plants sources that are used as flower remedies for various disorders or diseases (Athar and Siddiqi, 2004; Bhattacharjee and De, 2006; Chouhan *et al.*, 2002; 1999; Rizvi, 2001). The GC-MS of the methylated fatty acids (FAs) revealed the presence of 13 saturated and 24 unsaturated fatty acid i.e, 37 fatty acids (FAs) were isolated from *C.officinalis* flowers. These also showed the relative retention time (RRT) and relative percentage of occurrence of their methyl ester. The unsaturated fatty acids (UFAs) were present in higher amount (55.14%) than saturated fatty acid, SFAs (43.85%). The largest amount of (SFAs) 16.68% Hexadecanoate moderate % 9.51% n-Hexadecanoate and lowest relative % 0.20 %, 1-Octanoate the highest relative % of (UFAs) is 15.14 % n-Heptadecanoate, moderate 8.76%, 9-Eicosanoate and lowest relative % 0.10 %, Octatetraenoate.

**Elements analysis:** *C.officinalis* L. flowers collected from the area of Sindh were analyzed for the composition of Na, K, Pb, Cd, As, Cu, Fe, Mn, Zn and Mg elements potassium 71.79, zinc 15.90, cadmium 4.05, iron 2.66, copper 6.60, arsenic 40.23 µg, lead 0.44, sodium 2.84, magnesium 3.28 and manganese 0.37, The Such as zinc, copper and iron in human health. Ca, Mg, Fe and Zn point out the capability of some medicinal plants to maintain the body in strong resistant in different diseases.

The relativity low, but its how they develop, repair and maintenance of the body as the body that controls a variety of function, which can contribute to the formation of hormones.

**Table 1. Saturated fatty acids of *Calendula officinalis* L. flowers, analyzed as methyl ester.**

S.NO	SYSTEMATIC NAME	COMMON NAME	MOLECULAR FORMULA	Mo. Wt.	R.R.T	Rel. %ag
1.	Methyl-n-heptanoate	Methyl heptylate	C <sub>8</sub> H <sub>16</sub> O <sub>2</sub>	144	25.32	0.86
2.	2,3Dimethoxy propanoate	Propanoate	C <sub>6</sub> H <sub>12</sub> O <sub>2</sub>	148	15.15	0.23
3.	1-Octanoate	Caprylate	C <sub>9</sub> H <sub>18</sub> O <sub>2</sub>	158	18.37	0.20
4.	n-Nonanoate	Nonylate	C <sub>10</sub> H <sub>20</sub> O <sub>2</sub>	172	16.43	0.54
5.	Dodecanoate	Lauric acid	C <sub>12</sub> H <sub>24</sub> O <sub>2</sub>	200	21.28	0.22
6.	Nonanoate	Laurate	C <sub>13</sub> H <sub>26</sub> O <sub>2</sub>	214	19.67	5.40
7.	n-Tridecanoate	Tridecylat	C <sub>14</sub> H <sub>28</sub> O <sub>2</sub>	228	25.82	2.75
8.	n-Tetradecanoate	Myrislate	C <sub>15</sub> H <sub>30</sub> O <sub>2</sub>	242	24.53	2.27
9.	Hexadecanoate	Pantadecylate	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256	30.38	16.68
10.	n-Hexadecanoate	Palmilate	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270	28.73	9.51
11.	n-Heptadecanoate	Margorate	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	284	35.2	1.73
12.	n-Octadecanoate	Stearate	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	298	32.47	3.24
13.	n-Docosanoate	Behenate	C <sub>23</sub> H <sub>46</sub> O <sub>2</sub>	354	39.12	0.22
<b>Total</b>						<b>43.85</b>

**Table 2. Unsaturated fatty acids of *Calendula officinalis* L. flowers, analyzed as methyl ester.**

S.NO	SYSTEMATIC NAME	COMMON NAME	MOLECU LR FORMULA	MOLWt.	R.R.T	Rel. %ag
1.	Heptaenoate	Heptylenate	C <sub>8</sub> H <sub>14</sub> O <sub>2</sub>	142	18.03	0.75
2.	Octatetraenoate	Octatetraenoate	C <sub>9</sub> H <sub>10</sub> O <sub>2</sub>	150	15.58	0.10
3.	Nonatetraenoate	Eugenol	C <sub>10</sub> H <sub>12</sub> O <sub>2</sub>	164	21.4	0.22
4.	n-Decenoate	Decenoic acid	C <sub>10</sub> H <sub>18</sub> O <sub>2</sub>	170	15.73	0.11
5.	Decanotetraenoate	Decanotetraenoate	C <sub>11</sub> H <sub>14</sub> O <sub>2</sub>	178	19.88	0.54
6.	Methyl-decatrienoate	Decatrienoate	C <sub>11</sub> H <sub>16</sub> O <sub>2</sub>	180	24.68	0.32
7.	9-Decenoate	Decenoate	C <sub>11</sub> H <sub>20</sub> O <sub>2</sub>	184	18.67	0.21
8.	Undecatrienoate	Undecatrienoate	C <sub>12</sub> H <sub>18</sub> O <sub>2</sub>	194	14.57	0.54
9.	3,8-Dimethyl-27-nonadecanoate	Nonadecanoate	C <sub>12</sub> H <sub>20</sub> O <sub>2</sub>	196	11.07	0.11
10.	Dodecenoate	Lauroleic	C <sub>12</sub> H <sub>22</sub> O <sub>2</sub>	198	12.08	0.32
11.	9-Dodecenoate	Dodecenoate	C <sub>13</sub> H <sub>24</sub> O <sub>2</sub>	212	15.97	0.11
12.	Tridecadienoate	Tridecadienoate	C <sub>13</sub> H <sub>22</sub> O <sub>2</sub>	210	20.15	0.32
13.	Methyl-2Tridecynote	Methyl-2Tridecynote	C <sub>14</sub> H <sub>24</sub> O <sub>2</sub>	224	21.15	1.41
14.	Pentadecatrienoate	Pentadecatrienoate	C <sub>16</sub> H <sub>26</sub> O <sub>2</sub>	250	27.6	1.40
15.	Hexadecane-1,diol	Hexadecane-1,diol	C <sub>16</sub> H <sub>34</sub> O <sub>2</sub>	258	30.97	2.70
16.	Hexadecenoate	Palmileate	C <sub>17</sub> H <sub>32</sub> O <sub>2</sub>	268	39.35	0.65
17.	Heptadectrienate	Heptadectrienate	C <sub>18</sub> H <sub>30</sub> O <sub>2</sub>	278	33.42	15.14
18.	Heptadecadienoat	Heptadecadienoat	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	280	35.92	0.93
19.	Heptadecenoate	Heptadecenoate	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282	33.73	1.41
20.	9,12,15-Octadecatrienoate	Octadecatrienoate	C <sub>19</sub> H <sub>32</sub> O <sub>2</sub>	292	31.98	5.95
21.	Octadecadienoate	Octadecadienoate	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294	31.88	7.68
22.	Nonnadecenoate	Nonnadecenoate	C <sub>20</sub> H <sub>38</sub> O <sub>2</sub>	310	45.1	0.85
23.	9-Eicosenoate	Gadoleate	C <sub>21</sub> H <sub>40</sub> O <sub>2</sub>	324	36.35	8.76
24.	Methyldocosapentaenoate	Clupanodenoate	C <sub>23</sub> H <sub>36</sub> O <sub>2</sub>	344	43.13	0.97
<b>Total</b>					<b>55.14</b>	

13,Saturated, 24,Unsaturated, Total compounds=37  
Total %age of Saturated + Unsaturated fatty acid = 99.99  
Mol.wt = molecular weight ,R.R.T=relative retention time, Rel % age=relative percentage)

**Table 3- Elemental composition of *Calendul officinalis* L. flowers, mg /kg**

S. No	Name of Elements	Symbol	Amount Mg /Kg
1.	Sodium	Na	6.0
2.	Potassium	K	71.79
3.	Lead	Pb	0.37
4.	Cadmium	Cd	4.05
5.	Arsenic	As	40.23 µg/kg
6.	Copper	Cu	6.60
7.	Iron	Fe	2.66
8.	Manganese	Mn	0.42
9.	Zinc	Zn	15.90
10.	Magnesium	Mg	3.28

#### 4. CONCLUSION

The study showed that there is a great variation in fatty acids, elemental composition and total protein in *C.officinalis* (Tables 1, 2, 3). The seasonal variation as well as different ecological conditions in which they are growing e.g. soil, temperature, climatic factors and rainfall are major cases of increased or decreased in nutritional level in plants.

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