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Phytochemical Studies on *Tanacetum sinaicum* Delile ex DC. Plant and Isolation of Two Phenolic Compounds from Flowers

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Abstract

Recently, interest has increased in natural metabolites compounds from plants for industrial and pharmaceutical use rather than synthetic compounds. *Tanacetum sinaicum* Del. Is a wild plant found in many places in Egypt, especially south Sinai, in Saint Catherine, on rocky slopes and it has yellow tubular flowers and woolly leaves. Using different chromatographic techniques, extracted, isolated, purified, and structurally elucidated two phenolic compounds these compounds are4',5-dihydroxy-6,7-dimethoxyflavone, and 3,4-dihydroxy-cinnamic acid. The structures of these compounds were identified by using, UV, NMR, and mass spectroscopy. The quantitative estimation of total phenolics, total flavonoids, total alkaloids, total saponins, and total tannins showed their presence in a good amount in *Tanacetum sinaicum* flowers when compared to the other plant parts. The phytochemical screening of crude extract and the corresponding sub-fractions to investigate presence of alkaloids, anthraquinones, carbohydrates, cardiac glycosides, flavonoids, glycosides, reducing sugars, resin, saponin, starch, sterols, tannins, and terpenoids in different parts of *Tanacetum sinaicum* showed that crude extract of all parts and chloroform fraction of flowers are rich in all tested active constituents when compared to other parts and fractions.

Keywords: Tanacetum sinaicum, phytochemical composition, flavonoids, phenolic acids, Chloroform extract, Chromatographic Techniques

1. Introduction

The Egyptian lands, especially the desert, are very rich in medicinal plants, and those plants belong to many plant families [1]. Phytochemicals found inside these plants, for example, flavonoids, alkaloids, terpenoids, and phenolics have many medical benefits and get into a lot of food industries [2]. The Asteraceae or sunflower family is the largest and richest family of flowering plants in the world there are 1,600 genera included 23,000 species, many of which are of medical, industrial, and nutritional importance [3]. Tanacetum genera belong to this family, and it is one of the most important species used in the manufacture of food oils and many medicines [4]. *Tanacetum sinaicum* Delile ex DC.is a wild plant found in many places in Egypt, especially south Sinai, in Saint Catherine, on rocky slopes, and it has yellow tubular flowers and woolly leaves [5]. This plant is rich in many phytochemical compounds belonging to the most important chemical groups, for example, volaille oils [6], terpenes [7], and flavonoids [8]. This plant has been used in folk medicine to treat many diseases. Besides to using intreating high body temperature, treating stomach problems, treating pneumonia, and treating osteoarthritis [9-10]. In some cases, this plant is used as an antiviral [11]. In addition, several studies have shown that this plant has antinociceptive activities [12]. Knowing the chemical composition of compounds separated helps scientists use them to treat many diseases, including cancer [13]. Isolation of the main active constituents in this plant might help in explaining the aforementioned benefits.

2. Experiments

2.1 Plant material

Different parts(roots, stem, leaves, and flowers) of *Tanacetum sinaicum* were collected at flowering

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time(April 2019) from Saint Catherine, South Sinai, Egypt. After collecting the different plant parts were washed with water, left in a dry place, put on some clean paper in the open air, dried in the oven at 50 degrees Celsius, and then grounded to a fine powder. The powder was kept in a dark place at room temperature for further study.The taxonomic identification of plant materials was confirmed by Cairo University Herbarium

2.2 Preparation of different extracts.
Two hundred grams of dry powder ofeach part was extracted by using methanol 95 %. The waste of the crude material was washed with benzol to remove plant pigments then were dissolved in chemicals cyclohexane, pet. ether, trichloromethane, theethyl acetate of acetic acid, methyl alcohol seventy percent, and finally using distilled water.

2.3 Phytochemical assessment performing a phytochemical screening, isolation of aromatic oils [14].test for aglycon compounds and/or glycosides, resins[15].,saponins[16].,tannins[17].**,** flavonoids[18].**,** phytosterols and terpenes[19] and test for alkaloids[20].

2.4Quantitative estimation of phytochemicals, estimation of phenolics in*Tanacetum sinaicum*, were determined spectrophotometrically and calculated as gallic acid[20].estimation of flavonoids in *Tanacetumsinaicum*, was determined spectrophotometrically and calculated as quercetin[21]. Assessment of tannins using Cupric acetate method according to[22].**,** estimation of total saponins depending on[23]., and assessment of alkaloids were determined depending on the method in away[24].

2.5. Isolation of phenolic acids andflavonoids:
Estimation of phenolic acids andflavonoidsof *Tanacetum sinaicum*is carried out by using paper and column chromatography[25]of polyamide column[26]and Sephadex LH-20 column chromatography[27]. Identification techniques of flavonoid compounds by chemical analysis[28].includes: ultraviolet (UV) [30], nuclear magnetic resonance (^{1}H-NMR measurement using a Joel Ex500 spectroscopy; 500MHertz (^{1}NMR), 125 MHertz (^{13}C-NMR) or Joel JNMEX 270 spectroscopy; 270 MHertz (^{1}H-NMR), [31].

2. Results and discussion

Table 1: Total active constituents (%) in different parts of *Tanacetum sinaicum* plant.

	Total active constituents in different parts {Mean ±SE (n= 3 replicate per parts)}			
Item	Roots	Stems	Leaves	Flowers
Tanacetum sinaicum				
Total phenolics (mg/gm GAE)	0.87±0.03	1.39±0.08	1.44±0.08	2.59±0.14
Total flavonoids (mg/gm QE)	0.55±0.06	1.07±0.07	1.18±0.04	1.96±0.09
Total tannins (%)	1.77±0.04	2.02±0.13	0.79±0.03	2.56±0.07
Total saponins (%)	1.22±0.01	1.97±0.06	2.87±0.11	1.17±0.03
Total alkaloids (%)	0.94±0.02	1.17±0.06	1.03±0.02	0.69±0.04

Fig 1: Total active constituents (%) in different parts of *Tanacetum sinaicum* plant.

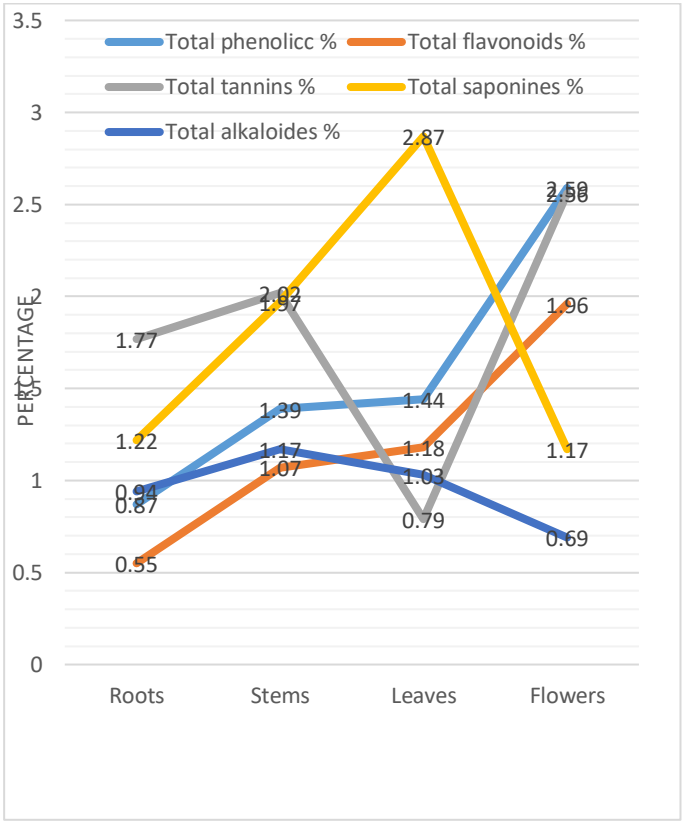


Fig 1

The percentages of total phenolics, tannins, and flavonoids table (1) and fig. (1) have increased value in flowers and decreased values in roots and stems respectively while the percentage of total saponins and alkaloids have maximum values in leaves and stem and decreased values in flowers. And that's

probably because the Asteraceae family has an unlimited capacity to produce aromatic materials, especially secondary metabolites from which at least 12,000 have been isolated so far, and these compounds have boosted the plant's ability to resist microorganisms, insects [30].

Table 2: Phytochemical screening of crude extract and subfractions for roots and stems.

S. No	Crude extract and fractions / Chemical constituents	Roots							Stems					
		Crude extract	*n*-hexane fraction	Pet.ether fraction	Trichloromethanfr	EtOAc fraction	Aqueous fraction		Crude extract,	*n*-hexane fraction	Pet..ether fraction	Trichloromethanfr	EtOAc fraction	Aqueous fraction
1	Alkaloids	+	+	+	-	-	-		+	+	-	+	+	+
2	Anthraquinones	+	-	-	-	-	-		+	+	-	-	+	+
3	Carbohydrates	+	-	-	+	+	+		+	-	+	+	-	-
4	Cardiac glycosides	+	+	-	-	-	-		+	-	+	-	+	-
5	Flavonoids	+	+	+	+	+	+		+	+	+	+	-	+
6	Glycosides	+	-	-	+	-	+		+	-	-	-	+	-
7	Reducingsugars	+	-	-	-	-	-		+	-	-	-	+	-
8	Resin	+	-	-	+	+	-		+	-	+	-	-	-
9	Saponin	+	+	+	-	-	+		+	+	-	+	-	+
10	Starch	+	-	-	+	-	-		+	+	+	-	-	+
11	Sterols	+	+	-	-	+	+		+	-	-	+	+	-
12	Tannins	+	-	+	+	-	-		+	+	-	-	-	+
13	Terpenoids	+	+	-	-	+	-		+	+	-	-	-	+

[a] (+) present, (-) absent, (EtOAc) Ethyl acetate

Table 3: Phytochemical screening of crude extract and subfractions for leaves and flowers.

S. No	Crude extract and fractions / Chemical constituents	leaves							Flowers					
		Crude extract	*n*-hexane fraction	Pet..ether fraction	Trichloromethanfr	EtOAc fraction	Aqueous fraction		Crude extract,	*n*-hexane fraction	Pet..ether fraction	Trichloromethanfr	EtOAc fraction	Aqueous fraction
1	Alkaloids	+	+	-	+	-	+		+	+	-	-	+	-
2	Anthraquinones	+	-	-	-	-	-		+	+	+	+	+	+
3	Carbohydrates	+	+	+	-	-	+		+	-	-	-	+	+
4	Cardiac glycosides	+	-	+	-	-	+		+	+	-	-	+	-
5	Flavonoids	+	+	-	+	+	-		+	+	+	+	+	-
6	Glycosides	+	-	-	-	-	+		+	-	+	-	+	+
7	Reducingsugars	+	-	+	-	+	-		+	+	-	+	+	+
8	Resin	+	-	-	-	-	+		+	-	-	-	+	-
9	Saponin	+	+	+	+	+	+		+	+	+	-	+	+
10	Starch	+	-	-	+	-	-		+	+	+	+	+	-
11	Sterols	+	+	-	-	+	+		+	+	-	-	+	+
12	Tannins	+	-	+	+	-	-		+	+	+	+	+	+
13	Terpenoids	+	-	+	-	-	+		+	+	-	-	+	+

(+) present, (-) absent, (EtOAc) Ethyl acetate

The preliminary Phytochemical screening of different extracts of *Tanacetum sinaicum* plant of different parts (roots, stems, leaves, and flowers) to investigate alkaloids,anthraquinones,carbohydrates,

cardiac glycosides, flavonoids, glycosides, saponins, phenol, sterol, tannins,Reducingsugars,resin, saponins, starch, sterols, tannins, and terpenoids in plant under investigationwas illustrated in tables (3&4) which showed that crude extract of all parts and chloroform fraction of flowers are rich in all tested active constituents when compared to other parts and fractions.The results of the research show that the plant is rich in all the activeconstituents, and that's due to the plant being rich with primary metabolites, such as amino acids, carbohydrates, and lipids [31].

Isolation, purification, and identification of phenolics

Phenolic compounds that present in great amounts in theplant flowers according to the first investigation using paper chromatography. Some of these phenolics were isolated from the flowers of the plant according to the results that showed in phytochemical screening experiments. Two compounds were isolated from chloroform extract. Results of separation processes explained that the chloroform fraction contained high percentage of phenolic compounds when screened by using paper and thin-layer chromatography.

Removing impurities from chloroform fragment

Chloroform fragment kneaded with silica gel and then placed at the top of a silica gel column, elution was performed in essence with butane then by butane/chloroform to increase polarity until chloroform only. It was come next by chloroform/ethyl acetate until ethyl acetate only, then it was come next by ethyl acetate/methanol until finally methanol only, where two main fragments I and II were.picked up. Fraction I was applied in paper chromatography in two ways using butanol: acetic acid: water (4: 1:5) and AcOH-15%, respectively as a mobile phase one spot waspicked up. and when applied on preparative paper chromatography using the following solvents as a mobile phase butanol: acetic acid: water (4: 1:5) for 24 hours deliver one band (compound 1), which was slice gingerly and pack up with seventy percent ethanol.

Fraction I when subjected to two-dimension paper chromatography using B: A: W (4: 1:5) and AcOH-15%, one spot was obtained.Fraction II when subjected topreparative paper chromatography using the following solvent, s as a mobile phase butanol: acetic acid: water (4: 1:5) for 24 hours deliver one band (compound 2), which was sliced gingerly and pack up with seventy percent ethanol.

Identification of compound 1

The purified compound 1 was obtainedan amorphous powder, soluble in methanol and its Rf-values and color reactions were outlined in table (4).

Table (4): Rf-values and color reaction com.1

Solvents	R_f values	Color		
		Visible	UV	UV + ammonia
BAW	0.61	-	Dark yellow	pale yellow
AcOH-15%	0.22	-	Dark yellow	Pale yellow

From R_f-values and color reaction of compound 1 seemed to be aglycone.

UV spectral data, λ_{max}, nm of compound (1) (fig. 2, 3&4) was:

MeOH: 234, 355 (sh), 361
NaOMe : 264, 318 (sh), 328, 407

NaOAc : 252, 306 (sh), 310, 344
NaOAc + H_3BO_3: 238, 316, 360, 415 (sh)
$AlCl_3$: 251 (sh), 254, 312, 404
$AlCl_3$ + HCl: 246 (sh), 254, 323, 418

From data of UV spectrum:

UV spectral analysis in methanol and after the addition of different shift reagents showed that the absorption maxima in methanol, a band I (333nm) and band II (330nm), indicate that compound 1 is isoflavone (Figs. 3, 4 & 5). Bathochromic shift of band II with an increase of intensity by addition of NaOMe indicating the presence of free OH at ring A. Bathochromic shift in a band II (+14nm) occurred on the addition of NaOAc indicating the presence of free OH group at position 7, this shift was not affected after addition of H3BO3 because the B ring in these flavonoid lacks' effective configuration with the major chromophore this confirmed with [32].

On the other hand, when adding aluminum chloride, the chromatic spectrum advertised a bath chromic shift when adding HCl nothing occurred, and this indicates a hydroxyl group at position four [33].

^{1}H-NMR data of compound 1 in $CDCl_3$ (Fig. 5):

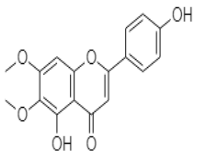
The spectrum of the compound 1 in DMSO, showed signals at δppm2.97, and 3.24 (4H, d, J= 2Hertz, H-2, and Hertz-5), 6.18 (2H, m, J= 3Hertz, H-4\, 3\, 6\, 5\, 7), 5.31 (H, s, H-2), a sharp singlet for H-3 which appeared at δ 6.10ppm (characteristic as flavonoids) [31].From data of the 1H-NMR spectrum of compound 1, the presence of hydroxylation at C-6, C-3 was indicated by the meta-coupling between H-3 and H-7. Thus H-2 and H-5 appeared as 4 doublets at δ 4.16, 2.26 respectively. The presence of hydroxylation in ring B was indicated by multiple signals at δ 6.34 for H-5\, 3\, 3\, 2\, and 7.

Mass spectrum (fig. 6):

Mass spectrum of compound 1 (Fig.6) showed a peak at m/z 243 ascribable to flavone and showed the fragment m/z 136 characteristics for aglycon, from

From the previously obtained data,compound1 was identified as 4',5-dihydroxy-6,7dimethoxyflavone.

Table (5) and scheme (1) for compound 1

Com. No.	Name	Structure
1	4',5-dihydroxy-6,7 dimethoxyflavone	 **Scheme (1)**

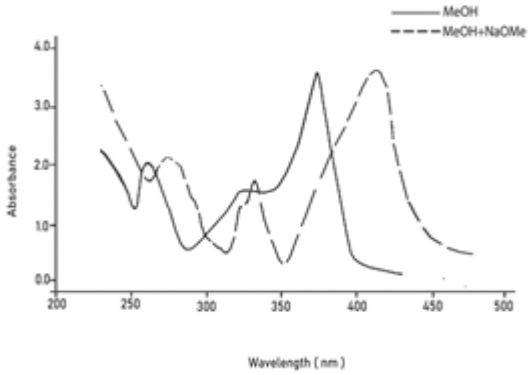


Fig 2

Solvents	R_f values	Color		
		Visible	UV	UV + ammonia
BAW	0.59	-	Bright blue	Dark blue
AcOH-15%	0.17	-	Bright blue	Dark blue

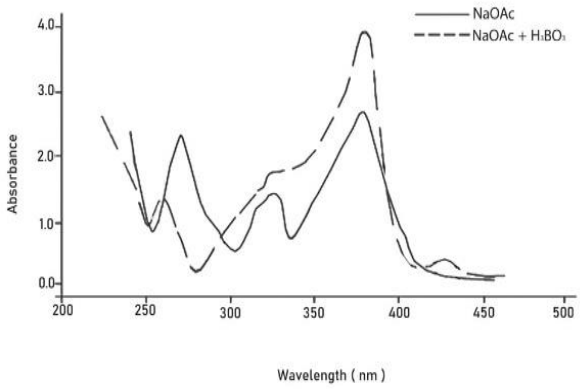


Fig 3

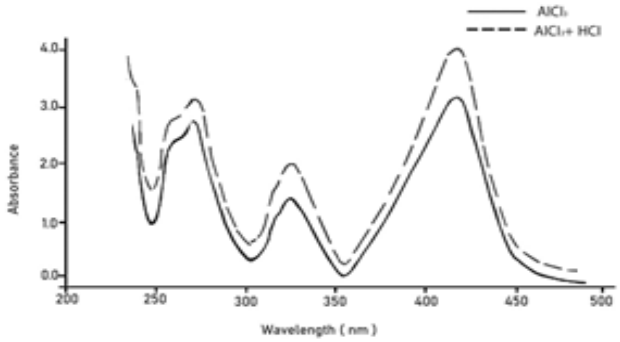


Fig 3

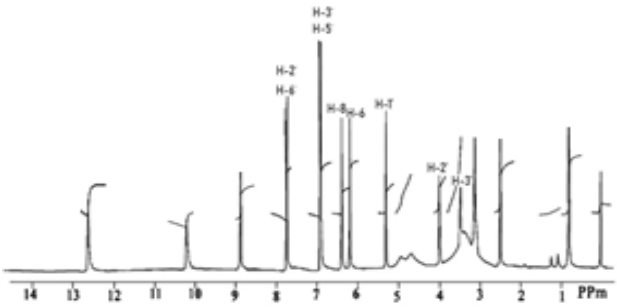


Fig 5

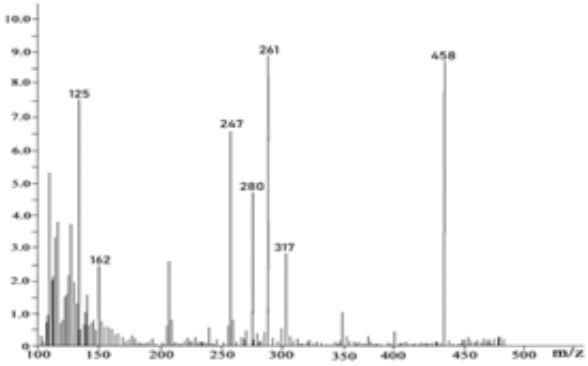


Fig 6

Isolation and identification of compound 2

The purified compound 1 was shown to be an amorphous powder, soluble in methanol and its Rf-values and color reactions were outlined infollowing

Table (6): Rf-values and color reaction com.2

UV spectral data, λmax, nm of compound 2 (fig. 7) was:

The obtained results of UV spectral data were:

MeOH : 251, 302

NaOMe : 223 (sh), 269, 317

UV spectral analysis in methanol and after the addition of different reagents showed that the absorption maxima in methanol, a band I (251nm) and band II (302nm), indicate that compound 1 phenylpropanoids(Fig. 7). Bathochromic shift after addition of sodium methoxide in theband I and II (317 and 269 nm) exhibits this com. Contains free hydroxyl group from the Rf-values and UV spectral analysis compound 2 phenolics in nature [32].

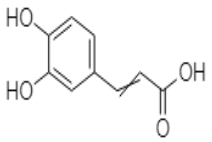
1H-NMR spectrum fig. 8

1H-NMR Spectrum of the compound 2 in dimethyl sulfoxide showed that signal at δ 3.91ppm 3.85 (2H, s, -OCH3), 5.22 (1H, d, J= 15Hertz, H-7), 7.45 (1H, d, J=6.5Hertz, H-5), 6.95 (1H, dd, J= 5.25 and 3.5Hertz, H-6), 6.95 (1H, d, J= 2.5Hz, H-2), 6.55 (1H, d, J= 17Hertz, H-7) and 6.893(s, OH) and 10.92 (-COOH broad band). The 1H-NMR spectral analysis exhibits two doublets at δ 6.95 and 6.15ppm, J=17Hz which are characteristic for trans olefinic double band (H-5 and H-6), respectively. The presence of two doublets at δ6.03 and 5.92 and the doublet of doublet signal at δ 4.89 are corresponding to ortho and meta coupling which isgood evidence for the presence of disubstituted benzene. The presence of singlet at δ 5.26 confirmed the ultraviolet analysis for the presence of free hydroxyl group. The presence of a signal at δ2.49 for –COOH group.

Mass spectrum (fig. 9)

Mass spectrum (Fig. 9) of compound 2 showed the molecular ion peak M+ at m/e 178, M+ -11, and C6H6 at m/e 67, which indicated the compound 2 may be Caffeic acid (3,4-dihydroxy-cinnamic acid). From the previous data, compound 2 is identified as ferulic acid (3,4-dihydroxy-cinnamic acid).

Table (7) and scheme (2) for compound 2

Com. no.	Name	Structure
2	3,4-dihydroxy-cinnamic acid	 **Scheme 2**

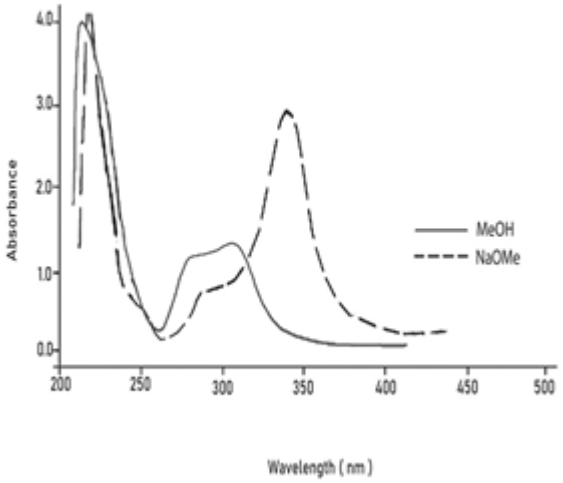


Fig 7

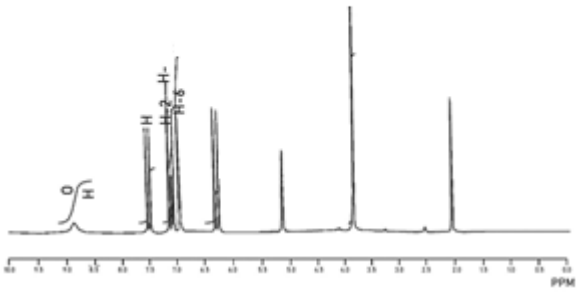


Fig 8

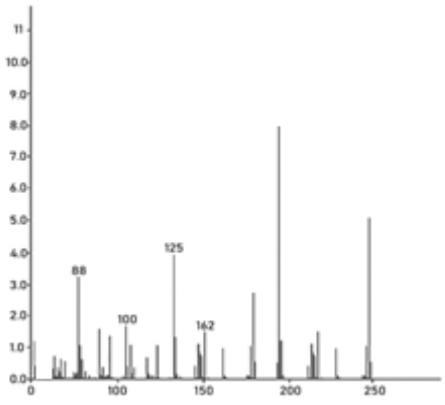


Fig 9

In a study, two compounds belonging to phenolics were registered. And the phytochemical screening exhibits this plant is rich in many chemical compounds that have medical, industrial, and nutritional importance. The compounds separated in this study could be used to classify the plants belonging to the family Solanaceae independent of the internal chemical composition and thus suggesting that these genera belong to Solanaceae and doesn't mean that we need to do more studies to prove that. Several studies have proven that these isolated compounds have antibacterial, antioxidant, and anti-cancer effects, and they are used to preserve food in many factories. A lot of research it's suggests that it's possible to use the isolated compounds that have been detected to develop a variety of drugs that will help you to treat many diseases like cancer.

4. Conclusions

This study included the chemical evaluation of the different parts of *Tanacetumsinaicum*. From the obtained results could be concluded the following, Preliminary phytochemical screening of the plant, which revealed the presence of tannins, flavonoids, chlorides, sulphates, carbohydrates and/or glycosides, resins, cardiac glycosides and coumarins.The phenolic constituents included elucidation of the structure of flavonoids were achieved using Rf-values, UV and 1H-NMR spectral analysis which concluded the presence of twophenolic compounds4',5-dihydroxy-6,7-dimethoxyflavone, and 3,4-dihydroxy-cinnamic acid. The total phenolics, total flavonoids,total tannins, total saponin, and total alkaloids of different plant parts of*Tanacetum sinaicum* were determined which concluded that presence in a good amount.

Conflicts of interest: There are no conflicts interested

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