GC/MS ANALYSIS OF THE FATTY ACIDS OF THREE *CLEMATIS* SPECIES GROWING IN SAUDI ARABIA AND THEIR ANTI-INFLAMMATORY ACTIVITY

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يشتمل هذا البحث على دراسة مقارنة لمحتوى الأحماض الدهنية لثلاثة نباتات من جنس كلاميتس التي تنمو في المملكة العربية السعودية وهي كلاميتس هيرسوتا، كلاميتس سيمنيس، كلاميتس ويختيانا. وقد أجريت الدراسة باستخدام كروماتوجرافيا الغاز المتصلة بمطياف الكتلة. وقد وجد أن حمض بالمتيك يمثل المكون الأكبر في الأجناس الثلاثة. كما تم تقييم التأثر المضاد للالتهابات للأجناس الثلاثة ووجد أن جزء إيثير البترول من الخلاصات الكحولية كان له الفاعلية الأكبر.

A comparative study of the fatty acid contents of three *Clematis* species growing in Saudi Arabia namely *Clematis hirsuta, C. simensis* and *C. wightiana* was carried out This comparison was preformed by GC/MS technique. Plamitic acid represented the major fatty acid in the three species. The anti-inflammatory activity of the three species was also conducted. The petroleum ether fraction of the ethanol extracts exhibited the highest activity.

Key Words: *Clematis hirsuta, C. simensis, C. wightiana,* fatty acids, anti-inflammatory.

Introduction

Family Ranunculaceae comprises 59 genera and about 1900 species distributed throughout temperate and cold regions. The genus *Clematis* is represented by 250 species (1). According to some references, two *Clematis* species are found in Saudi Arabia, namely *Clematis hirsuta* Guillemin & Perr, and *C. simensis* Fresen (2, 3). Another reference reported a third *Clematis* species as the only species in Saudi Arabia namely *C. wightiana* Wallich (4). Several *Clematis* species including *Clematis hirsuta* are used in folk medicine as analgesics and anti-rheumatics (5- 9). We were able to obtain samples of the three species mentioned above from the Kingdom of Saudi Arabia. Nothing was reported concerning the fatty acid contents of these species. This encourage us to conduct the present study.

The aerial parts of *Clematis hirsuta* Guillemin & Perr were collected from "Tanuma" region in March 2000**.** Whereas the aerial parts of *C. simensis* Fresen were collected from "Abha" in April 2005. The aerial parts of *C. wightiana* Wallich were collected from "Bany Sheher" region in June 2006. The plant materials were identified by the Taxonomist Dr. M. Atiqur Rahman, College of Pharmacy, King Saud University. A voucher specimen (#11411, 12582 and 10880, respectively) were deposited at the herbarium of the College of Pharmacy, King Saud University.

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Materials and Methods

Plant materials:

Extraction and Fractionation:

The air dried powdered aerial parts of *Clematis hirsuta, C. simensis* and *C. wightiana* (300 g, each) were separately extracted by percolation with ethanol (96% v/v) to exhaustion. The solvent in each case was evaporated under reduced pressure yielding 25.5, 27.0 and 23.3 g of the total alcohol extracts respectively.

Parts of the alcohol extracts, 20 g each were separately dissolved in 200 ml of distilled water/ ethanol mixture (2:1) and successively extracted with petroleum ether (3X 300 ml), $CHCl_3$ (3X 300 ml), EtOAc (3X 200 ml) and butanol (2X 200 ml).

Each extract was separately evaporated to dryness under reduced pressure at a temperature not exceeding 45 °C. The resulted extracts were petroleum ether (11.6, 6.3 and 6.9 g respectively); $CHCl_3$ (0.37, 0.55 and 1.8 g respectively); EtOAc (1.6, 1.3 and 2.2 g respectively) and butanol (3.94, 8.9 and 6.4 g respectively).

Preparation of the Fatty acids:

The ether-soluble parts of the petroleum ether extracts of each of the three *Clematis* species were subjected to saponification to obtain the fatty acids. About 3 g of the residue in each case were saponified by refluxing with100 ml of 10% alcoholic KOH for 8 hrs. The solvents were separately distilled off nearly to dryness. Dilution was done with 100ml water and the mixtures were extracted with ether till complete extraction of the unsaponifiable matters. The aqueous alkaline solutions left were acidified with dilute 10% HCl and the liberated fatty acids were extracted with ether yielding 1.73, 1.29 and 1.18% respectively. The fatty acids were subjected to methylation using methanol and dry H_2SO_4 (10, 11).

GC/MS Analyses of the Fatty acids:

The fatty acid methyl esters of the three species were analyzed by GC/MS adopting the following conditions: Column: cross-linked 5% phenyl methyl silicone (HP-5 M.S); 30 m X 0.25 mm i.d.; Carrier gas: helium, at flow rate 0.90 ml/min.; injector temp. program was 100°C for 2 min then increased to 190 °C at 4°C/ min.

Identification of the fatty acids was performed by the aid of computer library search (CAS No. 5989-27-5, Entry 8747, LIB# 1). The relative percentage of each fatty acid was determined based on peak area measurements. Results are recorded in Table 1.

Anti-inflammatory activity:

Rat paw edema as a model of acute inflammation induced in male Wistar rats using 2% aqueous carragenan was used to evaluate the anti-inflammatory activity of the total extracts, fractions and fatty acids contents of the three plants (12). The animals were divided into two groups. The first group (6 animals) served as negative control while the animals in the second group were divided into subgroups 6 animals each and treated with the total alcohol extracts, fractions and fatty acids contents of the three *Clematis* species. All tested materials were suspended in 0.25% sodium carboxy methylcellulose using sonicator and injection via intraperitoneal route one hour before carragenan. Edema was induced by injecting 0.2 ml carragenan solution into the rat hind paws. The doses of each fraction were chosen based on the % of each fraction relative to the total extract. The volume of the rat paws before and 2 hours after injection of carragenan were measured using a Hydro-Plethysmograph (Model 7150, Ug0, Basile, Haly). The rats held firmly and the right paw was immersed into the pool of mercury up to the tibiotarsic articulation. The pressure increase due to the slight rise in mercury level was transmitted to the pressure transducer. After amplification the transduced signal was increased as a deflection in the pen on the chart. The volume of the paw was then calculated. The volume of edema is expressed in ml (mean ± SEM). Protection against inflammation is expressed as % inhibition of edema 2 hours after carragenan injection in comparison with the control group. Difference between the control and the treated groups was subjected to Student's *t*-test and *P* values evaluation. The difference in results were considered significant when $P < 0.001$. The results of the anti-inflammatory testing are presented in Table 2.

Results and Discussion

Fatty acids Constituents:

The identified fatty acids amounted to 99.97, 86.75 and 87.98 in *Clematis hirsuta*, *C. simensis* and *C. wightiana* representing 14, 7 and 6 components in the three species respectively are presented in Table 1.

Palmitic acid represented the major fatty acid in the three species (47.08, 23.47 and 23.33%, respectively) followed by linoleic (8.92% & 12.98 and 6.67%) and stearic acid (5.84& 2.88 and 19.36) that has the highest percentage in *C. wightiana*.

Linolenic acid was detected in *C. hirsuta* (31.77%) and *C. wightiana* (16.93%) while *C. simensis* was free from this acid.

This is the first report for the fatty acids profile of *Clematis hirsuta*, *C. simensis* and *C. wightiana* growing in Saudi Arabia. The results could be important from chemotaxonomic point of view.

Table 1: Identified fatty acid methyl esters of *C. hirsuta*, *C. simensis* and *C. wightiana*.

Compound name	*Relative %*			M^+	*Base peak*
	C. hirsuta	*C. simensis*	*C. wightiana*		
Myristic acid	0.81	-	13.17	242	74.10
Palmitic acid	47.08	23.47	23.33	270	74.05
Heptadecanoic acid	0.50	-	-	284	74.10
6,9,12-Octadecatrienoic acid	0.04	-	-	292	79.10
Linoleic acid	8.92	12.98	6.67	294	67.10
Linolenic acid	31.77	-	16.93	292	79.05
Stearic acid	5.84	2.88	19.36	298	74.10
8,11,14-Eicosatrienoic acid	0.11	21.67	-	306	79.10
9, 12-Octadecadienoic acid	-	16.92	-	280	67.10
2-undecyl cyclopropanepentanoic acid	0.10	-	-	310	74.10
Arachidic acid	2.98	5.29	-	326	74.10
Heneicosanoic acid	0.20	-	8.52	340	74.10
Docosanoic acid	1.36	3.54	-	354	74.10
Tricosanoic acid	0.10	-	-	368	74.10
Tetracosanoic acid	0.16	-	-	382	74.10

Table 2: Effect of the total extracts and fractions of liquid-liquid partition of the three *Clematis* species on carregeenan-induced rat paw edema.

Groups	**Increase in paw edema (ml) ± SEM[a,b]**	**% Protection**
C. hirsute:		
Control	0.91 ± 0.08	-
1-Total extract (2g/kg)	0.64 ± 0.06	30.1 ± 2.6[b]
2- Petroleum ether (240 mg/kg)	0.45 ± 0.09	50.8 ± 4.5[b]
3-EtOAc (30 mg/kg)	1.05 ± 0.1	zero
4- Butanol (85 mg/kg)	0.44 ± 0.06	51.4 ± 4.6[b]
Control	0.97 ± 0.04	-
5- Fatty acids (240 mg/kg)	0.42 ± 0.04	56.7 ± 5.3[b]
C. simensis:		
Control	0.97 ± 0.04	-
1- Total extracts (2g/kg)	0.55 ± 0.08	43.29 ± 3.4[b]
2- Petroleum ether (240 mg/kg)	0.65 ± 0.08	32.99± 3.1[b]
Control	1.15± 0.20	
3-EtOAc (30 mg/kg)	0.91± 0.15	20.7± 4.8
Control	0.97 ± 0.04	-
4- Butanol (70 mg/kg)	0.6 ± 0.08	38.14 ± 3.6[b]
5- Fatty acids (240 mg/kg)	0.76 ± 0.23	21.6 ± 8.9
C. wightiana:		
Control	0.97 ± 0.04	-
1- Total extract (2g/kg)	0.41 ± 0.03	57.73 ± 4.1[b]
2- Petroleum ether (240 mg/kg)	0.76 ± 0.23	20.6 ± 9.8
Control	1.15± 0.20	
3-EtOAc (30 mg/kg)	0.83± 0.07	27.2± 2.5[b]
Control	0.97 ± 0.04	-
4- Butanol (70 mg/kg)	0.86 ± 0.10	11.3 ± 6.9
5- Fatty acids (240 mg/kg)	0.66 ± 0.09	31.9 ± 2.8[b]

Data are presented as mean ± SEM (n=6).
[a]SEM denotes the standard error of the mean.
[b]Data are significantly different from control.

Anti-inflammatory activity:

Table 2 showed the effect of the total alcoholic extracts, petroleum ether, ethyl acetate, butanol extracts and fatty acids fractions of the three *Clematis* species. The total extracts were tested at 2g/kg i.p. They inhibited the induced inflammation by 30.1% ± 2.6, 43.29% ± 3.4 and 57.73% ± 4.1, respectively, 2 hrs following carrageenan injection.

The petroleum ether fraction of *C. hirsuta* in dose of 240 mg/kg i.p. and butanol fraction in a dose of 85mg/kg i.p. showed the highest anti-inflammatory activity (50.8% ± 4.5, 51.4% ± 4.6, respectively), while the total extract was the least active. Although the total extracts of *C. simensis* and *C. wightiana* displayed high anti-inflammatory activity, their corresponding fractions did not.

The fatty acids of the three *Clematis* species at doses of 240 mg/kg did not induce any change in the volume of inflammation when measured 1 hr after carragenan injection. However, a significant inhibition was observed after 2 hrs. *C. hirsuta* fatty acids fraction was the most active (56.7% ± 5.3), followed by *C. wightiana* (31. 9% ± 2.8), while that of *C. simensis* was the least active (21.6% ± 8.9). It is worth to mention that *C. simensis* fatty acids fraction was free from linolenic, acid. Linolenic acid is the precursor for prostaglandin E1 (PGE1) which is reported to have anti-inflammatory properties (13, 14). The anti-inflammatory properties of some oils, like Evening Primrose oil (EPO), have been linked to their high contents of linolenic acid (15). The activity of the petroleum ether fractions are expected to be due to their fatty acids contents as well as sterols and triterpenes commonly present in the non polar fractions.

The $CHCl_3$ fractions were not tested due to their small quantities. The EtOAc fraction of *C. hirsuta* did not show any protective effect against carragenan induced inflammation. While that of *C. simensis* were not significant, however, EtOAc fraction of *C. wightiana* resulted in 27.2 ± 2.5 % inhibition of inflammation comparing to the control group.

The obtained results explain the use of *C. hirsuta* in folk medicine for the treatment of rheumatism. *C. wightiana* is also suggested to be used for this purpose. Safety studies of these plants should be carried out.

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