Study and Valorization of Dovyalis Caffra Seeds

I. Lakhtib[1], A. Ihihi[2], F. Rouda[3], M. Khifa[4], A. Bahloul[5], B. Himmi[6] and S. Kitane[7]

[1,2,3,4,5] Laboratory of Biomolecular and Organic Synthesis, Faculty of Sciences Ben M'Sik, Avenue CdtDriss ElHarti, B.P. 7955, SidiOthmane Casablanca, Tél : 05 22 70 46 71, Fax : 05 22 70 46 75,(University Hassan II Mohammedia – Casablanca, Morocco)

[6] Higher Institute of Nurses professions and Technics of health, Rabat, Morocco

[7]National School of Mineral Industry, Rabat, Morocco

[1]*Corresponding author's email: imane.lakhtib {at} gmail.com*

ABSTRACT— ***In this present work, the characterization of Dovyalis caffra seeds (region Rabat-Morocco) wasdone throughthe identification and quantification of all components containing inthe oil extracted from Dovyalis Caffra seedsincluding Fatty acid and sterol composition.***

This characterization was carried out using a technicalinstrumentsgas chromatography, In one hand the mean values of fattyacid composition found in the oil extracted from Dovyalis caffra seeds was: Linoleic acid (C18:2) with 54.98 %,Palmiticacid (C16:0) with 21.36% and oleicacid (C18:1) with 12.17%, In the other hand, β-sitosterol is the major sterol in the oil.

The mean objective of this work is the valorization of Dovyalis caffra seeds.

Keywords— Dovyalis caffra, seeds, valorization, fatty acid, sterol

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1. INTRODUCTION

This work is a continuation of the work undertaken in our laboratory concerning the agro-alimentary productsespecially the carob[1].

Dovyalis caffra is commonly known as *Kei apple*, [2] its common name is deriving from the Kei River of southwest Africa [3], the fruit is originates from South Africaaround the Eastern Cape, Kaffraria and Natal[4]and it was introduced to other African countries, Australia, southern USA, Middle East and Europe to the Mediterranean countries [5].

The fruits look something like small golden apples[6] almost round, fleshy berry, up to 4 cm in diameter, orange when ripe[5],containing 5 to 10 small seeds which are 10 mm long. The fruit is juicy, tasty and acidic [7].

The fresh ripe *Dovyalis caffra* fruits are rich in vitamin C (80-120 mg and more per 100 g), as well as potassium (more than 600 mg). Sugars generally exceed 15 percent, with pectin levels nearly 4 percent. Although the protein content is low, generally below 1 percent, the balance of essential amino acids is reported good [6], so that this fruit has been identified as one of the indigenous forest product which have a potential to improve people's health [8].

Picture1:*Dovyalis caffra*

To our knowledge, there is no literature report of chemical treatment of *Dovyalis caffra* seeds. Fruits of *Dovyalis caffra* have been investigated for their composition of pectin and amino acids (Abdel-Fattah et al., 1975), and for the antioxidant activity of the polyphenols present in the fruit juice (Loots et al., 2006). Leaves of *Dovyalis caffra* have been investigated for their content of tannins (Saleh et al., 1969), and the extracts of fruits, leaves, stems, and roots have shown antibacterial activity (Basile et al., 1997; Zaki, 1975). Although alkaloids are generally uncommon in Salicaceae, two alkaloids have been identified in *Dovyalis caffra* by Sayed et al.(2000), and the effects of various pre-sowing treatments on germination of *Kei apple* Seeds harvested was studies at different maturity stages by Hae. M and Funnah S.M (2011) [2].

The objective of this study was initially the valorization of *Dovyalis caffra* seeds.

In a second step, we will study *Dovyalis caffra* growing conditions.

2. MATERIAL AND METHODS

2-1- Seed source and collection

Ripe *Dovyalis caffra* fruits were collected from trees located in Rabat as an ornamental tree, the fruits were immediately put in plastic bags and frozen in the experimental laboratory of the department of chemistry.

2-2- Sample preparation

After thawing, 1Kg of the fruits was washed using a stream of running tap water, then peeled and pulped. Seeds were then immersed in 500 mL beaker filled with water. Some of the seeds which failed to sink were eliminated from the trial. After thorough cleaning, the seeds were then dried in steaming at 70°C during 24H, and then ground in the electric mixer, the quantity of powder was weighted (22,1g).

2-3-Sensoriel properties

Sensorial properties were done based on visual observation. The seed's oil was analyzed for its texture, colour and smell based on its physical appearances.

2-4- Chemical analysis

2-4-1- Solid-liquid extraction of oil

After the final process in preparation, the extraction of 11g dried seed powder was done by adding 50 mL of dichloromethane, the mixture was magnetically stirred for 2H and filtered using vacuum filtration. The filtrate obtained was dried using the anhydrous agent (Na_2SO_4) and the solvent evaporation was carried out by rota vapor. The oil obtained was weighted (3, 44 g).

2-4-2- Liebermann–Burchard test

1 mL of the oil extract was dissolved in 3 mL of chloroform, treated with 1 mL of acetic anhydride and 1 mL of concentrated sulphuric acid in a test tube, the contents was mixed. Formation of green colour indicates the presence of sterols.

2-4-3- Determination of fatty acid compositions

Fatty acid composition was determined by gas chromatography and for that purpose they were transformed to volatile fatty acid methyl esters.

2-4-3-1- Transformation to methyl ester

0.4g of the oil extract was placed in test tube; 3mL of methanolic sodium hydroxide and 3mL of isooctane were added to the oil and agitated vigorously for 30 second.

After settling, a layer was formed in the up of the solution which contains the methyl esters.

The methyl ester (volatile component) was analyzed using gas chromatography.

2-4-3-2- Gas chromatography

The fatty acid composition was analyzed using aClarus 580 GC_G12086 gas chromatography

The columnused is 30 m * 320 µm HP-5 Phenyl Methyl Siloxane capillary colomn.

The injection Temperature was 260 °C

The flame ionization detector temperature was 280 °C and the oven temperature was held at 200 °C for 50 min.

A split ratio was used with 20 mL / min column flow rate of azote

The injection volume was 1 µl

2-4-4- Determination of sterol

2-4-4-1- Extraction of the unsaponifiable fraction

3.5 g of the oil placed in a flask equipped with a reflux condenser was saponified with 35 mL of KOH 1M, the mixture was heated on water bath with stirring for 1H. After cooling to a temperature below 25 °C, The content was transferred in a separator funnel with 40 mL of water and the liquid was stirred 3 times with 25 mL of hexane.

The ethereal phase was recovered in round-bottom flask, and was refluxed for 90 min at 70 °C (water bath 85-90°C) in order to evaporate the solvent.

The unsaponifiable fraction was recovered (1.64 g).

2-4-4-2- Isolation of sterols

1 mL of the unsaponifiable fraction was diluted in hexane; 100µL of this solution was spotted at 2 cm above the bottom of silica gel TLC plate in continuous line. The plate was then placed in the developing tank containing hexane and diethyl ether (65/35) as a developing solvent. The plate was left in the tank until the solvent reached 1cm from the upper edge.

After that, the solvent was evaporated in the open air, the plate was then pulverized with dichloromethane, and the band of the sterol (lower band) was delimited on the plate.

The band of the sterol was scraped from the plate into 100 mL-flask. To extract the sterol 10 mL of chloroform was added and the mixture was filtered through filter paper into 10mL-test tube and the solvent was evaporated.

In the same tube, 100 µL of silylating reagent was added, the solvent was evaporated , diluted with 50 µL of hexane and injected into the gas chromatograph for sterol composition analysis.

2-4-4-3- Gas chromatography

The sterol composition was analyzed using an Agilent 19091J-436 gas chromatography.

The column used is 60 m * 250 µm HP-5 Phenyl Methyl Siloxane capillary colomn.

The injection Temperature was 260 °C.

The flame ionization detector temperature was 300°C and the oven temperature was held at 325 °C for 3 min.

A split ratio 5:1 was used with 7.5 mL / min column flow rate of Helium.

3. RESULTS AND DISCUSSION

2-1- Sensorial properties of oil

The seed oil obtained by extraction is a clear liquid, with a brown color and a fruity smell.

2-2- Lieberman's Test

A greenish colour is developed when the oil extract in chloroform is treated with concentrated sulphuric acid and acetic anhydride. This colour change from violet to blue to green indicates the presence of sterols.

2-3- Determination of fatty acid composition

Table 1 gives an overview of the composition and content of fatty acids in the analyzed samples of *Dovyalis caffra* seeds oil; we notice the presence of a predominant polyunsaturated omega-6:linoleicacidC18:2) with 54.98 % , Two fatty acids were also significantly present: the saturated palmiticacid (C16:0) with 21.36% and the unsaturated oleicacid (C18:1) with 12.17%. In addition, traces of stearic acid (C18:0), decenoic acid (C10:1), alpha linolenic (C18:2), arachidic acid (C20:0), eicosenoic acid (C20:1), docosanoic acid (C22:0) and docosenoic acid (C22:1) were detected.

2-4- Determination of sterol

Gas chromatography analysis of the unsaponifiable fraction of *Dovyalis caffra* seeds oil showed the presence of a series of sterols (Table2) (Campesterol, β-clerosterol, β-sitosterol, Δ-5 avenasterol, Δ-7-stigmasterol, Δ-7-avenasterol).

The β-sitosterol recognized as the reducing agent of cholesterol in blood and also as anticarcinogenic,[9]:is the major sterol found in the unsaponifiable portion with a yield of 84% from 1.64 g of the unsaponifiable fraction.

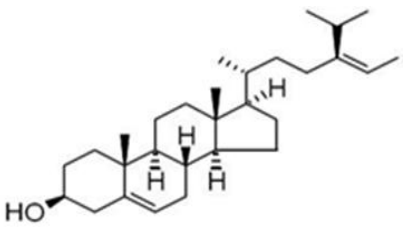

Figure 1:β- sitosterol

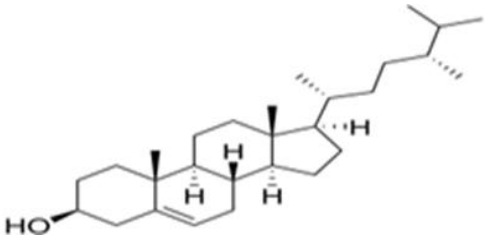

Figure 2 :Δ-5- Avenasterol

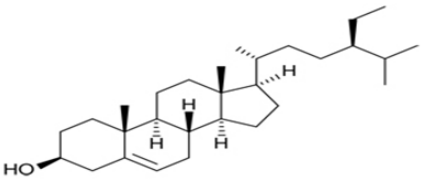

Figure 3: Campsterol

Table1: Fatty acid composition of *Dovyalis caffra* seeds oil

Peak	RetTime (min)	Type	Width (min)	Area (pA*s)	Height (pA)	Area %	Identification
1	4.534	MM	0.0451	6.57753	2.42822	0.13200	
2	5.862	MM	0.0466	8.81305	3.15026	0.17686	
3	7.428	MM	0.0445	5.92305	2.22081	0.11886	
4	7.762	MM	0.0535	9.69571	3.02014	0.19457	
5	9.736	MF	0.0543	1064.62964	326.52057	21.36452	C16:0
6	10.022	FM	0.0591	14.32621	4.04352	0.28749	C10:1
7	10.882	MM	0.0436	6.58420	2.51463	0.13213	
8	12.151	MF	0.0621	457.00015	122.72741	9.17088	C18:0
9	12.444	FM	0.0598	606.44635	168.94308	12.16990	C18:1
10	13.204	MM	0.0929	2739.71802	491.38425	54.97947	C18:2
11	14.091	MM	0.0542	28.68427	8.81456	0.57562	C18:3
12	15.154	MM	0.0649	18.98668	4.87953	0.38102	C20:0
13	15.563	MM	0.1185	8.19618	1.15321	0.16448	C20:1
14	19.769	MM	0.1034	5.97486	9.62618e-1	0.11990	C22:0
15	20.255	MM	0.0911	1.60993	2.94402e-1	0.03231	C22:1
				4983.16582	1143.05723		

Table2: Sterol composition of *Dovyalis caffra* seeds oil

Peak	RotTime (min)	Type	Width (min)	Area (pA*s)	Height (pA)	Area %	*Identification*
1	28.428	MM	0.2043	157.67955	12.86552	0.61667	
2	30.246	MM	0.2874	26.10785	1.51386	0.10210	
3	32.580	MF	0.0635	11.33781	2.97515	0.04434	
4	32.947	FM	0.3149	1262.50806	66.81354	4.93751	Campesterol
5	33.308	FM	0.2348	55.22059	3.92010	0.21596	
6	34.355	MM	0.3002	164.75017	9.14531	0.64432	
7	35.481	MM	0.2829	93.12323	5.48643	0.36419	
8	36.530	MF	0.3712	114.68978	5.14953	0.44854	β-clerosterol
9	38.166	FM	0.5928	2.15051e4	604.57147	84.10386	β-sitosterol
10	38.653	FM	0.2706	1785.10120	109.95548	6.98131	Δ5avenasterol
11	39.904	MM	0.3308	134.08353	6.75534	0.52438	
12	40.842	MM	0.3344	134.20200	6.68852	0.52485	Δ7stigrasterol
13	41.827	MM	0.3912	125.79405	5.35927	0.49197	Δ7avenasterol
				2.55697e4	841.19953		

4. CONCLUSION

Published data about valorization of *Dovyalis caffra* seeds are rare, and there are no reports about composition *Dovyalis caffra* seeds oil, in this work, we characterize this oil in terms of fatty acid composition and sterol composition. These analyses shows in one hand the presence of linoleic acid (C18:2) with 54%, palmitic acid (C16:0) with 21%, and oleic acid (C18:1) 12% . In the other hand *Dovyalis caffra* seeds oil showed the presence of a series of sterolswhosethe β-sitosterol recognized as the reducing agent of cholesterol in blood and also as anticarcinogenic is the major sterol.

In perspective, we intend to compare the *Dovyalis caffra* obtained in Morocco with this plant obtained in other countries in terms of nutrient, botanical description…

We start studying the Dovyalis caffra growing conditions in order to introduce it in Morocco. The picture below shows the plant after 8 mouths.

Picture 2:*Dovyalis caffra* plants after 8 Mouths.

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