Phytochemical Screening of Essential Oil of *Citrus sinensis* by Gas Chromatography-Flame Ionization Detector

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Abstract: *Citrus sinensis* commonly called sweet orange belongs to the family Rutaceae. Nutritionally, it is highly recommended due to its high content of micronutrients. However, the rejection of a large amount of epicarp in nature contributes to the emission of greenhouse gas and the development of leachate which contaminate surface water and groundwater. The aim of this work was to identify the essential oil components from *Citrus sinensis* epicarp, and then look after the biological activity of these components in order to underline the worth to reuse the *Citrus sinensis* epicarp as a gainful mean. The essential oil of 4,000 g of *Citrus sinensis* epicarp was done through the water steam distillation and 0.0287 g of essential oil was obtained; so a yield of 0.0007%. The essential oil was then submitted to gas chromatography-flame ionization detector (GC-FID). The result revealed that the essential oil was teemed with 28 volatile compounds, including terpene compounds (50%), aldehydes (32%) and alcohols (18%) whose anti-inflammatory, anti-diabetic, larvicidal and antioxidant activities were underlined.

Key words: Environment, *Citrus sinensis*, essential oil.

1. Introduction

Citrus sinensis commonly called sweet orange belongs to the family Rutaceae. Morphologically, the epicarp is made up of oil glands, which contain essential oil and thus cause its characteristic odor.

Biochemically, it has a high content of micronutrients, especially ascorbic acid (80 mg/100 g), vitamin P, B1, B9, E, provitamin A, calcium (40 mg/100 g) and pectins. Besides, it contributes to the regulation of intestinal transit, then their flora is rich of microorganisms, namely yeast and lactobacillus, essential for proper digestion [1]; this could justify the fact that it is nutritionally recommended. This results in an increased number of outlets in the city of Douala.

It is generally sold peeled throughout the year, and the finding of the rejection of a large amount of

epicarp in nature, particularly along the markets and along sidewalks, is obvious. Thus, this lucrative activity contributes on one hand to the urban unhealthy; on the other hand, the landfill biodegradation of these epicarps takes about two years and contributes to greenhouse gas emissions and the development of leachate contaminating surface water and groundwater [2].

Yet, these epicarps could be an important source of secondary metabolites. Indeed, numerous studies have attributed bioactive activities to volatile compounds of essential oils derived from citrus fruit epicarp. Indeed, anti-microbial activity of *Citrus limellioides* Tanaka was stressed [3].

In order to better manage organic waste in the city of Douala, the value of *Citrus sinensis* epicarp through the extraction of their essential oil was considered. Identification of volatile compounds of this essential oil is determined to support its bioactive nature and

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justify its use as a gainful mean. An investigation of the therapeutic properties of this essential oil had been conducted through a similar reasoning based on a comparison between the therapeutic properties already assigned to many volatile compounds and the compounds identified in the essential oil of interest.

2. Materials and Methods

2.1 Plant Materials

Fresh epicarps of *Citrus sinensis* were collected in January 2014 from vendors in four districts of the city of Douala—Bepanda, Deido, Central Market, Carrefour Elf. They were identified at the Cameroon Forestry Research Service and the voucher number was 25859 SRF/Cam.

2.2 Extraction of Essential Oil

The extraction was carried out using a semi industrial steam distillation apparatus locally manufactured; it was made up of stripping vessel provided with an extraction basket, a tank provided with a cooling coil outlet and an improved furnace.

The distillation of 4,000 g epicarp was performed in four steps of 1,000 g each based on the principle of water steam distillation. Distillates obtained after each distillation were mixed. The resulting mixture was dried by anhydrous sodium sulfate and the essential oil was collected and stored at 4 °C in dark bottles.

2.3 Analysis of Compounds by Gas Chromatography-Flame Ionization Detector (GC-FID)

Chemical analysis of the essential oil obtained was performed using a chromatograph (HP 6850) through the injection of 0.2 µL of a solution which is made up of essential oil and hexane. The carrier gas was helium at a flow rate of 0.7 mL/min. The column used is a capillary column DB-WAX 20 m in length and 0.1 mm in internal diameter. The thickness of stationary phase was 0.25 µm. The programming of oven temperature was 60 °C for 2 min, and then it was in

increments of 12 °C/min to 248 °C in 5 min. The detector used in this study was flame ionization detector (FID). All of that was controlled by a computer with National Institute of Standards and Technology (NIST) database which allowed the identification of compounds.

2.4 Ethics

This research had been done in an ethical manner. It did not harm people, to whom the epicarp of *Citrus sinensis* was gathered. Neither their privacy nor their health was exposed. They were mentioned anonymously. They were not robbed of their property, the epicarp of *Citrus sinensis* or orange peel was collected for the trash.

3. Results

The essential oil extracted has a clear appearance, orange yellow color (Fig. 1), with a characteristic odor of orange peel, sweet and fruity.

3.1 Extraction Yield

The extraction yield was estimated as a percentage of relative weight of the essential oil extracted from the peel weight. As shown in Table 1, the yield of the extraction was 0.0007%; it is really low in comparison to initial epicarp weight collected.

3.2 Chemical Composition of the Essential Oil of *Citrus sinensis*

Analyses revealed that the essential oil of *Citrus sinensis* is teemed of 28 volatiles compounds. Fifty percent of them are terpene compounds, 18% are alcohol and 32% are formaldehyde. These compounds are listed in Table 2.

Fig. 1 Essential oil extracted.

Table 1 Extraction yield.

Specie name	Epicarp weight (g)	Essential oil weight (g)	Yield (%)
<i>Citrus sinensis</i>	4,000	0.0287	0.0007

Table 2 Chemical composition of the essential oil of *Citrus sinensis* epicarp.

Compounds	Content (%)	Retention time (min)
α pinene	0.53	2.478
β pinene	Trace	3.629
Sabinene	0.33	3.989
δ -3-carene	0.05	4.131
Myrcene + α phellandrene	1.92	4.022
Limonene	94.40	4.850
β phellandrene	0.23	5.719
γ terpinene	Trace	6.959
Trans β ocimene	Trace	7.060
Terpinolene	Trace	8.146
Octanal	0.46	8.200
Nonanal	0.08	8.583
δ elemene	0.05	8.714
Citronellal	0.05	9.268
Decanal	0.38	10.031
Linalol	0.45	10.123
β caryophyllene	Trace	10.311
Neral	0.06	10.509
α terpineol	0.03	10.346
Valencene + geranial	0.12	10.834
Dodecanal	0.03	11.085
δ cadinene + citronellol	0.04	14.039
Nerol	0.03	14.300
Geraniol	Trace	14.400
β sinensal	Trace	15.538
α sinensal	0.03	19.796

4. Discussion

Twenty-eight volatile compounds were identified in the essential oil extracted from *Citrus sinensis* epicarp with a predominance of terpene compounds (50%), aldehydes (32%) and alcohols (18%). Hydrocarbon monoterpenes limonene (94.4%) and the association of phellandrene and myrcene (1.92%) were the major compounds.

The extraction yield was 0.0007%. This is insignificant compared to the range of 0.2%-2%, to which the yield of a good extraction of essential oil should belong [4]. This low yield could be associated with the extraction technique, including the water

steam distillation. Moreover, the defaults on the locally manufactured apparatus contribute to the loss of steam during the distillation process. After investigating previous work on essential oils from *Citrus* genus, it is clear that limonene is provided with anti-inflammatory properties. Indeed, limonene reduces the production of pro-inflammatory cytokine (TNF- α) and nitrate oxide regulator of inflammation by inhibiting the activity of polysaccharides that stimulate excessive production. So limonene looks like an effective anti-inflammatory, especially in the case of inflammation of skin [5, 6].

Overall, monoterpenes have several therapeutic properties, especially insecticidal and antidiabetic

properties. It turned out that the monoterpenes from the essential oil of *Citrus sinensis* epicarp have larvicidal activity against larvae of the mosquito—*Aedes aegypti* that causes yellow fever [7] in the fourth larval stage *Culex pipiens* [8, 9]. In the case of diabetes type 1 called insulin dependent diabetes, monoterpenes have the ability to mimic the properties of insulin, induce the secretion of the latter by regenerating dead pancreatic beta cells, or in further stimulate their secretion by alive pancreatic beta cells [10, 11].

The antioxidant activity of the essential oil of *Citrus sinensis* epicarp has recently been highlighted and it was attributed to the presence of phenolic compound [12].

5. Conclusions

At the end of this work, the authors could consider the essential oil of *Citrus sinensis* epicarp as an environmentally friendly product and so reuse its epicarp as a gainful mean. Indeed, due to its anti-inflammatory and antioxidant properties, it is used in the composition of anti-aging, anti-pain, anti-rheumatism and cosmetic products. Moreover, it could be a good product against insects, since it has larvicidal properties.

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