Constituents and Biological Activity of *Citrus aurantium amara* L. Essential Oil

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Abstract

***Citrus aurantium amara* L. belongs to the order Geraniales and family Rutaceae. It is native to South East Asia, and it is a wild crop in Venezuela . It is a tree 6 to 8 m high bearing fruits with a thick, rugged and easily detachable cortex. The essential oil obtained from the cortex of *C. aurantium amara* has been used to add aroma to beverages and liquors and as an ingredient to give fragrance to soaps, detergents, cosmetics and perfumes. The fruits were collected in several sites of Táchira State, Venezuela. The oil was extracted from the cortex by cold pressing. Its components were analyzed by gas chromatography with flame ionization detector and gas chromatography-mass spectrometry. The main constituents found were the following: monoterpenes (limonene, 77.90%; β-pinene, 3.40%; myrcene, 1.81%; and trans-ocimene, 1.16%), sesquiterpenes (valencene, 0.52%), aldehydes (decanal, 3.51%; dodecanal, 0.36% and geranial, 0.29%), alcohols (β-nerolidol, 0.85% and linalool, 0.89%), and nootkatone as the only ketone. The extraction procedure can be considered as adequate since the oil obtained does not contain p-cimene, which is an indicator of oxidation of monoterpenes in citrus essential oils. Terpinene-4-ol, a product of limonene degradation, was found in traces and thus no unpleasant odor was present. The biological activity of *C. aurantium amara* essential oil against *Escherichia coli, Stapylococcus aureus* and *Pseudomonas* was determined using filter paper disks impregnated with 20 µl of essential oil placed on agar plates inoculated with these bacteria (10^7 UFC). The biological activity was evaluated after 48 hours, being inactive against *E. coli* and *Pseudomonas* and moderately active (17 mm) against *S. aureus.* The results obtained confirmed the traditional properties of the essential oil studied as savoring, odor, and a perfume base, as well as a natural antiseptic to inhibit *S. aureus* growth.**

INTRODUCTION

Citrus aurantium amara L (Rutaceae) is a tree 6 to 8 meters high which has fruits with a thick outer coat. This rind is wrinkled, full of points, and peels off easily. The pulp is acid and very bitter. The flowers are white and are located, alone or in groups, at the axillas of the leaves. It is native to Southeast Asia and it has been introduced into all tropical countries. The essential oil of its rind is used to aromatize liquors, beverages, soaps, and detergents, and as an ingredient of cosmetics and perfumes.

Venezuela is one of the main citrus producing countries in South America and it has a well developed industry of juices and citrus concentrates but it imports every kind of citrus byproduct, including essential oils. In Táchira State a great variety of citrus is cultivated and *Citrus aurantium* grows quite well.

The aim of the present work is to identify the components of the essential oil from the rind of *Citrus aurantium* growing in Táchira State and to assess its biological activity.

MATERIALS AND METHODS

The essential oils were obtained by cold pressing the fruits (Mondelo et al, 1995). GC-FID analysis was performed on two capillary columns of different polarity: dimethyl-

polysiloxane and 20M-polyethylenglycol (Carbowax). Both columns were 60 m long with 0.25 mm diameter and 0.25 μm film. The oven temperature was programmed from 60° C (5 min) to 200° C at 4° C/min and the final temperature kept for 20 min. Injector and detector temperatures were 200° C and 220° C, respectively. The Kovats retention indices were determined relative to the retention times of n-paraffin hydrocarbons with a logarithmic scale.

GC-MS analysis was carried out on a model 5973 Hewlett Packard system fitted with a 5% diphenyl-dimethyl-polysiloxane column 30 m x 0.25 mm x 0.25μm film. The oven temperature was programmed from 60° C (3min) to 200° C (4 min) at 4° C/min. The injector and transference line temperatures were kept at 200° C and 280° C respectively. The ionization voltage used was 70 ev. Identification of oil components was established using a Wiley MS Data Library and retention indices.

Biological activity of *Citrus aurantium amara* oil against *Escherichia coli, Staphylococcus aureus, and Pseudomonas sp,* was determined by means of triplicate assays performed according to Janssen et al. (1987). Filter paper discs impregnated with 20 μL of the oil were located on agar plates inoculated with the above mentioned microorganisms (10^7 UFC). After 48 hours the activity was evaluated by measuring the inhibition halos (mm).

RESULTS AND DISCUSSION

Table 1 presents the constituents of the essential oil of *Citrus aurantium amara* distributed according to functional class. A total of 23 compounds were identified which represent 94.8% of the oil. Monoterpene hydrocarbons represent the main oil fraction (85.4%) and limonene (77.9%) is the most abundant constituent. All monoterpene hydrocarbons found have been reported for the oil of *Citrus aurantium amara* and similar species (Pino et al. 1999), and their relative concentrations agree with previous studies (Njoroge et al. 1995; Dugo et al 1993; Lawrence 1994). Carvone, which is indicative of decomposition, was not detected. This means that extraction and storage were oxidation free (Ojeda et al. 1998).

The essential oil of *C. aurantium amara* was inactive against *Escherichia coli* and *Pseudomonas sp* and moderately active (17 mm) against *Staphylococcus aureus*. These results agree with those reported by Mazzanti et al (1998) who found that limonene inhibits *S. aureus*.

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Tables

Table 1. Constituents of the essential oil from *Citrus aurantium amara* L.

Compound	Percentage		Compound	Percentage
Monoterpene Hydrocarbons	**85,42**		**Alcohols**	**2,09**
α-Pinene	0,48		Linalool	0,89
Sabinene	0,62		Terpinen-4-ol	0,11
β-Pinene	3,40		Nerol	0,24
Myrcene	1,81		β-Nerolidol	0,85
Limonene	77,90			
Trans-Ocimene	1,16		**Esters**	**4,92**
γ-Terpinene	0,05		Octyl Acetate	0,27
			Linalyl Acetate	4,01
Sesquiterpene Hidrocarbons	**0,70**		Neryl Acetate	0,22
β-Copaene	0,18		Geranyl Acetate	0,42
Valencene	0,52			
			Ketone	**0,41**
Aldehydes	**1,23**		Nootkatone	0,41
Citronellal	0,02			
Decanal	0,51			
Neral	0,05			
Geranial	0,29			
Dodecanal	0,36			