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Original article

Chemical Composition and Antibacterial Activity of the Essential Oil of *Citrus aurantium* L. Growing in Eastern Algeria [1]

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Abstract

Citrus aurantium (Bitter Orange) is a Rutaceae known for its extremely bitter and sour taste. Its leaves are rich in essential oil (EO).

The purpose of this study was to extract, analyse and evaluate the antibacterial activity of this EO in vitro, against 10 bacterial strains responsible for nosocomial infections (05 *Escherichia coli*, 03 *Staphylococcus aureus* and 02 Klebsiella ssp).

The extraction of the essential oil was carried out on fresh leaves harvested in Annaba (Eastern Algeria) using a Clevenger type device. The analysis was performed by GC/MS and it was tested on 10 bacterial strains by the dilution method in agar medium.

The results showed that the EO is composed mainly of linalool (44.52%). Among the ten strains tested, eight were sensitive to this EO with inhibition diameters ranging from 12.1 mm to 21.45 mm and minimum inhibitory concentration (MIC) between 0.1% and 1%, however, both *Klebsiella pneumoniae* and *Escherichia coli* ATCC strains were resistant.

The antibacterial activity of Bitter Orange EO seems to be largely due to the major component linalool.

Keywords: *Citrus aurantium* L., Leaves, Essential oil, GC/MS, Antibacterial activity, Eastern Algeria.

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INTRODUCTION

The Bitter Orange (*Citrus aurantium*) is a small evergreen tree, belonging to the family Rutaceae and the genus Citrus, up to 10 m tall with thorny branches and leaves 4 cm to 10 cm long. The bitter orange is very widespread in hot and humid countries especially in Spain, Sicily, North Africa, United States, which are also big producers. Its leaves are rich in essential oil which treat insomnia, anxiety (Carvalho-Freitas et al., 2002) and stomach gastritis (Moraes et al., 2009).

In Algeria this species is cultivated especially as a decorative tree and a rootstock. Its multiplication by sowing or grafting is very easy, it presents a large flexibility of adaptation to edaphic conditions and it is drought resistant (ITAFV, 2011). Like all citrus fruits, the bitter orange tree is an evergreen tree with high water requirements that varies between 900 and 1200 mm per year. These needs are more pronounced, especially during the magnification stage coinciding with the summer period. The optimum growth temperature would be 25°C to 26 °C; beyond, the activity decreases to stop at around 38 to 40 °C (ITAFV, 2011). All these conditions are met in the region of Annaba.

The aim of this study was to extract, analyze and evaluate *in vitro* the antibacterial activity of the essential oil extracted from the leaves of the Bitter Orange on 10 strains of bacteria responsible for nosocomial infections.

Materials and methods

Bacterial strains

Escherichia coli (ATCC22, S45, S55, S145, S102)

Klebsiella oxytoca S 113

Klebsiella pneumoniae +2815

Staphylococcus aureus S 47

Staphylococcus aureus 93

Staphylococcus aureus ATCC 23

They were provided by the laboratory of Microbiology of the Faculty of Medicine (Annaba).

E. coli S 145, *S. aureus* S 47 and Kpc + are multiresistant strains.

Extraction of the essential oil

The quality of the oil obtained from particular species will be influenced by where it is grown and how it has been processed. The EO was extracted from the bitter orange grown under the climatic conditions of Annaba (Eastern Algeria), and harvested in February 2013 from the garden of the faculty of Medicine.

It was carried out on fresh leaves by the steam training method using a Clevenger type device, for 1h 30 min; relatively short duration because the anatomical secretory structures are superficial (Schizolysigenic secretory pockets), characteristics of the genus "Citrus".

Chemical characterization

It was done by GC/MS Shimadzu. The column type was QP 2010 S, with a length of 25 m and an inside diameter of 0.25 mm. The carrier gas used was helium at a flow rate of 1.5 ml / min. The temperature of the column was maintained at 60ºC for 5 min and then increased 5ºC per min to 220ºC.

Aromatogramme

This technique, inspired by that of antibiograms, has been generalized to essential oils. The principle of this method is the migration of EO by diffusion in agar. A bacterial suspension of each of the strains studied is seeded on a Muller - Hinton agar medium. Calibrated discs of 6 mm diameter, impregnated with 10 µl of pure essential are placed on the sown media; after an incubation of 24 hours at 37 °C, the reading is performed by measuring the inhibition diameter in mm.

In addition to the disks impregnated with the essential oil in question, a control disk (sterile without oil) is deposited and incubated under the same conditions to ensure that the disks are devoid of antibacterial activity.

Minimum inhibitory concentration (MIC)

It was determined by the dilution method in a solid medium. In dilution tests, the microorganisms are tested for their ability to produce visible growth on a series of agar plates (diluted on agar) containing dilutions of the antimicrobial agent. The lowest concentration of an antimicrobial agent (in mg/l) that, under defined *in vitro* conditions, prevents the onset of visible growth of a microorganism within a defined period of time, is known as the MIC (EUCAST, 2003).

Results and discussion

Composition of the EO

The chemical analysis revealed a total of 23 constituents. This analysis showed that the major component of the EO is beta linalool at 44.52%. This analysis also showed that most of the substances identified were monoterpene hydrocarbons including a geraniol ester (3.48%) and α terpineol at an appreciable content of 8.34% (Table 1).

Table 1. Results of the analysis of *C. aurantium* EO by GC/MS

Components	**Time**	**Area**	**Area (%)**
Alpha.-Pinene	3.491	5100222	2.31
Camphene	3.687	269550	0.12
Sabinen	4.088	501392	0.23
Beta.-Pinene	4.152	2846872	1.29
Beta.-Myrcene	4.443	1993026	0.90
3-Carene	4.856	894239	0.41
p-Cymene	5.026	323866	0.15
Eucalyptol	5.199	3634440	1.65
Beta.-trans-Ocimene	5.449	213616	0.10
Beta.-cis-Ocimene	5.717	321444	0.15
Linalooloxidetrans	6.201	3902180	1.77
Linalooloxidetrans	6.599	3718171	1.69
Beta.-Linalool	7.095	98209137	44.52
Camphor	7.855	1842315	0.84
Isoborneol	8.818	429731	0.19
Norborneol	8.913	802988	0.36
Alpha.-Terpineol	9.697	18385660	8.34
Trans-Geraniol	11.234	1112266	0.50
Beta.-Citral	11.320	489511	0.22
Cis-Geraniol	12.262	3051081	1.38
Bergamol	12.497	60689598	27.52
Nerolacetate	16.439	4153888	1.88
Geraniol ester	17.165	7674333	3.48

Antibacterial activity

It was noted that the greater activity of the EO was recorded on Klebsiella oxytoca S 113 whose inhibition diameter is greater than 21 mm with a MIC equal to 1%, the oil had also significant activity on Staphylococcus aureus which was of the order of 20.19 mm and a MIC of the order of 1%, the inhibition diameter was 17.2 mm with S. aureus ATCC and a MIC of 0.3%.

There was also an inhibition zone greater than 15 mm with the following Escherichia coli strains: S 55, S 45 and S 145 with a MIC of 0.3% for the three strains.

The oil had a lower activity on Staphylococcus aureus S 47 and Escherichia coli S102, which generated an inhibition diameter of 13.6 mm and 12.1 mm respectively and a MIC of 0.4% for the first strain and 0.3% for the second one. E. coli ATCC 2815 and Kpc + have been insensitive to this EO.

It is known that the sensitivity of a microorganism to EO depends on the properties of EO and the microorganism itself (Kalemba and Kunicka, 2003). The antibacterial activity of the bitter orange EO

appears to be largely due to the major components, especially linalool. The bacterial sensitivities to linalool is partly related to its lipophilic character leading to the accumulation at the level of the bacterial walls thus disturbing the functioning of the permeability of the cell membranes, degradation of the cell wall (Helander et al., 1998), damage of the cytoplasmic membrane (Ultee et al., 2002), damage to membrane proteins (Juven et al., 1994) leakage of cellular content (Lamber and Pearson, 2001) coagulation of cytoplasm and proton motive force exhaustion (Ultee and Smid, 2001). It could be attributed also, to the presence of minor compounds present at low non-negligible levels; this is implied in the phenomena of synergy between the different constituents which can be at the origin of a much more pronounced antimicrobial activity than that predicted by the majority compounds (Zhiria, 2006).

Studies have shown that citrus essential oils are effective against photogenic bacteria, bacterial spores, but also certain bacteria responsible for food poisoning (Belletti et al., 2004; Fisher et al. 2007).

Table 2. Aromatogramme and MICs of *C. aurantium* EO

Strains	**Diameters (mm)**	**MIC (%)**
Kpc+	6	/
E.coli ATCC 2815	6	/
K.oxytoca S 113	21.45	0.1
S. aureus 93	20.19	1
S. aureus S 47	13.6	0.4
S. aureus ATCC 23	17.2	0.3
E.coli S 145	15.95	0.3
E.coli S102	12.1	0.3
E.coli S 45	15.6	0.3
E.coli S 55	15.25	0.3

Conclusion

The bitter orange (*Citrus aurantium*) has many qualities, it is self-fertile, hardy, vigorous, fast growing and resistant to many diseases. All parts of the bitter orange (fruit, flower, leaves, and twigs) have multiple applications (cooking and perfume, herbal medicine, aromatherapy).

The aim of this study was to extract the essential oil from fresh leaves harvested in Annaba (Eastern Algeria), analysed it and evaluate its antibacterial activity *in vitro*, against 10 bacterial strains responsible for nosocomial infections. The study showed that the essential oil was composed mainly of linalool (44.52%). Among the ten strains tested, eight were sensitive to this essential oil with inhibition diameters ranging from 12.1 mm to 21.45 mm and MICs between 0.1 and 1%; however, both *Klebsiella pneumoniae* and *Escherichia coli* ATCC strains were resistant.

The antibacterial activity of Bitter Orange EO seems to be largely due especially to the major component: linalool. This oil already, known for its sedative and anxiolytic properties, proved to be also antibacterial.

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