Journal of Clinical Toxicology
ISSN: 2161-0495

Mostaphi et al., J Clin Toxicol 2018, 8:2
DOI: 10.4172/2161-0495.1000381

Research Article Open Access

Antimicrobial Activity of the Essential Oil of an Endemic Plant in Morocco, *Impatiens balsamina*

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Received date: March 20, 2018; **Accepted date:** April 24, 2018; **Published date:** May 15, 2018

Abstract

The purpose of this present work is to study the yield of the essential oil of an aromatic and endemic plant *I. balsamina*. Additionally, we intend to determine the chemical composition of the essential oil stemming from this species, as well as the determination of its antibacterial activity towards five microorganisms. Our samples result from Morocco, mainly from region Marrakech. 13 constituents were identified by chromatographic analysis (GC) in essential oil of this plant among which 3 are preponderant: β–thujone (66.75%), camphor (17.52%), and camphene (1.67%). The yield in essential oil of the *Impatiens balsamina* is in the order of 1,2% (mg/100 g). The essential oil of *Impatiens balsamina* showed a significant antimicrobial activity against all seven tested species of bacteria. To the best of our knowledge.

Keywords: *Impatiens balsamina*; Essential oil; Chemical composition; Antibacterial activities; Endemic

Introduction

Herbal medicine has a long history in the treatment of several types of diseases. Their use for the treatment of the disease has been practiced by man for many years and is still widely practiced even today. For many years, people have developed a stock of empirical information about the therapeutic values of local plants prior to the appearance of orthodox medicine. Through periods of trial, error and success, these herbalists and their apprentices have accumulated a large body of knowledge about medicinal plants [1].

This study describes the antibacterial properties for *I. balsamina* (member of the balsaminaceous family). The plant grows throughout South Asia, India and Myanmar, the *I. balsamina* plant seeds are commonly used in tea. It is an annual plant that grows 20 to 75 cm tall, with a thick but soft stem. The leaves are spirally 2.5 to 9 cm long and 1 to 2.5 cm wide, with a deeply toothed margin. The flowers are red, purple pink or white and 2.5 to 5 cm in diameter [2]. The plants are used in traditional medicine. *Impatiens balsamina* root is also used to treat jaundice and digestive disorders [3]. The leaves, seeds and stems of *Impatiens balsamina* are also edible if cooked. The sap of the fir leaves bathes the warts and also the snake bites, while the flower can be applied on the burns to cool the skin [4]. *I. balsamina* has traditionally been used for the treatment of thorny wounds or glass wounds, abscesses, growing nails and chronic ulcers caused by the allergic reaction of detergents. The groups of compounds commonly found in this plant are naphthoquinones, coumarins, phenolic acids, flavonoids, and steroids [5].

The present study was conducted to test the antibacterial efficacy and minimal inhibitory concentration of the extract of *Impatiens balsamina* against bacterial strains.

Materials and Methods

Collection of plants

The plant of *I. balsamina* was collected at random during the month of January from a village near the town of Marrakeche. Plant species have been identified using morphological characteristics.

Hydrodistillation

The extraction of essential oils was carried out by hydrodistillation in a device of the Clevenger type. Three distillations were carried out by boiling during two hours of 200 g fresh vegetable equipment with 1 liter of water in a balloon of 2 L surmounted by column a 60 cm length connected to a cooling agent. The essential oil yield was given compared to the dry matter, evaluated starting from 3 samples of 30 g dried until the constant weight during 48 h with the drying oven with 60°C [6].

HE was stored to 4°C with the darkness in the presence of anhydrous sodium sulphate before proceeding to the operations of analyses by CG.

Analysis chromatographic

Operating conditions of Gas chromatography (CPG): The chromatographic analysis of HE was carried out with a chromatograph in gas phase type chromatography in gas phase 7890b-Agilent technologies the fragmentation is carried out by electronic impact with 70 eV. The column used is a capillary tube HP 5MS (30 m × 0,25 mm). The thickness of the film is of 0,25 µm. The temperature of the column is programmed of 50 with 250°C at a rate of 4°C.min^{-1}. The carrier gas is the helium whose flow is fixed at 1.5 ml.min^{-1}. The mode of injection is mode Split (report of escape: 1/70). The device is connected to a computer system managing a library of spectrum of mass NIST 98 and is controlled by software "HP ChemStation" making it possible to follow the evolution of the chromatographic analyses. The

identification of the components was made on the basis of comparison between their indices of retention and those of the standard compounds of the computerized data bank (NIST 98) [7].

Bacterial strains

For the determination of the antimicrobic activity of essential oils of Impatiens balsamina, 6 bacterial strains (*Escherichia coli, Klebsiella, Citrobacter, Bacillus, Staphylococcus aureus and Acinetobacter*). These bacteria are pathogenic and are known for their strong antibacterial resistance and their invasive and toxic power at the Man. They are frequently met in many infections in Morocco and pose a clinical and therapeutic problem [8].

The bacterial strains are maintained by road repair on agar nutritive favorable to their growth during 24 h with the darkness with 37˚C.

Microbiological procedure

The inhibiting minimal concentrations (CMI) of essential oils were given according to the method by Chericoni and Flamini. Because of no miscibility of oils essential with water and thus in the middle of culture, the setting in emulsion was carried out thanks to an agar solution with 0,2% in order to support the contact germinates/made up. Dilutions are prepared with the 1/10 e, 1/25 e, 1/50 e, 1/100 e, 1/200 e, 1/300 e and 1/500 e in this agar solution. In test tubes containing each one 9 ml of agar medium of TSA (Tryptic Soybean Agar) for the bacteria and of malt (2%) for the moulds, sterilized with the autoclave (20 min with 121°C) and cooled with 45°C, one adds 1 ml of each dilution in order to obtain the final concentrations of 1/100, 1/250, 1/500, 1/1000, 1/2000, 1/3000 and 1/5000 (v/v). Then one suitably agitates the tubes before pouring them in boxes of Petri. Witnesses, containing the culture medium and the agar solution with 0,2% only, are also prepared.

Sowing is done by scratches using a handle of turntable gauged in order to take the same volume of inoculum. This last is presented in the form of culture medium of 24:00 for the bacteria and in the form of a suspension in the physiological water of spores coming from a culture of 7 J in malt for the moulds. Incubation is done with 37°C with the darkness during 24:00 for the bacteria and with 25°C during 7 J for mushrooms. Each test is repeated three times in order to minimize the experimental error.

Results and Discussion

Output

The medium output in essential oil was calculated according to the dry plant material of the air part of the plant. The output in essential oil of *I. balsamina* gave a 1.2 % rate.

This output can be considered to be means in comparison with some plants which are industrially exploited as source of essential oils. It is higher than that of pink (0,1-0,35%) and weaker than that of the mint added pepper (0,5-1%), the neroli (0,5-1%), anise (1-3%), lavender (0,8-2,8%), rosemary (1-2,5%) and thyme (2-2,75%).

Chemical composition

The chromatography analysis of HE of *I. Balsamina* in the region of Marrakech revealed the presence of 13 volatile compounds. These constituents of the essential oil of the plant represent about 95,7%.

The combination in volatile compounds of this kind is variable, in term of diversity and of concentration. The essential oil of *I. balsamina* (Table 1) includes terpene esters with the acetate of terpinyle, terpene alcohols as Eucalyptol and of monoterpenes such as the camphene, Thujene, Terpinene and Pinene. There are also oxides, sesquiterpenes Ketones and sesquiterpenes [9].

N°	Tr	component	Air %
1	3.150	α -Pinene	0.48
2	4.397	α-Thujene	0.48
3	4.569	2-Pinene	0.96
4	5.003	Camphene	1.67
5	5.429	Sabinene	1.54
6	6.414	D-Limonene	0.74
7	6.664	β-Cymene	1.63
8	6.724	Eucalyptol	0.92
9	7.023	γ-Terpinene	0.69
10	8.444	Thujone	1.42
11	8.606	β.-Thujone	66.75
12	9.522	Camphor	17.52
13	10.994	2-Isopropenyl-5-methylcyclohexanone	0.90
Tr: retention time			

Table 1: Chemical composition of the essential oil of *I. balsamina* harvested in Morocco.

The classification of the identified components based on the functional groupings shows dominance of β-Thujone, Its distribution is relatively high (66,75%). monitoring of the camphor (17,52%). They are accompanied with other minority constituents which are not lacking of importance: camphene (1,67%), β-Cymene (1,63%), Sabinene (1,54%). What compounds with a previous study of Holeman which showed that, by hydrodistillation, ß-thujone constitutes 60 % plants [10].

Antimicrobial activity of essential oils

Essential oils are admitted by their natural components, as monoterpenes, diterpenes and hydrocarbons with various functional groups. In 1990, Muanza and its collaborators searched potentially bioactive extracts of plants against bacteria. Since, many other researchers brought back antimicrobic effect and antifongique of the essential oils in agroindustrial application, pharmaceutical research and in other domains. Several compounds are often named as representative for the antiseptic properties of essential oils: the thymol, the carvacrol, the cinnamaldéhyde, the eugénol, the 1,8-cinéole, the camphor and thujones [11] .

For essential oils of I.B, the results of antibacterial activity and the antifongique are summed up in the Table 2.

Dilution v/v								
	1/100	1/250	1/500	1/1000	1/2000	1/3000	1/5000	Temoin
Bacteria								
Escherichia coli	-	-	+	+	+	+	+	+
Klebsiella	-	-	+	+	+	+	+	+
Citrobacter	-	-	+	+	+	+	+	+
Staphylococcus aureus	-	-	+	+	+	+	+	+
Acinetobacter	-	-	+	+	+	+	+	+
(-) inhibition; (+) growth/development								

Table 2: Antibacterial Activity of the essential oil of *I. balsamina*.

In general, the pathogenic germs used in this biotest were sensitive to the essential oil of *I. balsamina*. The thresholds of antibacterial activity range between 1/100 and 1/250 v/v.

In against part, for the bacteria, the concentration of 1/250 v/v was sufficient to stop the growth of all the bacteria which was shown most vulnerable to this essential oil.

The presence of an important content of monoterpenes oxygenated (thujones, camphene, camphor) in the essential oil of *I. balsamina* can be responsible for its activity pronounced against *Staphylococcus aureus* and its high activity against *Acinetobacter*. Indeed, it was shown that *S. aureus* is most affected by the monoterpenes ketones like the thujones [12].

Previous studies showed that the majority of the essential oils tested for their antibacterial properties have an effect more pronounced against Gram positive. The negative resistance of Gram is allotted to their absorbent external membrane which can block the penetration of hydrophobic compounds in the target cellular membrane. The relative action of the thujones was associated with their low Hydro-solubility and the capacity to form hydrogen bonds, which limits their entry in Gram negative which has hydrophobic ways inoperative in the external membrane [13].

Moreover, stereochemistry influences the antibacterial activity. It was observed that the α-isomer is less active compared to ß-isomers. Since the β-thujone, is the majority compound (66.75%) oil of *I. balsamina* of Morocco, it confers to him a strong property disinfectant besides other physiological characteristics; it is abortive, antibacterial, insecticide and larvicide [14].

In addition to that others composed of this oil have interesting biological activities. The camphor, for example, presents an antibacterial, anti-dysenterique activity and also fungicide. The percentage of the camphor is of 17.52%. What could be potentially a reason of microbial over-sensitiveness towards essential oil of *I. balsamina* [15].

This essential activity antibacterial of oils could be explained by the molecular interaction of the functional groupings of the components of essential oils with the wall of the bacteria what causes deep lesions. One can thus conclude that this activity can be the result of a synergistic effect between several compounds of this HE [16].

Conclusion

This work is devoted to the determination of the output, the chemical composition and the properties antibacterial of the essential oil of extracted from *I. balsamina* collected in the area of Marrakech (Morocco). The average essential oil yield is 1,2%. The chemical analyses, by CG, made it possible to identify approximately 95.7% of the products total birds of this petrol. The β-thujone (66.75%) constitutes the principal compound identified among the 13 characterized followed by the camphor (17.52%) and of the camphene (1,67%). The got results, in this study, show that the essential oil of Impatiens balsamina presents, *in vitro*, an important inhibiting activity on the five bacteria tested. The major components like the β-thujone, the camphor can be responsible for the differentiation of the antimicrobic activity. Moreover, all these compounds are well-known for their antimicrobic properties. The performances antibacterial put in obviousness deserve to be to study with more details in order to consider prospects for application of this petrol like agent for organic-conservation.

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