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Alessandra Piras[a], Hanen Marzouki[b], Andrea Maxia[c], Arianna Marengo[c],Silvia Porcedda[a]† , Danilo Falconieric,[d], Maria José Gonçalves[e], Carlos Cavaleiro[e] and Ligia Salgueiro[e]

[a]department of chemical and Geological science, university of cagliari, cittadella universitaria, Monserrato, Italy;

[b]laboratory of transmissible diseases and Biologically active substances, Faculty of Pharmacy, university of Monastir, Monastir, tunisia;

[c]department of life and environmental sciences, university of cagliari, cagliari,
Italy;

[d]state Institute of higher education "Michele Giua", cagliari, Italy;

[e]cNc.IBIlI, Faculty of Pharmacy,university of coimbra, coimbra, Portugal

ABSTRACT

In the present work the chemical compositions, measured by GC and GC-MS, of the essential oils obtained by hydrodistillation from leaves of Pistacia terebinthus collected in Bizerte (Tunisia) and Baunei (Italy) are reported. Both essential oils possessed high content of monoterpene hydrocarbons (86.3% and 90.9%, respectively), being α-pinene (62.4 vs. 35.0)%, camphene (3.0 vs. 2.4)%, β-pinene (12.1 vs. 4.5)%, terpinolene (1.7 vs. 35.2)% and β-phellandrene (3.8 vs. 4.5)% the main components. The Tunisian essential oil exhibited higher antifungal activity than the Italian one. Cryptococcus neoformans and the majority of dermatophyte strains showed more sensitivity to the Tunisian oil, when compared to Candida strains, in particular Trichophyton rubrum, Microsporum canis and Epidermophyton floccosum, with MIC and MLC values in the range (0.16–0.32) μL/mL. The results obtained support the use of the oil from Tunisia for the treatment of dermatophytosis.

1. Introduction

The genus *Pistacia* L. (*Anacardiaceae*) includes evergreen and deciduous shrubs and trees with resinous bark (Bozorgi et al. 2013). This genus was originated in Central Asia (Turkistan) and approximately at the beginning of the Christian Era, it was introduced into Southern Europe through the Balkans and the Mediterranean Sea. At present, it is widely distributed, from the Mediterranean Basin (Italy, Greece, Turkey, Syria, Lebanon, Tunisia, Jordan, Cyprus, Morocco) to the United States (California, Texas, Arizona) (Buffa et al. 2009; Senyay-Oncel et al. 2011). Among the 20 different species belonging to this genus, *Pistacia vera* has been extensively studied; in particular it have been investigated its fatty acid content (Pantano et al. 2016) and their metabolites soluble in aqueous solution (Sciubba et al. 2017). This genus is also a source of extracts having promising pharmacological activity in the treatment of the Alzheimer disease. For example,

the triterpenic compound pistagremic acid, isolated from *Pistacia integerrima*, in addition to their many different biological activities (Rauf & Patel 2017), it is able to inhibit β-secretase enzyme (Rauf et al. 2015) and the ethanolic extracts prepared from fruits of *Pistacia terebinthus* and from seeds of *P. khinjuk* have showed a very high anticholinesterase activity (Hacıbekiroğlu et al. 2015). *P. terebinthus* L., is a small deciduous tree or shrub up to 5 m tall with impairpinnate, aromatic, dark green leaves. It blossoms in early spring between March and May. The inflorescences have long branches and red-brownish flowers. The fruits are globose and obovate drupes (5÷7) mm long × (4÷6) mm wide, at first of a reddish colour that turns to brown when ripe. This species is located in dry and open woods and in rocky, usually calcareous, slopes. In Sardinia it is present in a restricted, calcareous area of the East Coast (Tutin et al. 1968; Usai et al. 2006; Dogru et al. 2014). *P. terebinthus* is known as a source of turpentine and different traditional uses of this plant, in the Mediterranean area, have been reported. Leaves are consumed either fresh or cooked while the resin is used as a chewing gum and as food additive (Schoina et al. 2015). Leaves, shoots, resins and fruits have been traditionally used for the treatment of several ailments like respiratory and urological diseases, stomach ache, headache, asthma, sunstroke, rheumatism. Hypotensive, anti-inflammatory, antiseptic, antitussive, diuretic properties of *P. terebinthus* extracts have also been documented (Ozcan et al. 2009; Orhan et al. 2012; Bozorgi et al. 2013; Pulaj et al. 2016). In Sardinian, tradition *Pistacia* species are used both for dietary and for medicinal purposes (Loi et al. 2005; Sanna et al. 2006; Maxia et al. 2011). The resin of *P. terebinthus* is used as expectorant, diaphoretic, analgesic, tonic, to treat renal stones and to obtain an ointment used for the treatment of bladders (Atzei 2003). The galls are used to treat asthma and the leaf decoction is employed as expectorant (Atzei 2003). The extracts of *P. terebinthus* are also used in eczema treatment, as anti-inflammatory and as antibacterial (Topcu et al. 2007). The antimicrobial activity of *P. terebinthus* essential oil against bacteria strains (Dhifi et al. 2012; Bozorgi et al. 2013; Pulaj et al. 2016) and phyto-pathogenic fungi (Ismail et al. 2013) was previously reported. Nevertheless, to our knowledge, no studies have been carried out concerning the putative antifungal activity of *P. terebinthus* oil against fungi pathogenic for humans. The use of medicinal herbs in the treatment of skin diseases including mycotic infections is ancient practice in many parts of the world (Ugurlu & Secmen 2008). This therapeutic practice can be easily adopted even in developing countries where mycotic infections are still common diseases. Recently, the efficacy of the soap obtained from this plant in the treatment of cetuximab-induced skin toxicity has been documented (Tastekin et al. 2014). The purpose of this study was to ascertain the chemical composition and to evaluate the antifungal activity of the essential oils obtained from leaves of *P. terebinthus*, collected in Tunisia and Italy, against several yeasts and dermatophytes; concomitantly the mechanisms behind the antifungal activity against *Candida albicans* were also disclosed, with a special focus on the inhibition of the germ tube formation.

2. Results and discussion

The investigation of the leaf essential oil composition, expressed as chromatographic area percentage, of *P. terebinthus* collected in Tunisia and Italy was carried out by GC-FID and GC-MS. Twenty-nine constituents,

representing 96.2% and 97.9% of the total essential oils of *P. terebinthus* from Tunisia and Italy, respectively, were identified (Table S1). Both the essential oils were characterised by a high percentage of monoterpene hydrocarbons (86.3 and 90.9)%, followed by sesquiterpene hydrocarbons (7.5 and 3.0)% and oxygenated monoterpenes (2.4 and 4.0)% (Table S1). However, the percentages of the individual constituents differed significantly. The major components of Tunisian *P. terebinthus* essential oil were α-pinene (62.4%), β-pinene (12.1%), germacrene D (5.5%), β-phellandrene (3.8%) and camphene (3.0%). In the Italian *P. terebinthus* essential oil, the two major components were terpinolene (35.2%) and α-pinene (35.0%); other important components were β-pinene (4.5%), β-phellandrene (4.5%) and α-terpinene (3.6%). In the Tunisian sample, the concentration value of α-pinene, its main constituent, was almost twice the corresponding value of the Italian sample. In addition, terpinolene, the major constituent of the Italian oil, reached only 1.7% in the Tunisian oil. The chemical profile of our Italian sample differs remarkably from the one derived from leaves of *P. terebinthus* collected in the same area by Usai et al. (2006). According to that report, α-pinene (16.4%) and β-pinene (13.5%) were the major constituents, followed by a remarkable high concentration of β-phellandrene (8.4%) and α-terpineol (8.0%). Also our Tunisian sample composition is different from the Tunisian leaf essential oil obtained by Ismail et al. (2013) that was dominated by α-pinene (19.2%), α-terpinene (41.3%), α-terpinolene (8.0%) and δ-terpinene (4.4%). Our results are in agreement with previous studies that demonstrated a great variability in the chemical composition of essential oils depending on the location where plants have grown (Duru et al. 2003; Kıvcak et al. 2004). Recently, Pulaj et al. (2016) found that the main constituents of the essential oil, obtained from wild, Kosovan *P. terebinthus*, were α-pinene (27.2–32.8)%, d-limonene (13.9– 46.3)%, β-ocimene (0.0–40.5)%, β-pinene (2.6–20.5)%, sabinene (0.0–5.6)% and (Z)-β- ocimene (0.0–44.8)%. Environmental factors such as geography, temperature, day length, nutrients were considered to play a key role in the chemical composition of *P. terebinthus* oil. In a similar way, Mezni et al. 2014 have recently documented the influence of the geographical origin on tocopherols, carotrenoids and fatty acid composition of *P. lentiscus* fixed oil. On the other hand, Sifi et al. 2015 analysed 52 samples of essential oils from unripe galls of *P. atlantica* (male and female trees from different regions of Algeria) and found a great variability that being not related to the site of collection or to the tree gender it was ascribed to the existence of two different chemotypes. Concerning our results on Italian *P. terebinthus*, it is interesting to underline that it is the first time that terpinolene (35.2%) is designated as the major component in the oil extracted from the leaves. This compound was previously identified only in the essential oil extracted from fruits or galls of the species under investigation (Couladis et al. 2003; Ozcan et al. 2009; Pulaj et al. 2016). Evaluation of MIC and MLC of the essential oils showed a variability of inhibition among all the fungal strains tested (Table S2). Essential oil from Tunisia exhibited higher antifungal activity than the oil from Italy. It is reasonable to suppose that the higher inhibitory activity of the Tunisian oil can be related with the higher amount of α-pinene, a compound with marked activity against several fungi (Cavaleiro et al. 2006). *Cryptococcus neoformans* and the majority of dermatophyte strains showed more sensitivity to this oil when compared to *Candida* strains, particularly *Trichophyton rubrum*, *Microsporum canis* and *Epidermophyton floccosum*, with MIC and MLC values in the

range (0.16–0.32) μL/mL. Also, for each tested fungus, MIC and MLC have the same value, revealing the fungicidal activity of the oil. These results support the use of the oil from Tunisia for the treatment of dermatophytosis. In addition, this oil revealed an important inhibitory effect on germ tube formation in *C. albicans* at sub-inhibitory concentrations (Table S3). For a concentration of MIC/8 (0.16 μL/mL), the inhibition of filamentation was 100% in comparison to untreated control cells (1% DMSO without essential oil). The yeast-mycelium transition in *C. albicans* is described as an important virulence factor in this species and, importantly, it was previously reported that filamentation inhibition per se is sufficient to treat disseminated candidiasis (Saville et al. 2006). These findings indicate this oil as a good candidate for the treatment of disseminated candidiasis.

Supplementary material

The experimental section, including Tables, can be accessed as supplementary material.

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