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In vitro Antimicrobial Activities of Essential Oils from *Rosmarinus officinalis* on some pathogens.

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ABSTRACT

Rosemary (Rosmarinus officinalis L.), an evergreen plant belonging to the Lamiaceae family of herbs and spontaneously growing in the Mediterranean region. It has been reported to possess a number of therapeutic applications in folk medicines in curing or managing of a wide range of inflammatory and infectious diseases. This study was designated to evaluate the antimicrobial activity of the essential oils obtained from Rosmarinus officinalis against Coliform spp, Pseudomonas spp, Saccharomyces cerevisiae (EC1118), Zygosaccharomyces bailii (DSM 70492) and Lactobacillus plantarum (DSM2601) using agar well diffusion method. The results revealed that the oil showed moderate antibacterial activity toward all tested strains with a zone of inhibition ranging from 1 and 3 mm. Among the test microorganisms, essential oils exhibited maximum zone of inhibition against Coliform spp and L. plantarum (3 mm) and minimum zone of inhibition against all organisms – except Z. baillii – (1mm). This established a good support to the use of this essential oil in herbal medicine and as a base for the development of novel potent drugs and phytomedicine.

Keywords : *Rosmarinus officinalis* oils; antibacterial activity; organisms.

INTRODUCTION

Plants had been used for medicinal purposes long before recorded history. The World Health Organization estimated that 80% of the population of developing countries rely on traditional medicines, mostly plant drugs, for their primary health care needs (FAO, 1997). The genus *Rosmarinus* L., of the *Lamiaceae* family, as currently circumscribed encompasses only three species, namely *R. officinalis* L., *R.eriocalyx* Jordan & Fourr. and, *R.tomentosus* Huber-Morath & Maire (Segarra-Moragues and Gleiser, 2009). *Rosmarinus officinalis* is the most widespread species from this genus, which is endogenous to Europe, Asia and Africa, mainly in areas surrounding the Mediterranean Sea. However, this aromatic plant can also be found in other countries such as Argentina, Brazil, Uruguay, among others (Miguel et al.,2007; Segarra-Moragues and Gleiser, 2009). Rosemary (*Rosmarinus officinalis* L.) is of considerable importance in term of its great an important medicinal and aromatic value (Al Hussain et al., 2010). Rosemary herbs have been widely used in food, perfume, cosmetic and pharmaceutical industries (Miguel et al., 2007).

Rosmarinus officinalis essential oil is also important for its medicinal uses and its powerful antibacterial, cytotoxic, antimutagenic, antioxidant, antiphlogistic and chemopreventive properties (Al Hussain et al., 2010).

The aim of this study was to evaluation of antimicrobial effects of Rosemary (*R. officinalis* L.) essential oils against Coliform spp, *Pseudomonas* spp, *Saccharomyces cerevisiae, Zygosaccharomyces bailii* and *Lactobacillus plantarum*.

MATERIALS AND METHOD

Essential oils

We used commercial EO of *Rosmarinus officinalis* purchased from Farmalabor (Canosa di Puglia, Italy) as liquid extract.

Microorganisms used

The antimicrobial activity of *R. officinalis* EO was investigated against three strains of bacteria and two yeasts. Coliform spp and *Pseudomonas* spp belonging to the Culture Collection of the Laboratory of Applied Microbiology (University of Foggia, Italy), *Saccharomyces cerevisiae* EC1118 (Lallemand Inc.), *Zygosaccharomyces bailii* DSM 70492 and *Lactobacillus plantarum* DSM2601 were obtained from German Collection of Microorganisms and Cell Cultures (Deutsche SammLung von Mikroorganismen und Zellkulturen GmbH, DSMZ, Germany).

Evaluation of antimicrobial activity

The well diffusion assay technique was used, 10, 50 and 100 ppm of microbes cultures age 24 h were add to Petri plates and nutrient agar (except *L. plantarumin* MRS) were poured. After media were solidified, two holes were made by using a sterilized cork borer each hole was filled with 10, 50 or 100 ppm of plant extract. The control was cultured without essential oil. Plates were incubated at 37°C for the bacteria and 25°C for the yeast, for 24 h. The zones of inhibition were then recorded in millimeters.

RESULT AND DISCUSSION

The results of the in vitro antimicrobial activity of *R. officinalis* EOs estimated by the diameter of inhibition varied according to essentials oil concentrations and microorganisms strains were summarised in Table I.

Table I. Microorganisms growth in the presence of various concentrations of *R. officinalis* essential oil

	Coliform spp	*Pseudomonas* spp	*S. cereviceae*	*Z. baillii*	*L. plantarum*
10	2.5	1.5	1	2.3	3
50	3	1	2.5	2.25	2
100	1	2	2.5	2.5	1

NI: no inhibition

The diameters of growth inhibition zone ranged from 1 to 3 mm and were as follow: 1 to 3 mm

for Coliform spp and *L. plantarum*, 1 to 2 mm for *Pseudomonas* spp, 1 to 2.5 mm for *S. cereviceae*, and 2.3 to 2.5 mm for *Z. baillii*. The highest inhibition zone values (3 mm) observed against *L. plantarum* with 10 ppm and Coliform spp with 50ppm. The greatest level of resistance (the zone inhibition 1 mm) showed with Coliform spp (100 ppm), *Pseudomonas* spp (50 ppm), *S. cereviceae* (10 ppm) and *L. plantarum* (100 ppm). The essential oil showed weak or similar activity on bacteria and yeast.

For several years, essential oils of *R. officinalis* are known for their antimicrobial activity which reported in several studies (Panizzi et al., 1993; Mangena and Muyima, 1999; Angioni et al., 2004; Sacchetti et al., 2005; Santoyo et al., 2005; Bozin et al., 2007; Celiktas et al., 2007).

Interestingly, Mangena and Muyima (1999) tested essential oils of *Artemisia afra, Pteronia incana* and *Rosmarinus officinalis* against 41 microbial strains, and found that *R. officinalis* oil displayed similar antibacterial activity to *A. afra* oil. *Acinetobacter lwoffi*, *Shigella flexneri* and *S. pyogenes* showed the highest sensitivity to *R officinalis* oil. *Enterobacter aerogenes, Acinetobacter calcoaceticus, B. subtilis, Erwinia carotovora, S. aureus* and *Yersinia enterocolitica* showed a lower sensitivity to this oil.

In other study, Santoyo et al. (2005) found efficient antimicrobial activity of the essential oil of *R. officinalis* against the bacteria *S. aureus*, *B. subtilis, E. coli, P. aeruginosa, C albicans* and *A. niger* , with inhibition zones and minimal bactericidal and fungicidal concentration values in the range of 17 to 33 mm and 2.25 to 0.25 mg/ml, respectively.

Rashid (2010) used essential oils of *R. officinalis* to evaluate their activity on *B. cereus, E. coli, P. aeruginosa*. The author observed that essential oils were effective against all tested bacteria. The MIC values were 16 µg/ml for *B. cereus*, 32 µg/ml for *E. coli* and 64 µg/ml for *P. aeruginosa*. However, *P. aeruginosa* showed weak sensitivity to the oil.

Tavassoli et al. (2011) tested the essential oils of *R. officinalis* on *Leuconostoc mesenteroides, Lactobacillus delbruekii, Saccharomyces cerevisia* and *Issatchenikia orientalis*. The results indicated that the tested microbes were highly sensitive to this essential oil, mainly *L. mesenteroides* and *L. delbruekii* which their minimum inhibitory concentration values ranged between 0.5 and 1.0 mg/ml.

A good to moderate antimicrobial activity of rosemary essential oil against different microorganisms has been reported by other authors (Pintore et al., 2002; Gachkar et al., 2007; Genena et al., 2008; Al Hussain et al., 2010).

The antimicrobial activity of *R. officinalis* essential oils may be attributed to its composition. Some reports found that the mainly apolar phenolic compounds from rosemary extracts may be responsible of their antibacterial activity (Tavassoli et al., 2011).

The high antimicrobial capacity of Rosemary may be explained by the high content of phenolic compounds found in its essential oil analyzed in the present study. Activity of rosemary is mainly due to borneol and other phenolics in the terpene fraction (Tavassoli et al., 2011).

Miladi et al. (2013) attributed the antimicrobial action of *R. officinalis* essential oils to the dominant presence of 1,8-cineole. The inhibitory effects of 1,8-cineole have been recorded in a number of strains of bacteria and fungi, including *B. cereus, S. aureus, S. lutea, E. coli, P. aeruginosa, K. pneumoniae, S.epidermidis, S. enteritidis, Shigella* sp, *C. albicans, A. niger* and *A. fumigates* (Santoyo et al., 2005; Jiang et al., 2011; Ojeda-Sanaa et al., 2013; Radulović et al., 2013). However, Rashid. (2010) concluded that the antimicrobial activities of rosemary essential oils are not related only to the major compounds but also the minor components of the oil. The weak

antimicrobial activity of *R. officinalis* essential oil founded in our study could be attributed to the low contents of the active components.

CONCLUSION

In view of their antimicrobial activity, the essential oils of *R. officinalis* can be used in pharmaceutical industry for production of new synthetic agents in the treatment of the infection disease caused by these pathogens, or can be suggested as candidate natural conservation agents in the cosmetic and/or food industries.

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