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Inhibitory Effects of *Echinacea Angustifolia* Essential Oils on the Growth of Five Pathogenic Organisms: Coliform Spp, *Pseudomonas* Spp, *Saccharomyces Cerevisiae*, *Zygosaccharomyces Bailii* and *Lactobacillus Plantarum*

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Abstract: *Echinacea commonly called the Purple coneflowers, is a genus of nine species of herbaceous plants in the Family Asteraceae. Three of them are important in commerce, with the majority of wild harvest being E. angustifolia. It has been used for a variety of ailments, including toothache, coughs, colds, sore throats, snakebite, and as a painkiller. In the current study, in vitro inhibitory activity of Echinacea angustifolia essential oils were screened against Coliform spp, Pseudomonas spp, Saccharomyces cerevisiae (EC1118), Zygosaccharomyces bailii (DSM 70492) and Lactobacillus plantarum (DSM2601). Agar well diffusion assay was adopted for the study. E. angustifolia oils showed very weak antimicrobial activity against the microorganisms tested with diameter of inhibition zone not exceeding 3 mm. The highest activities were observed for Z. baillii and S. cereviceae at a concentration of 10 and 100 ppm respectively, while for the rest of the strains the diameter of inhibition zone were ranged 1 and 2.5 mm, except Coliform spp which was not affected by the presence of the essential oil at a concentration of 50 ppm. The low bacteriostatic effect of this plant essential oil against some of the most important causes of infections provides an exciting potential for the future, especially in the light of the shift away from commonly used antibiotics and the move towards more natural alternatives.*

Keywords: *Echinacea angustifolia* oils; antimicrobial activity; organisms.

1. INTRODUCTION

In the last three decades, although pharmacological industries have produced number of new-antibiotics, but microbial resistance to these drugs by microorganisms has increased because of genetic ability of the bacteria to acquire and transmit the resistance against to drugs, which are utilized as therapeutic agents (Nascimento et al ., 2000; Tajehmiri et al ., 2014). Herbal treatment is one possible way to treat diseases caused by multidrug resistant bacteria (Olukoya et al., 1993). Medicinal plants play a key role in the human health care. About 80% of the world population relies on the use of traditional medicine, which is predominantly based on plant material (Kumar, 2014). *Echinacea* belongs to the *Asteraceae*, a family important to commerce for its many medicinal and culinary herbs. There are nine species of *Echinacea* known this time, but only three are marked in the medicinal herb trade. *E. angustifolia*, *E. purpurea* and *Echinacea pallida* (Miller., 2000). *Echinacea* has a long history of medicinal use for a wide variety of conditions, mainly infections, such as syphilis and septic wounds, but also as an ‘‘anti-toxin’’ for snakebites and blood poisoning. Traditionally, *Echinacea* was described as an ‘‘anti-infective’’ agent, and was indicated in bacterial and viral infections, but the current interest in the medicinal use of *Echinacea* is focused on its immunostimulant (increasingly described as immunomodulatory) effects, particularly in the treatment and prevention of the common cold, influenza and other upper respiratory tract infections (Barnes et al ., 2005). The chemistry of *Echinacea* species is well known and caffeic acid derivatives, flavonoids, polyacetylenes, alkamides, pyrrolizidine alkaloids, polysaccharides and glycoproteins were isolated and characterized (Lucchesini et al ., 2009).

Therefore, this study was conducted to evaluate the antimicrobial activity of *Echinacea angustifolia* extracts against Coliform spp, *Pseudomonas* spp, *Saccharomyces cerevisiae*, *Zygosaccharomyces bailii* and *Lactobacillus plantarum*.

2. MATERIALS AND METHODS

Essential oils

The essential oils (EO) of *Echinacea angustifolia* were provided by a commercial company, Farmalabor (Canosa di Puglia, Italy) as liquid extract.

Tested microorganisms

To assess the antimicrobial properties of *E. angustifolia* EO, three strains of bacteria and two yeasts were used in the study: Coliform spp and *Pseudomonas* spp isolated by the Laboratory of Applied Microbiology (University of Foggia, Italy), while *Saccharomyces cerevisiae* EC1118 (Lallemand Inc.), *Zygosaccharomyces bailii* DSM 70492 and *Lactobacillus plantarum* DSM2601 were procured from the German Collection of Microorganisms and Cell Cultures (Deutsche SammLung von Mikroorganismen und Zellkulturen GmbH, DSMZ, Germany). Microbiological media were purchased from Oxoid Ltd (Basingstoke, UK) and Biolife (Milan, Italy).

Antimicrobial Activity Assay

The antimicrobial activity of the *E. angustifolia* EO was determined with the agar-well diffusion method. Cultures of microbes age 24 h were inoculated separately on the solidified Nutrient agar (except *L. plantarumin* MRS) on each Petri dish by streaking using sterilized cotton swabs. Two wells were made in the solidified agar using a sterile borer and each hole was filled with 10, 50 or 100 ppm of plant extract. The control was set in parallel without essential oil. The plates were then incubated at 37°C for the bacteria and 25°C for the yeast, for 24 h. The sensitivity of the test microbes to the extracts were determined by measuring the diameters of the zone of inhibition surrounding the wells in millimeter (mm).

3. RESULTS AND DISCUSSION

The in vitro antimicrobial activity carried out by agar-well diffusion method of the essential oil resulted in a range of growth inhibition pattern against tested microorganisms summarized in Table below.

Table. *Results of agar-well diffusion test of various concentration of E. angustifolia essential oil against bacteria and yeasts.*

		Microorganisms				
		Coliform spp	*Pseudomonas* spp	*S. cereviceae*	*Z. baillii*	*L. plantarum*
Oil concentration	10 ppm	1.5	1	2	3	1.25
	50 ppm	NI	1	2.5	1.5	1.5
	100 ppm	2.5	1.5	3	2	2

NI: no inhibition

Plants are important source of potentially useful structures for new chemotherapeutic agents. The first step towards this goal is the in vitro antibacterial activity assay (Abdel-Shafi, 2013). The *E. angustifolia* EOs showed a weak activity against all the tested microorganisms, especially for *Pseudomonas* spp, whose zones of inhibition ranged from 0 mm to 3 mm. The largest diameter (3 mm) was observed with 100 ppm of oils on *S. cereviceae* and 10 ppm on *Z. baillii* while the smallest (no inhibition) was recorded with to 50 ppm of this extract on Coliform spp. Generally, yeasts are more susceptible to the presence of EOs than the bacterial species confirming previous works (Hammerschmidt et al., 1993; Charai et al., 1995; Hili et al., 1997; Nzeako et al., 2006; Mahboubi and Kazempour, 2011). The reason why yeast is more susceptible to the extracts than bacteria is unclear but it may be that at any given time, these oils may break up the structural integrity of yeast faster than they dissociate bacteria (Nzeako et al., 2006). Among the test microorganisms, the most resistant was *Pseudomonas* spp, which correlated with previous data (Hili et al., 1997; Bergkvist, 2007). *Pseudomonas* is example of multiresistant bacteria that are becoming an alarming problem within the healthcare system (Bergkvist, 2007). Nazzaro et al. (2013) indicated that this resistance is due to the formation of exopolysaccharides that increase resistance to EOs. As shown in Table, it was found that a Gram positive bacterium (*L. plantarum*) was slightly more susceptible than

Gram negative bacteria (*Pseudomonas* spp). The weak antibacterial activity against the gram negative bacteria was ascribed to the presence of an outer membrane which possessed hydrophilic polysaccharides chains as a barrier for hydrophobic essential oils (Inouye et al., 2001). With increase in concentration of essential oil, increase in zone of inhibition was observed thus dose-dependent response was clear for essential oil, except *Z. baillii*.

As per the available literature, there is not much experimental evidence with regard to antimicrobial activities on *E. angustifolia* extract. In contrast to above obtain results, Wendakoon et al. (2012) reported that *E. angustifolia* extract did not show any antibacterial activity against *S. aureus, S. epidermidis, E. coli, P. aeruginosa* and *S. enteritidis*. Also, Izzo et al. (1995) in his study to the antibacterial activity of 68 plant extracts against eight bacteria (*Bacillus subtilis, Staphylococcus aureus, Streptococcus haemolyticus, Escherichia coli* 7075, *Klebsiella* Binns *pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhi* Z-Z) reported that Extracts of *E. angustifolia* D.C showed activity only against *Bacillus subtilis*.

Mir-Rashed et al. (2010) found that all *Echinacea* extracts tested had antifungal activity against the wild type *S. cerevisiae* S288C. Whereas, Binns et al. (2000) reported that hexane extracts of *Echinacea* variably inhibit growth of yeast strains of *Saccharomyces cerevisiae, Candida shehata, C. kefyr, C. albicans, C. steatulytica* and *C. tropicalis* under near UV irradiation (phototoxicity) and to a lower extent without irradiation (conventional antifungal activity). Sharma et al. (2008) had screened and tested six different commercial *Echinacea* extracts for their antibacterial activity against 15 different human pathogenic bacteria and two pathogenic fungi. They observed that *E. angustifolia* extracts exhibit strong growth inhibition against *Haemophilus infuenzae,* moderate activity against *Clostridium difficile* and *Legionella pneumophila* but inactive on other microorganisms (*Propionibacterium acne, Acinetobacter baumanii, Bacillus cereus, Bacillus subtilis, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Mycobacterium smegmatis, Pseudomonas aeruginosa, Candida albicans* and *Trichoderma viride*).

Similarly, Bírošová et al. (2010) studied antimicrobial activity of extracts of underground and above-ground parts of *E. angustifolia* against *Mycobacterium smegmatis, Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli* and *Salmonella Typhimurium, Alternaria alternata, Aspergillus fumigatus, Microsporum gypseum* and *Trichophyton terrestre.* Their results showed that Radix extract had the highest antimicrobial activity both against bacteria and filamentous fungi and no growth inhibition of all tested bacteria was observed at the extract from herba of *E. angustifolia*.

It was observed that the antimicrobial activity of plant extract varies from one plant to another in different studies carried out in different parts of the world. The variation in results of different researches may be due to many factors such as, the effect of climate, soil composition, age and vegetation cycle stage, on the quality, quantity and composition of extracted product, different bacterial strains and type of solvent used for extraction (Ababutain, 2011). The antimicrobial activity has been attributed to the presence of some active constituents in the extracts (Joshi et al., 2011). The chemical composition of various plant parts from the three *Echinacea* commonly used as medicines, *E. pallida* var *pallida, E. pallida* var *angustifolia* and *E. purpurea*, is well established (Binns et al., 2002). Three groups of compounds in these *Echinacea* species have pharmacological activity: the caffeic acid derivatives (CADs), the lipophilic alkamides, and the highly polar polysaccharides (Bauer, 2000; Clifford et al., 2002). In early research, Echinacoside - a caffeic acid derivative- demonstrated weak antimicrobial activity against *Staphylococcus aureus* in vitro (Stoll et al., 1950). The alkamides have shown strong inhibitory activity in vitro against *Saccharomyces cerevisiae* by disruption to the fungal cell wall/membrane complex (Cruz et al., 2014). In immunodeficient mice, treatment with *E. purpurea* polysaccharide led to enhanced production of TNF-a and enhanced cytotoxicity against *Leishmania enrietti*, and protected the mice against lethal infections with *Listeria monocytogenes* and *Candida albicans* (Goldhaber-Fiebert and Kemper, 1999). Some experts believe that the polysaccharides are primary active ingredients for immune modulating effects (Tubaro et al., 1987; Wagner et al., 1988). It appears that the immune-stimulating effects of *Echinacea* result from polysaccharides surrounding tissue cells and thereby providing protection from bacterial and pathogenic invasion (Newall et al., 1996).

The observed low antimicrobial activity of *E. angustifolia* essential oil founded in our study could be associated with the low amount of those active components.

4. CONCLUSION

This study has shown that essential oils from *E. angustifolia*, displayed inhibitory activity against the tested microorganisms to varying degree, higher against *L. plantarum* than *Pseudomonas* spp. The bioassay confirms that yeasts are more susceptible than the bacteria and Gram positive bacteria are more sensitive compared to Gram negative ones, *Pseudomonas* spp being in general the most resistant strain. Essential oils are potential agent against both bacteria and yeast. Similar experimentations can help to explore the potential role of essential oils as antimicrobial agents but requires further study.

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