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Antimicrobial Activity of Some Plant Essential Oils Compared with Propolis

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ABSTRACT

Antimicrobial activity of some plant essential oils namely, rosemary (*Rosmarinus officinalis*), ginger (*Zinger officinale Roscoe*), red pepper (*Capsicum annum L.*), marjoram (*Origanum marjorana L.*) oils and propolis were studied. About 24 different phenolic compounds were identified by HPLC which have the ability to act as antimicrobial agents. Results indicated that all examined oils and propolis were varied in their content of phenolic compounds. These oils and propolis were evaluated for their antimicrobial activities against four pathogenic bacteria strains (*Staph aureus*, *Listeria monocytogenes*, *Salmonella sp* and *E. coli*), two strains of fungi (*A. flavus* and *A. niger*) and yeast strain (*Rhodotorula sp.*). Results indicated that marjoram oil had inhibitory effect against all tested strains of bacteria, fungi and yeast. Ginger and rosemary oils affected strains of *Salmonella sp and Listeria monocytogenes*. Also, propolis had inhibitory effect against all tested strains of bacteria, fungi and yeast. In contrast, the red pepper essential oil showed no inhibition effect against any tested strain. Obtained results suggest the possibility to use the studied plant oils and propolis to control pathogens for food preservation.

Keywords: Antimcrobial, essential oils, rosemary, ginger, marjoram, red pepper and propolis.

INTRODUCTION

Food-borne diseases are considering a great problem all over world, even in advanced nations (Mead *et al*., 1999). An assortment of microorganisms also causes food deterioration which is one of the most significant concerns of the food technology. A variety of pathogenic microorganisms such as *Escherichia coli*, *staphylococcus aureus* and *Listeria monocytogenes* have been reported as the causal of food-borne disease and food deterioration (Deak and Beuchat 1996 and Betts *et al*.,1999).

In last years a great excess in the number of registered cases of food-borne disease has been observed, with 82041 registered cases in 1995 (Monitor 1996). So, there is a great attention in methods to stop this upward trend and decrease the occurrence of food poisoning. An important part of research is the improvement of new and developed procedure of food preservation. Plant extracts, including their essential oils are well established that these substances have anti-microbial effect against of bacteria, moulds and yeasts (Pai and Paltt 1995). However, it is only comparatively newly that great concern has been offered to possibility of application as food preservative substances (Smith-Plmer *et al*., 1998).

In food technology, the shelf life of food products is generally extended by the supplement of antibacterial chemical substances to these products. However, there is a necessity for natural preservative additives, which do not change the sensory characteristics of food products. Several of the essential oils are considered very good candidate for achievement that object to prevent food deterioration (Schelz *et al*., 2010).

Rosemary (*Rosmarinus officinalis L.*) originally grows in southern Europe. Its herb and oil are commonly used as spice and flavoring agents in food processing for its desirable flavor, high antioxidant activity and lately as antimicrobial agent (Ouattara *et al*., 1997; Lo *et al*., 2002). It is a complex botanical, containing over 240 medical and nutritional active compounds. Rosemary volatile oil is characterized by containig a high percentage of terpenoid oxygenated compounds like L-penene and 1.8-cineole as the major compounds, which are mainly responsible for their aromatic profiles (Celiktas, *et al*., 2007 and El-Bastawesy, *et al*., 2008).

Ginger (*Zingiber officinale*, *Zingiberaceae*) has widely been consumed as spices and food preservative. It is added to food products in a form of essential oils and various extracts (Yu *et al*., 2007).

Capsicum (red pepper) has its beginning since the beginning of civilizations. It is a part of human diet since 7500 BC (Bosland, 1996). Dorantes *et al*., (2000) have reported the inhibitory effect of pathogens by capsicum annum extract. Also, the inhibition of *salmonella typhimurium* and *Pseudomonas aeruginosa* inoculated in minced beef meat by extract of *capsicum annum* bell pepper has been recorded (Careaga *et al*., 2003).

Among several essential oils that may be useful as antimicrobial agents, marjoram oil (*Origanum majorana L.*) may have the greatest potential for use in industrial applications (Daferera *et al*., 2000; Ezzeddine *et al*., 2001).

Propolis is a rubbery and resinous substance produced by worker bees from buds of flowers, coat of trees and leave of plants. Bees used the enzymatic secretion-enriched substance to coat hive walls to secure a very clean environment. Propolis extract as a natural product have been used both inwardly and outwardly for thousands of the years as one of the cure factors in conventional medicine. The biological characteristics of propolis such as antiviral, antibacterial, antifugal and other properties have attracted the researcher's attention (Simone-Finstrom and Spivak, 2020). The precise chemical composition of propolis is coplex influenced by the phytogeographical origin, the season) of collection, and the type of bees foraging (Murad *et al*., 2002; Bankova, 2005 and Silici and Kutluca, 2005).

In general, propolis contains a variety of chemical compounds such as polyphenols (flavonoid aglycones, phinolic acids, and their esters, phenolic aldehydes, alcohols, and ketones), terpenoids, steroids, amino acids, and inorganic compounds (Moreno *et al*., 2000 and Osman, 2012).

The aim of this research is testing the antimicrobial activity of four plant essential oils against four prevalent food-borne pathogens (*Salmonella sp*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Escherichia coli*), two strains of fungi (*A. flavus* and *A. niger*) and yeast strain (*Rhodotorula sp.*).

MATERIALS AND METHODS

Materials:

Essential oils:

Four plant essential oils namely; rosemary, ginger, marjoram and red papper oils were obtained from Medical Plant and Agriculture Seeds Haraz Company. Cairo, Egypt.

Propolis:

Propolis was obtained from Egyptian Company for Production and Marketing of honey bee products. Cairo, Egypt.

Microorganisms:

Four pathogenic bacterial strains namely, *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella sp* and *E. coli* (O157:H7), two fungi strains (*A. flavus* and A. *niger*) and one yeast strain (*Rhodotorula sp*) were obtained from Food Hygiene Inspection Lab., Damietta Seaport. These strains were chosen for their economic importance regarding the human health.

Media:

`Tryptone soya agar (Oxoid, 2006, CM0131). Sabouraud dextrose agar (Oxoid, 2006, CM0041).

Methods:

Chemical analysis:

Extraction and Identificationof phenolic compounds:

Extraction of phenolic compounds of rosemary (*Rosmarinus officinalis*), ginger (*Zinger officinale* Roscoe), red pepper (*Capsicum annuum L.*) marjoram (*Origanum marjorana L.*) oils and propolis, was carried out according to the method described by Wojdylo *et al*., (2007), then determined according to the method described by Waskmundzka *et al*., (2007), which calculated as mg Gallic acid/100g of dry weight material. Phenolic compounds of studied oils were identified using High Performance Liquid Chromatography (HPLC), "HP1050" in Center Laboratory of Food Technology Research Institute, Giza, Egypt.

Preparation of propolis extract:

Ethanolic extract of propolis (EEP) which used in holes (in the plate diffusion method) was obtained by diluting 25 g crude propolis in 100 ml of 70% ethanol, and extracted at room temperature. After three days the extract was filtered (Whatman paper No. 1) and kept at refrigerator temperature at 4 °C.

Microbiological experiments:

Assessment of antimicrobial activities:

The plate diffusion method was used, holes with a cork borer were pushed in medium inoculated with a standard incolumn of 0.5 ml cell suspension of bacteria using vortex mixer (No 502550, Taiwan) under aseptic conditions. These cultivation media were tryptone soya agar for bacteria and sabouraud dextrose agar for both fungi and yeast. Each hole was filled with 10µl of the oil samples and propolis then left one hour to allow diffusion, the plates of *Salmonella sp*, *E. coli* and *Staphylococcus aureus* bacteria were incubated at 37 °C for 24-48 h. The plates of *Listeria monocytogenes* were incubated at 30 °C for 24-48 h. While, plates of fungi strains (*A. flavus* and *A. niger*) and yeast strain (*Rhodotorula sp*) were incubated at 25 °C for 4-5 days. At the end of incubation period, the inhibition zones of microbial growth were measured by ruler (as mm) and recorded (Bagamboula *et al*., 2003).

RESULTS AND DISCUSSION

Extraction and identification of phenolic compounds in plant essential oils and proplolis:

Data in Table (1) show the identification of different phenolic compounds of investigated plants oils. The obtained results indicate that phenolic acids were the most abundant compounds in all examined oils.

Table (1): Identification of phenolic compounds in plant oils compared with propolis (mg/100gm)

Phenolic compounds (mg/100gm)	Essential oils				propolis
	Rosemary	Ginger	Red pepper	Marjoram	
Gallic acid	1.56	0.60	0.61	0.60	ND
Chlorogenic	0.90	ND	1.05	0.32	5.52
Pyrogallol	ND	ND	3.32	ND	39.75
Protocatechuic	ND	ND	0.05	ND	1.72
Catechol	ND	0.19	ND	ND	21.07
Catechin	0.77	0.78	0.83	1.74	ND
Ferulic	1.05	4.17	0.41	ND	3.74
Caffeic	ND	0.36	0.53	1.01	ND
Vanillic	ND	ND	2.31	ND	1.46
P.Cumaric	ND	0.06	0.42	ND	ND
Cholchecien	ND	1.02	ND	ND	ND
Caffeine	ND	ND	1.79	ND	ND
Salicylic	2.65	1.09	ND	54.1	ND
Chrysin	1.05	0.32	0.07	ND	287.28
Cumarin	ND	ND	ND	ND	2.03
Cinnamic	ND	ND	ND	ND	142.59
Benzoic	ND	ND	ND	ND	5.45

ND: Non Detected

Results of HPLC indicate that fourteen phenolic compounds were separated, six of them were identified salicylic acid was the major phenolic compound in rosemary (2.65 mg/100gm) followed by gallic (1.56 mg/100gm) then both ferulic and chrysin (1.05 mg/100gm). More ever, chlorogenic and catechin also were detected (0.90 and 0.77 mg/100gm).

In ginger oil the main phenolic compound was ferulic acid (4.17 mg/100gm), followed by salicylic (1.09 mg/100gm) and cholchecien (1.02 mg/100gm), while there were lower amounts of catechin (0.78 mg/100gm), gallic (0.60 mg/100gm), caffeic (0.36 mg/100gm), chrysin (0.32 mg/100gm), catechol (0.19 mg/100gm) and p.cumaric (0.06 mg/100gm).

The phenolic compounds of red pepper oil were pyrogallol (3.32 mg/100gm), vanillic (2.31 mg/100gm), caffeine (1.79 mg/100gm), chlorogenic (1.05 mg/100gm), catechin (0.83 mg/100gm), gallic (0.61 mg/100gm), caffeic (0.53 mg/100gm), p.cumaric (0.42 mg/100gm), ferulic (0.41 mg/100gm), chrysin (0.07 mg/100gm) and protocatechuic (0.05 mg/100gm).

Marjoram oil is used also as natural flavoring agent, nearly five phenolic compound were identified. Data in the same table indicate that salicylic was the predominant phenolic compound (54.1 mg/100gm) followed by catechin (1.74 mg/100gm) and caffeic (1.01 mg/100gm). However, there were lower amounts of gallic and chlorogenic (0.60 and 0.32 mg/100gm, respectively).

The phenolic compounds of propolis are shown in Table (1). According to the results of HPLC analysis, the propolis sample contains about 10 identified phenolic compounds. Among these phenolic compounds chrysin (287.28 mg/100gm) followed by cinnamic (142.59 mg/100gm), pyrogallol (39.75 mg/100gm) and catechol (21.07). However, there were lower amount of protocatechuic (1.72 mg/100gm), chlorogenic (5.52 mg/100gm) and vanillic (1.46 mg/100gm).

Antimicrobial activity of plant essential oils and propolis:

The antimicrobial effect of the tested plant essential oils and propolis was assessed against *Staph aureus*, *Listeria monocytogenes*, *Salmonella sp* and *E. coli*, *A. flavus*, *A. niger* and *Rhodotorula sp* by diffusion method. Results are presented in Table (2). The results show the wide variation in the antimicrobial effect of the antimicrobial effect of the plant essential oils. From the microbial examination, it could be reported that ginger and rosemary essential oils had an effect against tested strains of *Salmonella sp and Listeria monocytogen*es.

The essential oil of red pepper as revealed in the present study showed no effects against gram-negative, gram-positive bacteria, fungi and yeast (no inhibition zone) comparing with those of other oils such as marjoram oil and propolis. These results are similar with those reported by Dorman and Deans (2000) and in contrary with those reported by Dorantes *et al*., (2000) and Careaga *et al*., (2003), which they found that there was an inhibition effect caused by the five capsainiods. It was observed that m-cumaric and cinnamic acids are responsible for the inhibitory effect of the bacteria. It can be seen that the inhibition effect against *Salmonella typhimurium* is peripheral but *Bacillus cereus* and *Staphylococcus aureus* were the most sensitive to these substances. Both of bacteria showing an inhibition zone of 10 and 9.8 mm respectively, for m-cumaric acid and 8.2 and 6 mm respectivly, for cinnamic acid. Also, there was strong inhibition of *Listeria monocytogens* by m-cumaric acid (6.2 mm) and cinnamic acid (5mm).

Table (2): Antimicrobial activity of some plant oils and propolis (inhibition zone/mm)

Essential oils and propolis (10 µl)	Examined Microorganisms						
	Gram negative bacteria (G -)		Gram positive bacteria (G+)		Fungi		Yeast
	Salmonella sp	*E.coli*	*Staph aureus*	*Listeria monocytogenes*	*A. flavus*	*A. niger*	*Rhodotorula sp*
Rosemary oil	11.50	NA	NA	15.00	NA	NA	NA
Ginger oil	10.00	NA	NA	19.50	NA	NA	NA
Red pepper oil	NA	NA	NA	NA	NA	NA	NA
Marjoram oil	15.75	15.00	12.5	16.75	13.50	13.50	17.5
Propolis	9.50	9.81	14.3	10.20	15.40	15.60	16.20

NA= non active
G -= gram negative cells
G += gram positive cells

Marjoram essential oil resulted in zones of 15.75, 15, 12.5 and 16.75 mm against gram negative bacteria *salmonella sp* and *E. coli* , gram positive bacteria *Staph aureus, Listeria monocytogenes*, respectively, and 13.5 , 13.5 and 17.5 against *A. flavus, A. niger* and *Rhodotorula sp.* So, such results indicate that marjoram oil had the superior compared with other oils. These results may be due to that marjoram oil was rich in some phenolic compounds namely salicylic, caffeic, catechin and gallic acid. Dwivedi (1990) reported that complete growth inhibition of *F. oxysporum f.sp.psidii* at 0.1% and 0.15% concentration of phenol and 0.15% concentration of salicylic acid due to strong toxicity of these compound against pathogen. Also, gallic acid has been shown to possess antibacterial activity against human pathogens (*Staphylococcus aureus*, *Corynobacterium accolans*), a plant pathogen (Fogliani *et al*., 2005). These results are in accordance with those given by Govaris *et al*., (2010) and El-Gammal and Omar (2012), which they mentioned that antimicrobial effect of marjoram oil constitutes were found to be phenols, alcohols, aldehydes, ketones, ethers and hydrocarbons can increase the antimicrobial activity and their bacteriostatic effect depending on their effectiveness concentration.

Marjoram essential oil has been tested as a natural preservative substance in food processing. Antimicrobial effects of marjoram essential oil occurs via cell membrane destroy, with envelop disruption, formation of bubble and lack of cytoplasmic substance. These changes correlate with the ability of hydrocarbons to react with hydrophobic structures, like bacterial membranes. These observations are based on examination of *E. coli* and *S. aureus* by transmission electron microscopy, which allow to studying the possible bacterial ultrastructural modifications Becerril *et al*., (2007).

Shan *et al*., (2007) studied a series of dietary spices and herbal medicines in order to confirm their antibacterial effects against pathogenic bacteria, of which infections are responsible for food toxicity. The results showed that phenolic compounds significantly participated to the antibacterial activity of the investigated herbs. This antibacterial activity may be due to the enzyme inhibition by the more oxidized phenolic compounds probably via interaction sulfhydryl compounds or via more non-specific reaction with the protein. Caffeic acid and cinnamic acid, pyrogallol and catechol were shown to be toxic to microorganisms (Cowan, 1999).

Results also, indicated that marjoram oil have also high levels of antifungal activity, it has been demonstrated that antimicrobial effect of volatile oils structural and functional damages to the bacterial cell membrane, it also, indicated that the optimum range of hydrophopicity is involved the toxicity of volatile oils (Goni *et al*., 2009).

A lot of investigations reporting the antibacterial effect of volatile oils against pathogenic bacteria accept that volatile oils are comparatively more active against Gram (+ve) than Gram (-ve) bacteria (Viuda-Martos *et al*., 2008). The cell structure of Gram (-ve) bacteria is constituted essentially with lipopolysaccharides. This structure averts the collection of oils on the membrane of bacterial cell (Bezic *et al*., 2003).

Also, the results of propolis effect as antimicrobial agent were shown in table (2). These results revealed that propolis had strong antibacterial activity against Gram (+ve) than Gram (-ve) bacteria. The largest inhibition zone was recorded against *Staphylococcus aureus* (by 14.3 mm). From these results it was observed that propolis results were nearly in accordance with that obtained by (Park *et al*., 2005), who reported that Hungarian propolis had greater inhibitory effect on growth of resistant Gram (+ve) bacteria and fungi than Gram (-ve) bacteria. Abd El Hady and Hegazi (2002) reported that *Staphylococcus aureus* were sensitive to Propolis which is in agreement with the results of the present study. Furthermore, the result of disc diffusion methods of propolis revealed that, *Staphylococcus aureus* was highly sensitive with 13 mm as zone of inhibition. In contrary with our results, the antibacterial activity of propolis was generally investigated on *Escherichia coli* by Hendi *et al*., (2011). The results confirmed a very inhibitory activity of propolis on the growth of *Escherichia coli* and these results were in complete accordance with the results of Sforcin *et al*., (2000), Drago *et al*., (2000). As a basic rule, an extract substance is conceder active against both bacteria and fungi if the zone of inhibition was greater than 6 mm (Muhammad and Muhammad, 2005). From otherwise, different researchers (Katircio and Nazime 2006; Yaghoubi. *et al*., 2007) have reported that propolis antibacterial activity is due to a number of phenolic compounds, mainly flavonoids, phenolic acids and their esters. Also, Kosalec *et al*., (2004) reported that, propolis and some of its cinnamic acid derivatives and flavonoids were responsible for uncoupling the energy transuding cytoplasmic membrane inhibiting bacterial motility, which might contribute to the antibacterial action.

Finally it could be concluded that the effective essential oils and propolis could be a natural source of reducing the total contamination level of foods beside on their effect against food pathogen microorganisms.

The obtained results suggest that, marjoram oil and propolis have a strong antimicrobial activity.

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الخواص المضادة للنشاط الميكروبى لبعض الزيوت النباتية العطرية بالمقارنة مع البروبوليس

أحمد عبدالعزيز الرفاعى*، أماليكا درويش الدهشان* ، محمد أبو طالب **و فيفى راغب أنيس محمود**
***قسم الصناعات الغذائية ـ كلية الزراعة- جامعة المنصورة ـ مصر.**
****معمل تكنولوجيا تصنيع الأسماك- المعهد القومى لعلوم البحار والمصايد- أكاديمية البحث العلمى- مصر.**

باستخدام جهاز الفصل الكروماتوجرافى (HPLC) تم تقدير المواد الفينولية للزيوت المستخلصة من بعض النباتات وهى حصا اللبان ،الزنجبيل، الفلفل الأحمر، البردقوش و البروبوليس وكانت حوالى ٢٤ مركب والتى لها القدرة أن تعمل كعوامل مضادة للميكروبات.وأظهرت النتائج ان الزيوت التى تم تحليلها وكذلك البروبوليس كانت مختلفة فى محتواه من المركبات الفينولية. كما تم تقدير النشاط الميكروبى لهذه الزيوت النباتية الطيارة وكذلك النشاط الميكروبى للبروبوليس على أربعة سلالات من البكتريا (*Staph aureus*, *Listeria monocytogenes*, *Salmonella sp* and *E.coli*) وسلالتين من الفطر (*A. flavus*, *A. niger*) وسلالة من الخميرة(*Rhodotorula sp*) وقد أظهرت النتائج ان كلا من زيت البردقوش أعطى أعلى فاعلية تجاه جميع سلالات البكتريا والفطريات والخميرة المختبرة تلاه زيت الزن9+جبيل وحصا البان حيث كان له تأثير على بكتريا *Listeria monocytogenes*, *Salmonella sp* كما أظهر البروبوليس تأثير واضح على جميع السلالات الميكروبية المختبرة وعلى العكس فلم يظهر الفلفل الأحمر أى تأثير على أى من السلالات المختبرة سواء كانت البكتريا أو الفطريات أوالخميرة لذلك نقترح من النتائج التى تم الوصول إليها إمكانية إستخدام الزيوت النباتيه الطيارة محل الدراسة التى أظهرت تأثير على السلات المختبرة وكذلك البروبوليس فى مجال حفظ الأغذية والحد من نمو الميكروبات الممرضة للإنسان.