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Determination of Phenolic compounds, Antimicrobial activity and Antioxidant Potential of Volatile Extracts Isolated from Various Spices

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Abstract:

Plants are potentially useful sources of aromatic, phenolic, antimicrobial and antioxidants compounds. The total aromatic contents, phenolic compounds and antioxidant activities of extracts from four aromatic plants (*Cinnamomum zeylan* blume, *Eiettaria Cardamorum* motan, *Citrus Aurantium* l and *Galanga Galangal Alpinia officinarum)* have been determined. Antibacterial activity was assayed against a variety of human pathogenic bacteria (Escherichia coli, Bacillus subtillus and Alternaria tenuis) and antifungal activity was assayed against Penicillum chrysogenum by Disc Diffusion Method.

Cinnamomum zeylan blume had the highest phenolic contents and the highest content of total volatile components. Tannins, Vit. E, Carotene and Vit. C recorded the highest values (300.2 mg / 100 ml sample, 0.95 micro Mol/ ml sample , 0.21 mg/ 100 ml sample and 7.2 mg/ 100 ml sample) for *Citrus Aurantium l, Cinnamomum zeylan blume, Eiettaria Cardamorum motan and Citrus Aurantium* l ; respectively. *Galanga Galangal Alpinia officinarum* recorded the lowest values in all components. These extracts showed good activity against the growth of food pathogenic Bacteria *(Escherichia coli* and *Bacillus subtilis)*. The four extracts did not inhibit an Alternaria tenuis or Penicillum chrysogenum.

The results of this study showed that *(Cinnamomum Cassia blume, Eiettaria Cardamorum maton, Citrus Aurantium* l and *Alpinia officinalis* L) extracts can be used as natural antimicrobial and antioxidant agents.

Keywords: Antioxidants, Linoleic acid; Medicinal plants; Phenolic content; antibacterial activity.

Introduction:

Plants have been utilized to treat disease and disease symptoms for their biologically active phytochemicals. Flavonoids have variety of pharmacological effects including antioxidant, anticancer, anti-inflammatory, hepatoprotective and immunostimulant (Harborne and Williams (2000), Benavente-Garcia *et al*., (997), Middleton et al., (2000) and Manthey et al., (2001)). Moreover, studies revealed that flavonoid rich diet reduces cho-

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lesterol and improves conditions of cardiovascular system (Wilcox *et al.,* (1999)). Elevated demand on phytochemical remedies open the search for flavonoid sources for the mass production of flavonoid compounds to use in commercial preparations. For this purpose plants containing high levels of flavonoids were preferred for the mass extraction. There are limited plants, which produce desired levels of flavonoids. Collecting plants from wild that produce flavonoids, and utilizing them for direct extraction is easier in short term but it may cause to reduction of the origin even though it may put the plant in danger of extinction (Ufuk Koca 2007)

Alpinia officinarum Hance (small galanga) is a wellknow pungent and aromatic plant whose rhizoma is frequently used as a medicinal herb in China. Its root contains volatile oil, resin, galangol, kaempferid, galangin and alpinin. The active components are the volatile oil and acrid resin. Galangin is dioxyflavanol and has been obtained synthetically. Many biological activities of galangal have been reported which include antitumor, antiulcer, antibacterial and antifungal properties. Some glycosides and anti-oxidative compounds in small galanga have been separated and identified (Ly *et al*., 2002, 2003).

Citrus fruits and juices are an important source of bioactive compounds including antioxidants such as ascorbic acid, flavonoids, phenolic compounds and pectins that are important to human nutrition (Fernandez- Lopez *et al.,* 2005; Jayaprakasha and Patil, 2007; Ebrahimzadeh *et al.,* 2004). Epidemiological studies on dietary Citrus flavonoids improved a reduction in risk of coronary heart disease (Di Majo et al., 2005; Hertog *et al.,* 1993) and are attracting more and more attention not only due to their antioxidant properties, but as anti-carcinogenic and anti-inflammatory agents because of their lipid anti-peroxidation effects (Stavric, 1993; Martın et al., 2002). The interest in these classes of compounds is due to their pharmacological activity as radical scavengers (Cotelle *et al.,* 1996). Several studies have demonstrated the antibacterial and/or antioxidant properties of these plants, mainly using in vitro assays. (Dean and Svoboda, 1989; Farag *et al.,* 1989). In addition, Citrus byproducts also represent a rich source of naturally occurring flavonoids (Horowitz, 1961).

Cinnamon has been a favorite spice around the world not only because of its health benefits but also because it flavors and preserves food. Cinnamon (Cinnamomum zeylan) is employed in aromatherapy as a rub to promote blood circulation. It also contains both anti-fungal and anti-bacterial principles that can be used to prevent food spoilage due to bacterial contamination (Fabio *et al.,* 2003). Some of the plant constituents have shown effects against fungi,

including the molds that produce the carcinogenic aflatoxins and 80% bacterial activity has been found (McCann, 2003). Even though cinnamon has antibacterial effects, clinical trials against Helicobacter pylori, associated with gastric ulcer, have shown contradictory results. It is proven to be particularly effective against some species of toxicogenic fungi as well as respiratory tract pathogens, including species belonging to the genera *Aspergillus, Candida, Cryptococcus and Histoplasma* (Inouye *et al.*, 2001).

Natural antimicrobial agent derived from sources such as plant oils have been recognized and used for centuries in food preservation Essential oils and spices were used by the early Egyptians and have been used for centuries in Asian countries such as China and India. Some of the spices, such as clove, cinnamon, mustard, garlic, ginger and mint are still applied as alternative health remedies in India. Essential oils production can be traced back over 2000 years to the Far East, with the beginnings of more modern technologies occurring in Arabia in the 9th century. However, it was also during this time period that the medical applications Essential oils became secondary to their use for flavor and aroma.

Plant extracts and spices, in addition to contributing to taste and flavor, can act against Gram-positive pathogens such as Listeria monocytogenes. They can also enhance storage stability by means of active componts including phenols, alcohols, aldehydes, ketones, ethers and hydrocarbons, especially in such spices as cinnamon, clove, garlic, mustard, and onion.

It has been demonstrated that antimicrobial effect of the Essential oils acts by causing structural and functional damages to the bacteriral cell membrane. It is also indicated that the optimum range of hydrophobicity is involved in the toxicity of the Essential oils (Goni *et al.*, 2009).

Objectives of this study were to investigate and comparison of four aromatic plant species *(Cinnamomum zeylan blume, Eiettaria Cardamorum motan, Citrus Aurantium l and Galanga Galangal Alpinia officinarum)* by determination of their aromatic and phenol compounds and their biological activated substances, studying the effect of their water-alcoholic extracts on antibacterial and fungicidal activities and antioxidant activity of spices extracts compared to α tocopherol activity

2. Materials and Methods:

2.1. Preparation of Extracts:

Four medicinal plant materials *(Cinnamomum zeylan blume, Eiettaria Cardamorum motan, Citrus Aurantium l and Galanga Galangal Alpinia officinarum)* were purchased from the local Herbal market in Jordan and were authenticated by National Center for Agricultural Research and Extension (NCARE) Jordan. All spices were washed under tap

water to remove the dirt and soil and dried separately in a vaccum drier at 25°C for 24 h and size reduced to coarse powder using a cutter mill. Powders of each rhizome (500 g) were extracted separately with petroleum ether, chloroform, methanol and water by Soxhlet extraction technique successively (Sunilson et al., 2009). All the extracts were concentrated using rotary vaccum evaporator and kept in dessicator until further studies.

2.2-Determination of Total Volatile Components in Extract.

The mass of each concentrate was determined according to the method reported by Lee and Shibamoto (2001a) and Lee & Shibamoto (2001b). Each concentrate was analyzed by gas chromatography (GC), using a flame ionization detector (FID). The total mass of volatile components was calculated by multiplying the percentage representing the total peak area of components by the total mass of extract

2.3 .Determination of total phenolic contents.

The total phenolic contents of each extract were determined using the Folin–Ciocalteu reagent (Zhou & Yu, 2006). The reaction mixture contained 50 ml of extract, 250 ml of the Folin–Ciocalteu reagent, and 0.75 ml of 20 g/100 ml sodium carbonate and 3ml of pure water. After 2 h of reaction at ambient temperature, the absorbance at 765nm was measured and used to calculate the phenolic contents using gallic acid as a standard. The total phenolic contents were then expressed as gallic acid equivalent (GAE), in mg/g dry sample.

2.4-Selection of Microorganisms:

Stock cultures of three different species of food-borne bacteria such as *Escherichia coli, Bacillus subtillus, Alternaria tenuis and (Penicillum chrysogenum) as* one of fungi species were obtained from the microbiology laboratory, Mutah University,Jordan. The food-borne pathogens were maintained separately in different stock culture for bacteria (nutrient broth) and fungi (sabouraud dextrose broth). A loop full of each culture was inoculated individually into the respective agar broth and incubated at 37±1 °C for 24 h for bacteria and 28±1 °C for 48 h for fungi. Anbu Jeba Sunilson *et al.,* (2009).

2.5-Antibacterial Assay by Disc Diffusion Method:

All the extracts were concentrated and dissolved in dimethyl sulfoxide (DMSO) to a final concentration of 10 mg mL^{-1} and sterilized by filtration through 0.45 um millipore filters. Screening of antimicrobial activity was carried out by disc diffusion method as described by Munay *et al.* (2007) using 100 uL of suspension containing 10^8 colony forming unit (cfu). of bacteria, 10^4 spore mL^{-1} of fungi spread on nutrient agar and SDA medium, respectively. Discs of 6 mm in diameter were impregnated with 10 uL of the extracts (300 ug

disc^{-1}) at a concentration of 100 ug mL^{-1} and placed on the inoculated agar. Ofloxacin (10 ug disc^{-1}) and fluconazole (10 ug disc^{-1}) were used as reference standards for the antibacterial and and fungal activities, respectively (Karaman *et al.,* 2003; Amerjothy *et al.,* 2007). All the inoculated plates were incubated at 37±1 °C for 24 h for the bacteria and at 28±1°C for 72 h for fungus. The zones of inhibition were measured for determining the antimicrobial activity and the findings were tabulated.

2.6- Determination of antioxidant activity:

The antioxidant activity of plant extracts against peroxidation of linoleic acid was determined by the method described by Furuta et al., (1997). Alpha-tocopherol was used as reference compound. For a typical assay an aliquot of 20 µl of three dilutions of each extract in ethanol (0.002, 0.02 and 0.2 mg/ml) and 20 µl of 2 mg/ml linoleic acid in ethanol were used. A spectrofluorimeter (Model RF-5000, Schimadzu, and Kyoto, Japan) at an excitation wavelength of 515 nm and an emission wavelength of 555 nm was used for measurements .The antioxidant activity was calculated as the percent of peroxidation inhibition. All extracts and reference substance were assayed in triplicates and averages of results were calculated. A percent inhibition versus log concentration curve was plotted and the concentration of sample required for 50 % inhibition was determined.

2.7- Statistical Analysis:

Data are expressed as Mean±SEM of triplicates.t-test was used to compare the antimicrobial activity of the extracts against the standard antimicrobial agents. All statistical analysis were conducted with SPSS software (V. 12, SPSS, USA) at significant levels of 0.05, 0.01 and 0.001 (Sunilson *et al*., 2008).

3.Results and discussion:

3.1.Total Volatile content and phenolic compounds of aromatic plant extracts :

Aromatic substances content, phenolic compounds mg/100ml sample (chlorogenic acid, Flavonol acids and catechine polyphenolic), Tannins mg / 100 ml, Vit. E micro Mol/ ml, Carotene micro Mol/ ml and Vit. C mg/ 100 ml are presented in Table (1).

The yields of total volatile components for *Cinnamomum zeylan blume, Eiettaria Cardamorum motan Citrus Aurantium l and Galanga Galangal Alpinia officinarum* were 154.2; 138.7; 128.0 and 120.0; respectively. It could be noticed from that *Cinnamomum zeylan* Blum had the highest content of total volatile components.

Chlorogenic acid contents mg/100ml sample were 280.4; 257.4; 146.4 and 200.6 however Phlavonol acids contents mg/100ml sample were 27.0; 51.9; 86.4; 74.6. On the other hand the contents of catechine polyphenolic mg/100ml sample

were 146.2 ; 61.4 ; 56.7 ; and 33. 8 for *Cinnamomum Cassia blume, Eiettaria Cardamorum maton, Citrus Aurantium l and Alpinia officinalis* L; respectively. The tabulated data showed that Cinnamomum Cassia blume had the highest contents of chlorogenic acid and catechine polyphenolic while the highest content of Phlavonol acids was found in Citrus Aurantium l.

These results are in good accordance with Kwang & Takayuki (2002), findings. Phenolic compounds contribute to the overall antioxidant activities of herbs and spices. Generally, the mechanisms of phenolic compounds for antioxidant activity are inactivating lipid free radicals and preventing decomposition of hydroperoxides into free radicals.

As shown in Table (1) the highest values of Tannins, Vit. E, Carotene and Vit. C were 300.2 mg / 100 ml sample, 0.95 micro Mol/ ml sample 0.21 mg/ 100 ml sample and 7.2 mg/ 100 ml sample for Citrus Aurantium l,; Cinnamomum zeylan blume, Eiettaria Cardamorum motan and Citrus Aurantium l ; respectively. Galanga Galangal Alpinia officinarum recorded the lowest values in all these contents.

Table (1): Total Volatile content and phenolic compounds of aromatic Plant extracts

Aromatic plant extracts	Aromatic substances	Phenol compound ,mg / 100 ml			Tannins mg / 100 ml	Vit. E micro Mol/ ml	Carotene micro Mol/ ml	Vit. C mg/ 100 ml
		(chlorogenic acid)	Phlavonol acids	catechine polyphenolic				
Cinnamomum zeylan blume	154.2	280.4	27.0	146.2	300.0	0.95	0.16	2.0
Eiettaria Cardamorum motan	138.7	257.4	51.9	61.4	250.2	0.94	0.21	4.2
Citrus Aurantium l	128.0	146.4	86.4	56.7	300.2	0.88	0.11	7.2
Galanga Galangal Alpinia officinarum	120.0	200.6	74.6	33.8	150.3	0.58	0.08	1.9

3.2 . Effect of water-alcoholic extracts from phytoadditives spices on antibacterial and fungicidal activities :

Table (2) shows the antibacterial activity against four pathogenic bacteria spices *(Esherichia coli, Bacillus Subtilis and Alternaria tenuis)* and antifungal avtivity against *Penicillum chrysogenum* as pathoginic fungi of four aromatic plant extracts (Cinnamomum Cassia blume, Eiettaria Cardamorum motan, Citrus Aurantium l and Alpinia officinalis L) presented as Zone of Growth Inhibition, mm. The zones recorded included the size of filter paper disc (6 mm in diameter) and potency for each disc was 10µl.

The results showed that the Cinnamomum *Cassia blume* extract posses great antimicrobial properties (18.0±1.1) against both of **Esherichia coli and Bacillus subtilis** because of cinnamaldehyde is major volatile and divers constituent present in it and also has variety of active components viz., eugenol, cinnamic acid, weitherhin, mucilage, diterpenes, proanthocyanidins. These active constituents posses both anti-fungal and antibacterial properties that could be used as a medicine to prevent the human health effecting disorders. (Bown, 1995).These results are similar with those reported by Ates and Eradogrul (2003) about *Cinnamomum cassia* that observed antimicrobial effect of essential oil of cinnamon and found remarkable inhibition against variety of tested bacterial and fungal strains.

The aqueous decoction of *Cinnamomum cassia* showed high antibacterial activity against *Streptococcus oralis* and *Streptococcus sanguis* (23mm), *Micrococcus roseus* (21mm), *Streptococcus intermedius* (20mm) and *Streptococcus mutans* (17mm), while *Pseudomonas aeruginosa*, *Salmonella typhi para A*, *Salmonella typhi para* B, *Klebsiella ozaenae*, Citrobacter sp., *Enterobacter aerogenes*, *Staphylococcus aureus*, *Plesiomonas shigelloides* and *Alcaligens* sp., were not inhibited. (Biavati *et al*., (1997)). It is noticeable that the four *extracts* did not inhibit an *Alternaria tenuis* or *Penicillum chrysogenum.*

Results reported in the present study contribute to the knowledge of the antimicrobial effect of *Cinnamomum cassia,* as well as confirm their potential application in the treatment and prevention of diseases caused by bacterial flora. Therefore, this study represents an inexpensive or cost effective source of natural mixtures of antibacterial compounds that exhibit potentials for use in food systems to prevent the food borne bacteria and extend the shelf life of the processed food. In addition, it may be effective in reducing the number or preventing the growth of pathogenic bacterial flora e.g., ***Escherichia Coli and Bacillus subtilis.***

The main component of galangal extract, has been previously reported to have an antibacterial

Activity against S. aureus (Gachkar et al., 2007).

Antimicrobial and antioxidant activities of plant extracts correlated well with their phenolic fraction (Puupponen-Pimia¨, et al., 2005).

Table (2) Effect of water-alcoholic extracts from phytoadditives spices on antibacterial and fungicidal activity

Aqueous-Alcoholic Extracts	Zone of Growth Inhibition, mm			
	Esherichia coli	*Bacillus subtilis*	*Alternaria tenuis*	*Penicillum chrysogenum*
Cinnamomum Zeylan blume	18.0±1.1	17.0±0.7	–	–
Eiettaria Cardamorum motan	15.1±0.8	14.5±0.5	–	–
Citrus Aurantium l Golanga galangal	13.0±0.5	7.5±0.4	–	–
Alpinia officinaram	7.0±0.3	6.5±0.3	–	–

3.3. Antioxidant activity of phytoadditives extaracted from natural spices compared with α tocopherol activity

Antioxidant activity of phytoadditives extaracted from natural spices compared with α tocopherol activity expressed as Rate of linoleic acid Oxidation are shown in Table (3).

As seen in Table (3), the antioxidant activities of four plant extracts *(Cinnamomum Cassia blume, Eiettaria Cardamorum maton, Citrus Aurantium l and Alpinia officinalis* L) were 0.40, 0.45, 0.50 and 0.57, respectively, where it was 0.95 for α-*tocopherol Alpinia officinalis* L extract exhibited stronger antioxidant activity than did other extracts. Phenolic compounds and some of their derivatives are very efficient in preventing autooxidation. The antioxidant activities of phenolic compounds are mainly of redox properties, which include free radical scavenging, hydrogen donating and singlet oxygen quenching. Since Indian gooseberry is rich in tannins and phenolic compounds, these compounds acted effectively as the antioxidant agents. The better antioxidative results might also correlate to the profile of phenolic contents in *Alpinia officinalis* L Indian gooseberry extract as well.

Table (3) Antioxidant activity of phytoadditives extaracted from natural spices Compared with α tocopherol activity.

Aqueous-Alcoholic Extracts Zeylan	Rate of linoleic Acid Oxidation V • 103, • mol/dm^3
Cinnamomum *Cassia blume*	0.40
Eiettaria *Cardamorum* motan	0.45
Citrus Aurantium l	0.50
Golanga galangal Alpinia officinalran L	0.57
α-tocopherol	0.95

The present study provides additional data for supporting the use of *(Cinnamomum Cassia blume, Eiettaria Cardamorum maton, Citrus Aurantium l and Alpinia officinalis* L) extracts as natural antimicrobial and antioxidant agents. Future work will be performed to encapsulate these extracts in edible films to prepare active packaging materials, which can release antimicrobial and antioxidant agents to extend the shelf life of foods.

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تقدير المركبات الفينولية ، والنشاط المضاد للميكروبات ومضادات الأكسدة في المستخلصات المتطايرة المعزولة من التوابل المختلفة

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تعتبر النباتات مصادر مفيدة للمركبات العطرية والفينولية،المضادة للميكروبات وكذلك المضادة للأكسدة .وقد تم تقدير المحتوى الكلي للمركبات العطرية والفينولية، وكذلك النشاط المضاد للاكسدة في مستخلصات اربعة انواع من النباتات العطرية شائعة الاستخدام بالاردن. كذلك تم تقدير النشاط المضاد للبكتيريا المرضية والمضاد للفطريات.

وقد أظهرت النتائج ان نبات القرفة إحتوى على أعلى نسبة من المواد الفينولية وكذلك أعلى نسبة من المركبات المتطايره الكلية . وقد سجلت نسبة كل من التانينات ، فيتامين E ،والكاروتين ، وفيتامين C أعلي قيم في العينات موضع الدراسة ، عدا عينات الخولجان التي سجلت أدني قيم لكافة المكونات .

أظهرت المستخلصات النباتية موضع الدراسة نشاطا جيدا ضد البكتريا المرضية ، بينما لم يكن لها تأثير مضاد لنشاط الفطريات .

وقد أوضحت الدراسة أن المستخلصات النباتية المستخدمة في الدراسة يمكن أن تستعمل كمضادات طبيعية للميكروبات المرضية ، وكذلك كمضادات أكسدة طبيعية للأغذية .