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Comparative Study of Composition, Antimicrobial and Antioxidative Properties of Cinnamon Bark Oil and Curry Leaf Oil

Humeera Tazeen*, N. Varadharaju and Z. John Kennedy

Department of Food and Agrl. Process Engg, Post-Harvest Technology Centre, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India
**Corresponding author*

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A B S T R A C T

Antimicrobial and antioxidant effects of essential oils from *Murraya koenigii* commonly known as curry leaf oil and *Cinnamon cassia* cinnamon bark oil were determined. These essential oils were taken at concentrations of 1, 1.5, 2, 2.5 and 3%. Antimicrobial activity was tested for both the oils were tested against 6 microbial strains of two gram positive, 2 gram negative and one yeast and mold. The antioxidant activity of the essential oils was determined by measuring in terms of ascorbic acid equivalent (µg/mL). Also GCMS analysis was done to check the chemical constituents present. On the basis of evaluation, it was concluded that cinnamon bark oil had more potential against microbial strains and exhibited more antioxidative property followed by that curry leaf oil.

Introduction

Essential oils from plants serve as flavoring agents in food products, and because of their chemical compositions consisting of antimicrobial and antioxidative compounds, they possess potential as natural preservatives for food (Conner, 1993; Wichtl, 1994).

The antimicrobial and antioxidative activity of plant oils and their derivatives has formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies. Phenolics and terpenoids are the major contributors to antimicrobial as well as antioxidant effects of essential oils on foods. Essential oils that contained a high percentage of monoterpenes, eugenol, cinnamaldehyde, thymol and carvacrol had been reported to have strong antibacterial activities (Lis-Balchin and Deans, 1997; Ouattara *et al.*, 1997). Apart from phenolics and terpenoids, essential oils contain a complex mixture of numerous compounds from various parts of the plants. Some of the main groups found in essential oils include alcohols, aldehydes, esters, ethers, ketones (Orav, 2001). Each of the group consists of numerous compounds. For example, terpenes include monoterpenes, diterpenes, sesquiterpenes, sesquiterpene lactones, etc,

which are an important class of volatile constituents and may have bioactive properties (Orav, 2001). Ozkan *et al.,* (2003) like antioxidative and antimicrobial properties.

Consumers demand lesser use of chemicals on minimally processed food products, thereby more attention has been paid to the search for naturally occurring substances able to act as alternative antimicrobials and antioxidants. Shahidi *et al., (*1992) stated that beside their antimicrobial activity, essential oils and various plant extracts have been widely evaluated to be used as natural antioxidants. Meena and Sethi (1994) studied antimicrobial activity of essential oils extracted from commonly used spices. Plant essential oils and their derivatives are becoming more popular as naturally derived antimicrobial agents. Studies have shown that essential oils of oregano (*Origanum vulgare*), thyme (*Thymus vulgaris*), lemongrass (*Cymbopogon citratus*), and clove (*Eugenia caryphyllata*) are among the most active against strains of *E. coli* (Smith *et al.,* 1998; Hammer *et al.,* 1999; Dorman and Dean, 2000). Cinnamon oil and its active compound (cinnamaldehyde) also have been tested for their inhibitory activity against *E. coli* (Helander *et al.,* 1999; Friedman, 2004). However, the most of the studies have focused on the activity *in vitro*, and only very few authors have documented their antimicrobial activity on food products (Ouattara *et al.,* 2000; Ruberto and Baratta, 2000) tested about 100 pure components of essential oils for their antioxidant effectiveness and found that phenols possess the highest antioxidant activity. The function was due to the presence of hydroxyl groups in their molecules.
Lambert *et al.,* (2001) stated that the antimicrobial activity of essential oils is considered to be by one (or more) of the proposed mechanisms. The proposed mechanisms include (1) interference with the phospholipids bilayer of the cell membrane, causing increased permeability and loss of cellular constituents; (2) interference with activity of variety of enzyme systems, including those involved in the production of cellular energy and synthesis of structural components; and/or (3) damaging of genetic material.

Essential oil of *Murraya koenigii* commonly known as curry leaf oil and *Cinnamon cassia* cinnamon bark oil are the aromatic liquids extracted from the plants. They are majorly used in culinary as flavoring agents as well as in some traditional medicines preparations. These oils are proved to have cholesterol lowering effect, anticancer activity, antimicrobial activity, antioxidant activity etc., and are very commonly available in most parts of India. Hence in this study, a comparative study is carried out to accesses the chemical composition, antimicrobial effect and antioxidative properties of curry leaf oil and cinnamon bark oil.

Materials and Methods

Essential oil, chemicals and media cultures

The essential oils, curry leaf oil and cinnamon bark oil were procured from M/s. Synthyte Industries, Kochi, India. All other chemicals for the study were of laboratory grade (Sigma-Aldrich, India) and used without further purification. Media cultures were purchased from MTCC-microbial Type Culture Collection and Gene Bank, Chandigarh, India and NCIM- National Collection of Industrial Microorganisms, Pune, India.

GCMS analysis of essential oils

GC-MS analyses of essential oil samples were performed on GC-MS Model: TRIPLUS

RSH, Thermo Fisher Scientific. The specification of column used in GC-MS was TR5MS, 0.25mm ID, 0.25uf, 30mm dimension. The temperature of ion source in DSQ II was maintained at 2000°C. Initial temperature in Trace GC Ultra was set to 400°C withhold time of one min. Temperature in inlet and MS transfer line was maintained at 207° C and 265°C, respectively. Full scanning mode was used with scan rate of 500 amu/s and mass range 15 to 120. Manual injection of sample was done in the sampling port in an air tight syringe. The volume of sample injected was 50 µl. Pre injection and post injection flush was given using nitrogen gas to avoid contamination. The time for pre injection and post injection flushing was 5 s and 30 s, respectively.

Antimicrobial activity of essential oils against various microbes

The inoculums for the experiment were prepared in fresh Nutrient broth for bacteria and Sabouraud's broth for yeast and mold from preserved slant cultures. Cotton wool swab were prepared and sterilized by autoclaving. Sterilized forceps by dipping in alcohol and burning off the alcohol were used.

The standardized inoculums were inoculated in the Petri plates prepared earlier and sterile swabs were used to spread the culture on top of petri plate to ensure uniform spreading of culture after which the inoculums were left to dry at room temperature with the lid closed. Each Petri dish was divided into 6 parts, on top of which sterile discs (discs are soaked overnight in essential oils) of different concentrations (1, 1.5, 2, 2.5 and 3 percent) along with standard Ciprofloxacin (10µg), were placed with the help of forceps. Then Petri dishes were placed in the incubator at 37 º C for 24 hours. Observation of zone of inhibition produced by different samples were measured and recorded.

Total antioxidative property of essential oils- phosphomolybdenum method

The antioxidant activity of curry leaf oil and cinnamon bark oil was evaluated by the phosphomolybdenum method according to the procedure described by Prieto *et al.*, 1999. The analysis is based on the reduction of M_o (V_I)–M_o (V) by the extract and subsequent formation of a green phosphate/M_o (V) complex at acid pH. A 0.3 ml sample of curry leaf oil and cinnamon oil were taken separately in different test tubes. Each was combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate).

In case of blank 0.3 mL of ethanol was used in place of extracts. The tubes containing the reaction solution were covered with aluminum foil and incubated in a hot water bath at 95°C for 90 min. After which the test tubes were cooling to room temperature, and absorbance of the solution was measured at 695 nm using a spectrophotometer. The antioxidant capacity of each sample was expressed as ascorbic acid (A.A) equivalent using the following linear equation established using ascorbic acid as standard: [$A = 0.0105\ C + 0.0125$; $R^2 = 0.986$] where A is the absorbance at 695 nm and C the concentration as ascorbic acid equivalent (µg/ml) (Fig. 1).

Results and Discussion

The results got by GC-MS analysis leads to the quantitative estimation of various numbers of compounds from the GC fractions of *Murraya Koenigii* oil and *Cinnamon cassia* (Bark) oil. These compounds were identified through mass spectrometry directory attached with GC. The various major bioactive chemical compositions of both curry leaf oil and cinnamon bark oil are presented in table 1 and the same is shown in figure 2 (a) and (b).

Antimicrobial activity of essential oils against various microbes

All the cultures related to the study were procured from MTCC and NCIM with the following catalogue numbers are given in table 2.

Disc diffusion method was used to evaluate the antimicrobial property of curry leaf oil and cinnamon bark oil against 6 different strains of microbes as listed in table 2.

Minimum inhibition zones formed against 6 different microbes were recorded and presented in table 3. It can clearly be seen that cinnamon bark oil had more inhibition zone formation compared to curry leaf oil against all the 6 strains of microbes. Both the essential oils showed inhibition activity for all the 6 strains of microbes for different concentrations ranging from 1 to 3 percent. The largest inhibition zones in case of curry leaf oil was found against *Bacillus substilis*, *E. coli* and *Saccharomyces cerevisiae*.

Where as in cinnamon bark oil, highest inhibition zone was formed in *Salmonella paratyphi* followed by *Aspergillus niger*, *Saccharomyces cerevisiae* and *Staphylococcus aureus.* Figure 3 shows inhibition zone against different microorganisms for essential oils.

Table.1 Chemical composition of curry leaf oil and cinnamon (bark) essential oils

	Relative area %		
Components	**Curry leaf oil**	**Components**	**Cinnamon bark oil**
α-Pinene	1.77	à-Pinene	1.25
α- Thujenene	0.05	Camphene	0.01
á-Myrcene	0.74	Sabinene	0.02
á-Ocimene	0.25	ç-Terpinene	0.57
α- Linalool	0.13	α- Terpinene	0.57
α- Terpineol	0.12	Cineol	5.02
Caryophyllene	32.04	Cinnamaldehyde,(E)	0.24
Humulene	6.44	.alfa.-Copaene	0.55
Spathulenol	0.60	Caryophyllene	1.79
Cubenol	0.60	ç-Muurolene	0.55
Caryophyllene oxide	4.20	cis-à-Bisabolene	0.16
á-Phellandrene	0.05	Benzaldehyde	0.43
3-Carene	28.15	cis-linool oxide	0.02
Longiborneol	0.59	trans-Linalool oxide	0.02
Linalyl acetate	0.13	α- Linalool	3.62
ç-Gurjunenepoxide	0.60	Phenylethyl Alcohol	0.79
Alloaromadendrene	2.17	3-phenyl Propanal	0.24
Aromandendrene	2.17	à-Terpineol	2.16
ç-Muurolene	1.00	Borneol	0.03
Globulol	0.60	Cinnamyl acetate	0.39
Selina-6-en-4-ol	0.88		

[a] Identification based on retention time authentic sample and mass spectrum data.
[b] Trace: relative area % is less than < 0.05%.

Table.2 Media cultures and respective catalogue numbers

CULTURE	Number
Bacillus subtilis	NCIM 2063
Staphylococcus aureus	NCIM 2079
Salmonella paratyphi	NCIM 2501
Escherichia coli	NCIM 2065
Saccharomyces cerevisiae	MTCC 1150
Aspergillus niger	MTCC 1344

Table.3 Minimum inhibitory concentrations (MICs) of curry leaf and cinnamon essential oils at different concentrations against 6 different micro-organisms

Culture	Con (%)	Zone in inhibition (mm)	
		Curry leaf oil	Cinnamon bark oil
Bacillus subtilis (Gram positive)	1.0	13	10
	1.5	14	12
	2.0	14	13
	2.5	16	13
	3.0	16	13
Staphylococcus aureus (Gram positive)	1.0	9	13
	1.5	10	13
	2.0	12	18
	2.5	12	20
	3.0	16	28
Salmonella paratyphi (Gram negative)	1.0	8	30
	1.5	9	32
	2.0	9	35
	2.5	10	35
	3.0	11	40
Escherichia coli (Gram negative)	1.0	12	11
	1.5	13	11
	2.0	13	13
	2.5	13	14
	3.0	14	18
Saccharomyces cerevisiae (Yeast)	1.0	13	12
	1.5	14	16
	2.0	14	24
	2.5	20	26
	3.0	30	28
Aspergillus niger (mold)	1.0	8	24
	1.5	8	24
	2.0	9	26
	2.5	12	28
	3.0	15	30

Table.4 Total antioxidant property of curry leaf oil and cinnamon bark oil

Essential oil	Total Antioxidative property (µg/mL of AAE)
Curry leaf oil	55.92±0.5
Cinnamon bark oil	82.76±0.5

[a] Each value in the table is mentioned as mean (n=3)
[b] All the mean values are significantly different at probability level P < 0.05.

Fig.1 Standard curve of ascorbic acid absorbance value at 695 nm

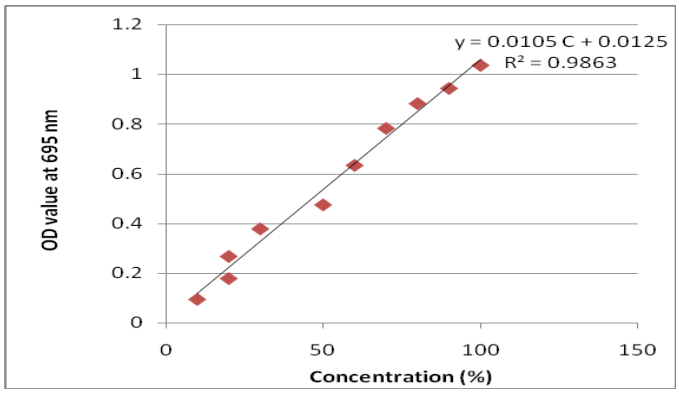


Fig.2 (a) Chromatogram of GCMS for curry leaf oil

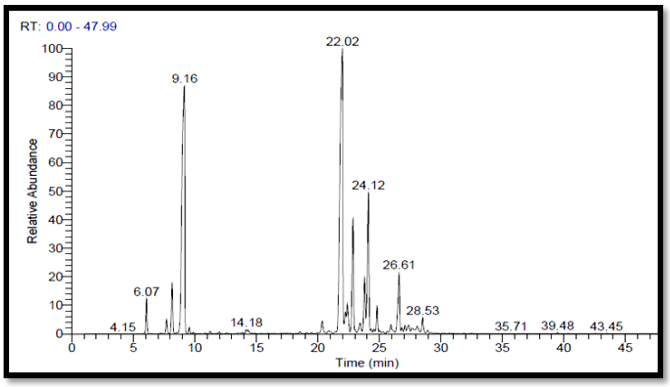


Fig.2 (b) Chromatogram of GCMS for cinnamon bark oil

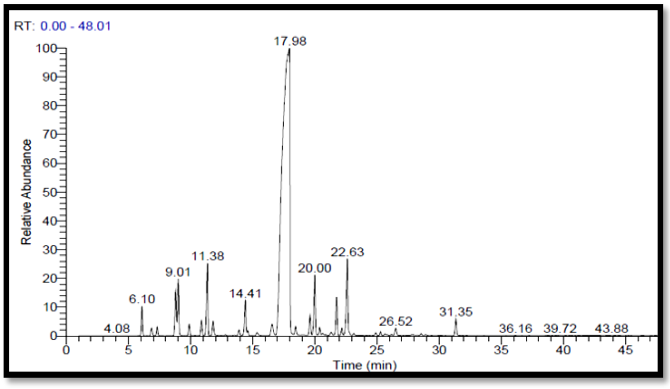
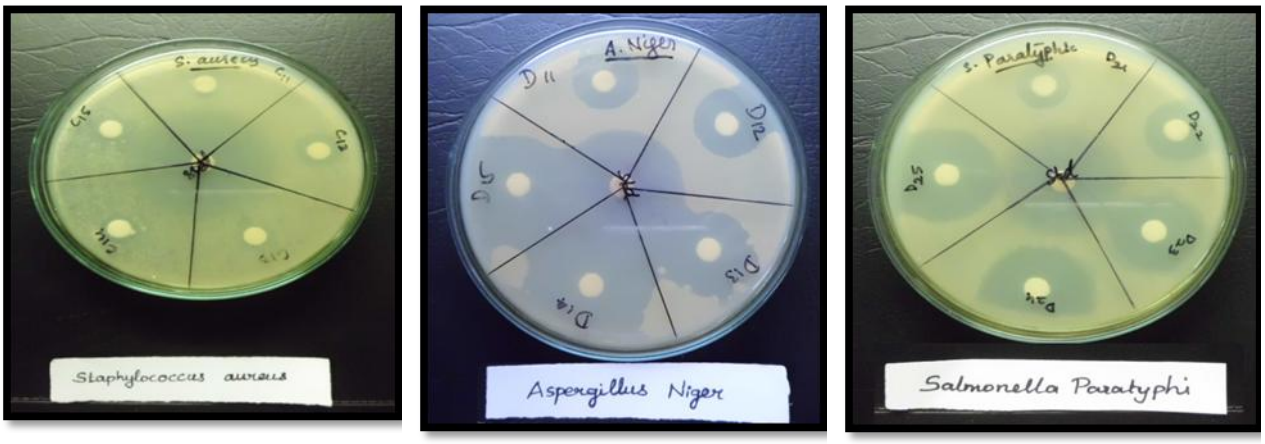


Fig.3 Zone of inhibition of cinnamon bark oil against *Salmonella*, *Aspergillus* and *Staphylococcus* for different concentrations



Total antioxidative property of essential oils- phosphomolybdenum method

The phosphomolybdate antioxidant assay is based on the reduction of Mo (VI) to Mo (V) by the antioxidant sample which is detected by the formation of green molybdenum (V) complex at an acidic pH (Baig *et al.*, 2011). In the present study, the antioxidant activity of the curry leaf oil was found to be 55.92 µg/mL of AAE (Ascorbic acid equivalent) and cinnamon bark oil was found to be 82.76 µg/mL of AAE (Ascorbic acid equivalent). The results are shown in table 4.

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