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Assessment of the Antimicrobial Effects of Some Ethiopian Aromatic Spice and Herb Hydrosols

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Abstract: The objective of the present study was to evaluate the *in vitro* antimicrobial effects of the hydrosols of basil (*O. basilicum)*, thyme (*T. schimperi*), cardamon (*E. cardamom)*, cinnamon (*C. Zeylanicum*), mustard (*B. nigra*) and clove (*S. aromaticum*) against *S. aureus*, *E. coli*, *S. typhi*, *P. aerugenosa* and *Candida albicans.* Hydrosols were obtained from the selected plant species after hydro distillation using Clevenger type apparatus. The antimicrobial effects of the hydrosols were determined by measuring the zone of microbial growth on agar plates treated with hydrosol and control agar (hydrosol untreated agar) plates and then the percentage of growth inhibition was determined. Accordingly, the percent inhibition of the hydrosols were found to range from 20 to 100% (against *S. aureus,* $p = 0.005$), 10 to 100% (against *E. coli*, $p = 0.005$), 0 to 35% (against *P. aerugenosa*, $p = 0.069$) and 15 to 100% *S. typhi*, $p = 0.00$). Complete (100%) growth inhibition was demonstrated at 15% hydrosol concentration of cardamom and thyme (against *E. coli*), cardamon and cinnamon (against *S. aureus*) and cardamom, thyme and cinnamon (against *S. typhi*). *Candida albicans* were inactive to the test hydrosols. From this study, it can be concluded that the hydrosols of basil, cardamom, clove, cinnamon and thyme were effective to elicit inhibitory effect against *S. typhi*, *S. aureus* and *E. coli*. Further study is recommended to verify the activity of the plant hydrosols against wide range of microbial strains, characterize the chemical constituents of the hydrosols and see if the biological property can be correlated to the constituents.

Key words: Aromatic spices, hydrosols, *in vitro*, antimicrobial, growth inhibition

INTRODUCTION

Despite the enormous advances made in health care, infectious diseases account for 25% of the mortality worldwide and 45% in low income countries. Moreover, the causative agents are also developing increasing resistance against many of the commonly used antibiotics and currently the costs of many drugs are not affordable for most people (Binder *et al*., 1999).

Traditional medicine and medicinal plants have been used in most developing countries as a normative basis for the maintenance of good health (Nagori and Solanki, 2011; Falodun, 2010; Abdel-Azim *et al*., 2011). In addition to economic constrains , reported side effects of the modern drugs encouraged the researchers to look for an economical and safe alternative that can cure the chronic diseases (Karim *et al*., 2011). In Ethiopia, traditional remedy represents not only part of the struggle of the people to fulfill their essential drug needs but also an integral component of the cultural beliefs and attitudes (Abebe and Ayehu, 1993). Plant remedies are still the most important and sometimes the sole source of therapeutics for nearly 80% of the population in the country (Giday, 2001).

The ability of spices and herbs to heal various health problems was widely reported and large numbers of the medicinal plants are utilized for their antimicrobial properties (Dagne, 1996). Cardamom, *Electtaria cardamom*, Zingiberaceae, known as "Hel" in Amharic, is among the spices with very high utilization in Ethiopia. It is used in a variety of special foods, vegetables and meat dishes, for flavoring tea, butter, coffee, bread and cakes in ground or whole forms as sole or mixed with other spices. Cardamon is also believed to have a carminative effect for gastrointestinal discomfort, as a digestive stimulant, for liver complaints, cold, fever and mouth inflammation (Bekele, 2007).

Cinnamon, *Cinnamon zeylanicum*, known as "Kerefa" in Amharic and its oil are used as carminative, spasm relieving, general tonic, antiseptic, anti diarrhea and ant diabetic, to lower cholesterol and lipids, for gum disease, to stimulate circulation and anti-inflammatory activities (Giday, 2001).

Clove, *Syzygium aromaticum*, Myrteaceae, known in Ethiopia by its vernacular name as "Kerunfud", is useful as carminative, spasm reliving; aphrodisiacs; antioxidant antipyretic; antimicrobial, anesthetic; anti-inflammatory for gum throat and tooth problems (Lemma *et al*., 2002).

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Basil, *Ocmium basilicum*, Lamiaceae, known by the local Amharic name as “Besso bila” grows in Ethiopia as a home garden. Its leaves and soft stems are considered to have medicinal value to treat stomach upset, colic, scabies, cough, asthma, irritated and inflamed bowl conditions, arthritis and menstrual problems. It is also used to increase breast milk and to help during child birth (Burt, 2004; Geyid *et al*., 2005; Mishra and Mishra, 2011).

Thyme, *Thymus schimperi*, Lameaceae, (commonly known as “Tosign” in Amharic) is one of the prominent potential aromatic herbs in Ethiopia. It is a useful condiment and is also reportedly used in the control of gonorrhea. When it is added to boiling water and drunk, it is used against cough and liver disease. It is also indicated for respiratory problems (cough, bronchitis, sore throat) gastrointestinal disorders, (colic, dyspepsia gastritis, flatulence, diarrhea); halitosis, rheumatism, diuretic antihelmintic (external, counter irritant, anti-inflammatory, certain skin disorders) (Abebe and Ayehu, 1993).

Mustard, *Brassica nigra*, known as “Senafich” in Amharic, has also been reported to contain a substantial quantity of tyrosine, an amino acid important in the prevention or treatment of hypothyroidism (Abebe and Ayehu, 1993).

The antimicrobial properties of plant essential oils and their constituents for inhibiting the growth of microbes have been demonstrated from previous studies of Desta *et al*. (1996), Dorman and Deans (2000), Malik and Singh (2010), Tajkarimi *et al*. (2010) and Butkhup and Samappito (2011). Little work, however, has been reported so far regarding the pharmacological properties of aromatic spices and herbs, under the condition they are used in traditional spiced food preparations and tea for flavor in general and Ethiopian aromatic spices in particular. The aim of this study was therefore to assess the antimicrobial effects of hydrosols obtained from Ethiopian basil, cardamom, cinnamon, clove, mustard and thyme against selected microbial pathogens.

MATERIALS AND METHODS

Study area and design: The study was conducted in Jimma zone from September to July, 2010. A laboratory based experimental study was conducted to assess the antimicrobial properties of hydrosols obtained from selected Ethiopian aromatic medicinal spices and herbs against selected human microbial pathogens. The primary outcome parameter was percent microbial growth inhibition by the hydrosols.

Materials

Plant material: The aromatic plants species: *Brassica nigra* (seeds), *Thymus schimperi* (leaves), *Ocimum basilicum* (leaves), *Syzygium aromaticum* (flower head), *Electtaria cardamom* (fruit) and *Cinnamon zeylanicum* (bark) were collected from the surroundings rural areas of Jimma town or were purchased form the local market in the month of October, 2010. The plant parts were taxonomically verified in Jimma University herbarium and specimen were deposited.

Chemicals and reagents: Distilled water, Alcohol (70%) and sterile normal saline were used in the study.

Media: Mueller Hinton Agar (MHA, JO0115, Oxoid, England) and Sabouraud’s Dextrose Agar, SDA (CM037, Oxoid, England) were prepared as per the manufacturer’s guideline and used for the assay.

Microbial test strains: Bacterial strains involving *Staphylococcus aureus* (ATCC 25923, Gram positive), *Escherichia coli* (ATCC 25922, Gram negative), *Salmonella typhi* (NTCC 83859, Gram negative) and *Pseudomonas aeruginosa* (ATCC 27853, Gram negative) and fungal strain *Candida albicans* (yeast, clinical isolate) were employed for activity testing. All the organisms were obtained from Clinical Bacteriology Laboratory, Ethiopian Health and Nutrition Research Institute (EHNRI).

Methods

Preparation of the hydrosols: Stock hydrosol solutions (1:10 w/v) were prepared from freshly collected or dried and pulverized plant materials using the method described by Boyraz and Ozcan (2005).

Antimicrobial assay of the hydrosols: The antimicrobial assays were performed on Muller Hinton agar (for bacteria) or Sabouraud’s dextrose agar (for fungi). Inoculums were prepared using a direct colony suspension method (CLSI, 2005). Well isolated colonies of bacteria (from overnight culture) or fungi (from 72 h old culture) were transferred into a test tube containing 5 mL of sterile normal saline. The density of the microbial suspension was equilibrated to the optical density of 0.5 McFarland turbidity standards, spectrophotometrically at 625nm. The antimicrobial assay of the hydrosols was determined employing the method described by Boyraz and Ozcan (2005) with slight modification. Briefly, test hydrosols of 5, 10 and 15% (v/v) were prepared separately by mixing specified amount of the stock hydrosol into the sterile molten agar media. The 20 mL of each of this hydrosol containing agar medium was dispensed into petri dishes (10 mm). Control media with no hydrosol incorporated were also dispensed into similar petridishes left to solidify. Sterile filter paper discs (5 mm diameter) were loaded with 10 μL of the bacterial inoculum suspensions of each micro organism and deposited onto the surface of the test agar plate (with hydrosol) and control agar plate (without hydrosol incorporated). The plates were allowed to stabilize for 15 min at room temperature and then kept for incubation at 37°C for 18-24 h (for bacteria) and at 26°C for 48 h (for fungi). The diameter of the zone of bacterial growth was then measured using a digital sliding caliper.

Statistical analysis: All the tests were done in duplicates for each test and control plates. Evaluation of the antimicrobial effect was done by determining the percentage of radial growth inhibition of the hydrosols using the following formula:

$$\%I = \frac{I_c - I_t}{I_c} \times 100$$

Where:

% I = Percent growth inhibition of the hydrosol

I_c = Diameter of the zone of microbial growth in the control plate

I_t = Diameter of the zone of microbial growth in test plate

The data obtained was analyzed using SPSS version 16 for Windows and General Linear Model Multivariate analysis was done to test the effect of concentration of the hydrosols on the mean inhibitory effects at 95% confidence level ($p < 0.05$). The results were presented as percent Mean±Standard Deviation (SD).

RESULTS

The antimicrobial effect of the hydrosols against selected bacterial strains is displayed in Table 1 below. The hydrosols showed, with varying degree, a concentration dependent increase in the antibacterial activity against most bacterial strains ($p<0.05$, Table 2). Basil, cardamon, cinnamon, clove and thyme exhibited broad spectrum growth inhibitory effect (Table 1). The percent inhibitions of the hydrosols were found to range from 20 (basil at 5% hydrosol) to 100% (cardamom and cinnamon at 15%) (against *S. aureus*, p = 0.001), 10 to 100% (against *E. coli*, p = 0.001), 0 to 35% (against *P. aerugenosa*, p = 0.026) and 15 to 100% (against *S. typhi*, p = 0.00). Complete (100%) growth inhibition was observed at 15% hydrosol concentration against *E. coli* (cardamom and thyme), *S. aureus* (cardamom and cinnamon) and *S. typhi* (cardamom, thyme and cinnamon). The hydrosols of basil and clove were also slightly active against *P. aeruginosa*. The 10 and 15% concentrations were more effective in terms of antibacterial effect against most of the tested bacterial strains. On the other hand, the fungal strain, *Candia albicans* was found to be inactive to the hydrosols at each concentration levels.

The overall mean percent effectiveness of the studied aromatic herbs/spices were 50.08±27.60 (p = 0 .042), 42.58±26.90 (p = 0 .009), 12.63±10.19 (p = 0 .066) and 51.76±26.41 (p = 0 .001) against *S. aureus*, *E. coli*, *P. aerugenosa* and *S. typhi*, respectively (Table 2). As can be seen, the general sensitivity patterns of the

Table 1: Percentage inhibitory effectiveness of hydrosols against selected bacterial strains (n =2)[§]

		The percent growth inhibition[†]			
Plant species	Concentration of hydrosol (% v/v)*	*S. aureus*	*E. coli*	*P. aeruginosa*	*S. typhi*
Mustard					
	5	25.0±1.1*	22.72±0.6	-	15.4±1.5
	10	33.3±1.5	22.72±1.2	-	33.8±1.2
	15	37.3±0.6	27.27±0.3	30.0±0.6	46.2±1.3
Cardamon					
	5	25.7±1.0	21.2±1.3	-	32.3±0.3
	10	72.3±0.6	49.8±0.6	10.1±1.1	48.5±0.3
	15	100.0±0.0	100.0±0.0	10.1±1	100.0±0.0
Basil					
	5	20.83±0.6	10.1±0.6	9.3±0.6	19.2±0.5
	10	29.16±1.5	45.3±1.5	10.3±0.6	41.3±0.3
	15	33.30±1.0	78.2±0.2	25.7±0.6	64.6±1.1
Thyme					
	5	33.3±0.6	27.7±1.0	5.0±0.2	39.2±0.6
	10	51.3±0.6	59.7±1.1	8.0±1.5	58.5±0.6
	15	86.3±1.0	100.0±0.0	10.0±1.0	100.0±0.0
Clove					
	5	33.3±1.5	27.27±1.5	20.6±0.6	30.8±0.3
	10	41.6±0.2	36.36±0.6	23.3±1.2	42.3±1.3
	15	87.3±0.6	56.36±0.6	35.0±0.6	72.3±1.3
Cinnamon	5	25.2±1.2	18.18±1	10.0±0.6	33.8±0.5
	10	66.5±0.6	27.27±0.6	10.0±1.2	53.9±0.7
	15	100.0±0.0	36.36±0.6	10.0±0.6	100.0±0.0

[§]The number of experiments. * The number of parts by volume of the hydrosol per 100 mL of agar solution. [†]Values are presented as Mean±Standard deviation, ($p<0.05$)

Table 2: Multivariate tests between-concentration effects

Dependent variable	Type III sum of squares	df[e]	Mean square	F	Sig.
The mean percent radial growth inhibition against *S. aureus*	6573.996[a]	1	6573.996	16.514	0.001
The mean percent radial growth inhibition against *E. coli*	6120.987[b]	1	6120.987	15.840	0.001
The mean percent radial growth inhibition against *P. aerugenusa*	480.068[c]	1	480.068	5.975	0.026
The mean percent radial growth inhibition against *S. typhi*	8132.813[d]	1	8132.813	34.958	0.000

[a] R Squared = 0.508 (Adjusted R Squared = 0.477), [b] R Squared = 0.497 (Adjusted R Squared = 0.466), [c] R Squared = 0.272 (Adjusted R Squared = 0.226), [d] R Squared = 0.686 (Adjusted R Squared = 0.666), [e] Difference

Table 3: The overall inhibitory effects of the spices investigated

	The overall mean percent radial inhibition of the plants[†]			
Plant species (spices) investigated	*S. aureus*	*E. coli*	*P. aerugenosa*	*S. typhi*
B. nigra	31.77±6.15[*]	24.24±2.63	10.00±17.32	31.80±15.49
E. cardamom	66.00±37.55	57.00±39.80	6.73±5.83	60.26±35.35
O. basilicum	27.76±6.35	44.53±34.06	15.10±9.19	41.70±22.70
T. schimperi	56.96±26.95	62.47±36.23	7.67±2.51	65.73±31.13
S. aromaticum	54.06±29.07	39.99±14.88	26.30±7.65	48.47±21.43
C. Zeylanicum	63.90±37.47	27.27±9.09	10.00±0.00	62.57±33.94
Total	50.08±27.60	42.58±26.90	12.63±10.19	51.76±26.41

[†] The overall percent inhibition of the plants species, making concentration as a control variable. [*] Results are presented as mean±standard deviation

organisms to the hydrosols were found to decrease in the following order: *S. typhi, S. aureus, E. coli* and *P. aeruginosa*. *P. aerugenosa* was the most resisted bacterial strain (Table 3).

DISCUSSION

As demonstrated above, the hydrosols obtained from basil, clove, cinnamon, cardamom, mustard and thyme were found to inhibit, with varying degree, the growth of most of the tested micro organisms (Table 1). As observed in this study, all the tested spice hydrosols were inactive against *Candidia albicans* (yeast). This result does not agree, in part, with previous finding by Inouye *et al*. (2009), in which growth inhibition of 10 and 100% were reported by the hydrosols of thyme (*Thymus mastichina*) and clove, respectively. In contradiction to our study, weaker antifungal property of basil against *Aspergillus parasiticus* (Ozcan, 2005) and *Aspergillus flavus* (Ozcalp and Ozcan, 2009) was also reported. Such variations could be due to, among other factors, the difference in the chemical composition of the hydrosols which might in turn be influenced by geographic sources and harvesting season (Senatore, 1996).

Cardamom, thyme and cinnamon were capable of complete inhibition against two or three bacterial strains. Relatively similar antibacterial properties have been demonstrated by basil and thyme hydrosols against *E. coli* and *S. aureus* (Sagdic and Ozcan, 2003), cinnamon aqueous extract against *Staphylococcus* species and *E. coli* (Puangpronpitag and Sittiwet, 2009) marking the antimicrobial potential of the plants.

Numerous publications have emerged regarding the chemical composition and antimicrobial effects of essential oils in which the major components have chiefly been implicated to the antibacterial activity (Senatore, 1996; Cox *et al*., 2000; Dorman and Deans, 2000; Tajkarimi *et al*., 2010). The antimicrobial properties of the hydrosol observed in this study could also be attributed to the chemical composition of the hydrosols.

Though, the chemical composition of hydrosols were not well elucidated, they are known to contain water soluble components and micronized droplets of essential oil molecules (Suzanne, 2001) dissolved in the hydrosol solution. This indicates that the chemical composition of spice hydrosols differs from those of pure essential oils. Few reports on the volatile component analysis in hydrosols and respective pure essential oils revealed the presence of extremely low concentration of the major volatile component in hydrosols (Inouye *et al*., 2008). This difference could suggest that the intensity of antimicrobial activity of hydrosols is not always the same as that of the essential oil. Thus, the non volatile chemical group primarily hydrophilic acidic constituents reported to occur in hydrosols could contribute for inhibitory effect possibly in synergy with the trace essential oils components. Essential oils of aromatic herbs and spices containing phenolic, aldehydes, ketones and alcohols components have been reported to show stronger antimicrobial effect than the hydrocarbon terpenes (Ozcan, 2005; Cosentino *et al*., 1999; Vaghasiya *et al*., 2011). This is in agreement with the studied plants basil, cardamom, clove, thyme and cinnamon which have been reported to contain such oxygenated constituents within their hydrosol (Inouye *et al*., 2008; Sanni *et al*., 2008).

From the current study, it can be concluded that the hydrosols of basil, cardamom, clove, cinnamon and thyme were active to elicit inhibitory effect against *S. typhi*, *S. aureus* and *E. coli*. Moreover, higher concentrations of the hydrosols were most effective to demonstrate improved inhibition of the bacterial growth. This observation marks the potential antibacterial potential of Ethiopian spice hydrosols, under the condition that they are added in foods or tea used for their flavor. Further study is recommended to verify the effectiveness of Ethiopian spice hydrosols against more diverse microbial strains. Attempts should also be made to characterize the chemical constituents of the hydrosols and see if the biological property can correlated to the constituents.

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