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Inhibitory effect of steam distillates of selected spices on the growth of bacteria and fungi

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ABSTRACT

Spices are some of the most commonly used natural antimicrobial agents in foods. Addition of spices in foods not only imparts flavor and pungency but also provides antimicrobial property. The present investigation describes the antimicrobial activity of steam distillates of four spices namely *Cuminum cyminun, Cinnamomum zeylanicum, Foeniculum vulgare* and *Alium sativum* against bacteria and fungi. Distillation process was employed to obtain steam distillate. Antibacterial activity was checked in liquid media by measuring cell crop in tubes added with steam distillate. Poison food technique was used to assess antifungal activity. The results clearly suggest the potential of distillates of selected spices against bacteria and fungi. Among distillates tested, *C.cyminum* was found to be more effective in most cases followed by others. The distillate of *F.vulgare* showed weak activity against bacteria while *A. sativum* showed less antifungal activity when compared to others. The findings give an indication of the presence of promising antimicrobial compounds in distillate fraction. The distillates could be used against bacteria causing food poisoning, enteric infections, nosocomial infections etc and fungi causing opportunistic mycotic infections. Further phytochemical studies are needed to elucidate the components responsible for antimicrobial activity.

KEYWORDS

Spices;
Antimicrobial activity;
Steam distillate;
Poison food technique;
Essential oil.

INTRODUCTION

It has been well known since ancient times that plants and spices have antimicrobial activity. There has been a considerable interest to use plants and spices for the elimination of microorganisms because of increasing antibiotic resistance of microorganisms[1]. Traditional cultures somehow understood that spices and herbs added more to food than flavor. They knew certain spices and herbs were important for health and longevity. Today, science has identified the unique compounds responsible for these benefits. Several studies have been conducted on the antimicrobial properties of herbs, spices and their derivates such as essential oils, extracts and decoctions. Some researches reported that there is a relationship between the chemical structures of the most abundant compounds in the tested extracts or essential oils and the antimicrobial activity[2]. Spices are some of the most commonly used natural antimicrobial agents in foods. Addition of spices in foods not

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TABLE 1: Percentage inhibition of test bacteria by steam distillates of spices

Distillate	Percentage inhibition when compared to control				
	E.coli	*B.subtilis*	*S.aureus*	*K.pneumoniae*	*P.aeruginosa*
Cuminum cyminun	99.00	72.59	91.56	86.66	87.93
Cinnamomum zeylanicum	81.18	84.13	89.58	84.57	91.09
Foeniculumvulgare	15.01	28.94	3.77	23.42	23.51
Alium sativum	96.50	94.23	87.79	78.47	92.02

only imparts flavor and pungency but also provides antimicrobial property. Natural antimicrobial compounds in spices were found to possess antimicrobial activity. The antimicrobial property of spices may differ depending on the forms of spices added, such as fresh, dried, or extracted forms. Antimicrobial activity of spices may differ between strains within the same species of bacteria. The aim and objective of the study is to evaluate the steam distillate of selected spices for antimicrobial activity against bacteria and fungi.

MATERIALS AND METHODS

Collection of plant material and distillation

The plant materials of *Cuminum cyminun, Cinnamomum zeylanicum, Foeniculum vulgare* and *Alium sativum* were collected from the local shops of Shivamogga city and mechanically grinded. A simple quick-fit apparatus with a 1000ml distilling flask (to boil the mixture of plant material and water), a condenser (to condense the steam to obtain the steam distillate), and a receiving vessel, was used for the steam distillation. A known weight of (100 grams) plant material was subjected to steam distillation and the volatile components were collected into the receiving flask during 3 hours of steam distillation[3,4].

Screening for antibacterial activity

The antibacterial activity of steam distillates was tested in liquid nutrient media with minor modifications[5]. The sterile nutrient broth tubes containing known volume of steam distillate were inoculated with standardized volumes of 24 hours old broth cultures of test bacteria namely *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa* followed by incubation at 37^0C for 24 hours. After incubation, the optical density was measured by spectrophotometer at a wavelength of 560 nm as a guide to microbial growth and compared with control to assess antibacterial activity. The experiment was carried in three trails and percentage inhibition was calculated.

TABLE 2: Percentage inhibition of test fungi by steam distillates

Steam distillate	Inhibition (in %)			
	A.niger	*A.oryzae*	*A.nidulans*	*A.terreus*
Cuminum cyminun	90.69	78.94	89.18	69.23
Cinnamomum zeylanicum	69.76	55.26	54.05	88.46
Foeniculum vulgare	55.80	44.73	37.83	65.38
Alium sativum	20.93	28.94	24.32	26.92

Screening for antifungal activity

In the study, four species of the genus *Aspergillus* namely *A.niger, A.oryzae, A.nidulans* and *A.terreus*, were selected as target fungi. The suspension of spores of the test fungi was prepared in a test tube containing 0.85% sterile normal saline containing 0.01% Tween 80 detergent[6]. The antifungal activity was assessed using Poison food technique[7]. The test fungi were allowed to grow in Sabouraud's dextrose agar plates poisoned with steam distillates (10% concentration). The effect of extract on fungal growth was determined by measuring the diameter of the colony obtained on poisoned plate and comparing with control plates which were not poisoned with the distillate. The experiment was carried in three trails and percentage inhibition was calculated.

RESULTS AND DISCUSSION

The distillates of spices exhibited potent antibacterial activity in terms of inhibition of bacterial growth (turbidity) in liquid media (TABLES 1 and 2). Among distillates tested, *C.cyminum* exhibited stronger antibacterial activity followed by *A.sativum, C.zeylanicum* and *F.vulgare*. An inhibition of over 70% was recorded in all bacteria by all distillates except *F.vulgare*. *S.aureus* was least inhibited (3.77%) by steam distillate of *F.vulgare*. The growth of *E. coli* was almost

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completely reduced by *C.cyminum* distillate. The results reveal the potential of these distillates to inhibit test bacteria. The spice steam distillates tested for antifungal activity have shown significant activity against fungi tested (**TABLES** 3 and 4). Among distillates tested, it was *C.cyminum* which showed more inhibition of test fungi. All the four species of *Aspergillus* were found to be susceptible to steam distillates. Next to *C.cyminum*, more activity was observed in case of *C.zeylanicum*, *F.vulgare* and *A.sativum*. Over 50% inhibition of test fungi was observed in case of *C.cyminum* and *C.zeylanicum*. Inhibition of test fungi was not more than 30% in case of *A.sativum*. Among distillates tested, *C.cyminum* was found to be more effective in most cases followed by others. The distillate of *F.vulgare* showed weak activity against bacteria while *A. sativum* showed less antifungal activity when compared to others. Since inhibition of *S.aureus* and *E.coli* was observed in the study, the spices could be added to foods to prevent contamination by these food poisoning bacteria. The distillate could also be used against antibiotic resistant bacteria as some strains of *E.coli*, *S.aureus*, *P.aeruginosa* etc have acquired antibiotic resistance against some antibiotics used against infections. As the study made use of enteric bacteria such as *E.coli* and *K.pneumoniae*, the distillate may be used for treating enteric infections caused by these bacteria. Aspergillosis is the serious complication caused in compromised individuals by species of *Aspgerillus* genus. The distillates have shown to inhibit the test fungi and thus may be used against opportunistic infections caused by *Aspergillus* species.

The steam distillates are known to contain essential oils. Essential oils are valuable natural products used as raw materials in many fields[8]. Essential oils are products, generally, of rather complex composition comprising the volatile principles contained in the plants[9]. The most popular method for extraction of essential oils is steam distillation in which water is heated to produce steam that carries the most volatile chemicals of the aromatic material with it. The steam is then chilled and the resulting distillate is collected. The Essential Oil normally float on top of the Hydrosol (the distilled water component) and may be separated off[10].

CONCLUSION

In conclusion, steam distillate of selected spices showed antimicrobial activity. This gives an indication of the presence of promising antimicrobial compounds in distillate fraction. Further phytochemical studies are needed to elucidate the components responsible for antimicrobial activity of these distillates against bacteria and fungi. Further experiments in animal models could possibly reveal the potential of plant to inhibit disease causing microorganisms.

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REFERENCES

[1] H.Durmaz, E.Sagun, Z.Tarakci, F.Ozgokce; Afr.J. Biotech., **5(19)**, 1795-1798 **(2006)**.
[2] B.Dulger; Afr.J.Trad.CAM., **2(3)**, 259-263 **(2005)**.
[3] P.Zeinsteger, A.Romero, P.Teibler, M.Montenegro, E.Rios, E.M.Ciotti, O. Acosta de Parez, N.Jorge; RIA., **32(2)**, 125-136 **(2003)**.
[4] H. Peng, X. Yang; J.Zhejiang Univ.Sci., **6B(2)**, 91-95 **(2005)**.
[5] C.M.M Ludin, J.M Radzi; Mal.J.Med.Sci., **8(2)**, 14-18 **(2001)**.
[6] Z.Rihakova, V.Filip, M.Plockova, J.Smidrkal; Czech.J.Food Sci., **20(2)**, 48-52 **(2002)**.
[7] G.Singh, S.Maurya, M.P.DeLampasona, C.Catalan; J.Food Sci., **70(4)**, 208-215 **(2005)**.
[8] G.Buchbaur; Perfumer and Flavorist., **25**, 64-67 **(2000)**.
[9] J.Bruneton; Pharmacognosy, Phytochemistry, Medicinal Plants Intercept, Ltd.: Hampshire, **(1995)**.
[10] K.F.Abed; Saudi Journal of Biological Sciences., **14(1)**, 53-60 **(2007)**.