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Antibacterial activity of Caraway essential oil against bacteria isolated from veterinary clinical cases

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ABSTRACT

Of the 257 strains of bacteria belonging to 75 species of 30 genera isolated from morbid or post-mortem samples of animals, fish, birds and human beings only 15 strains were sensitive to 2 mg discs of caraway essential oil (CEO). Fifteen CEO sensitive strains belonged to 13 species of bacteria namely *Bacillus cereus, Bordetella bronchiseptica, Brucella abortus, Dermatophilus congolensis, Erwinia ananas, Escherichia coli, Moraxella canis, Moraxella osloensis, Pasteurella multocida, Proteus penneri, Pseudomonas aeruginosa, Raoultella terrigena* and *Streptococcus pyogenes.* The MIC of CEO for all resistant strains was more than 2.0 mg/ mL while MIC of sensitive strains ranged between 0.20 mg/ mL to 2mg/ mL, minimum for *M. osloensis* (0.20 mg/ mL) strains. The study revealed only limited antimicrobial activity against clinically important bacteria causing disease or death. The antibacterial activity of CEO was more prominent for some of the strains of high zoonotic significance *viz*., *Brucella abortus, Burkholderia mallei* and *Bordetella bronchiseptica* which might be important in designing antimicrobials for their therapeutic control.

KEYWORDS

Caraway essential oil;
Bacillus cereus;
Bordetella bronchiseptica;
Brucella abortus;
Dermatophilus congolensis;
Erwinia ananas;
Escherichia coli;
Moraxella canis;
Moraxella osloensis;
Pasteurella multocida;
Proteus penneri;
Pseudomonas aeruginosa;
Raoultella terrigena;
Streptococcus pyogenes.

INTRODUCTION

Caraway (*Carum carvi* L.) also known as meridian fennel, or Persian cumin or Shahjeera, is grown in many countries of Europe, Asia and Africa. Caraway or Shahjeera is an important medicinal plant known for its wide spectrum therapeutic uses[1-4]. In Syria, Nigella and Caraway seeds are extolled as being "A cure for every disease except death" including the treatment of skin conditions, respiratory infections, intestinal disorders and parasites, headaches, toothaches, agalactia, uterine-tonic[2] and also an potent insect repellent[3]. Caraway essential oil (CEO) is reported to possess mild antimicrobial activity[1, 5] depending on its cultivar (MIC 0.16 mg/ mL to 1.75 mg/ mL/). However, in some other studies caraway essential oil had MIC >2 mg/mL against reference *Staphylococcus aureus* and *Klebsiella pneumoniae* strains[4]. In a study on food-borne pathogens CEO inhibited growth of most of the pathogens at 0.12% concentration[5] while in other study on food-borne pathogenic and spoilage

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bacteria including *Salmonella* Typhimurium, *Escherichia coli, Listeria monocytogenes, Pseudomonas* spp. and *Staphylococcus aureus* MIC ranged between 6 to 10 mg/ mL[6]. Friedman et al.[7] reported inhibitory concentration of CEO for *Listeria monocytogenes, Escherichia coli* and *Salmonella enterica* in range of 0.33% to 0.47%. However, CEO could not inhibit growth of most of the common phytopathogenic bacteria except of *Erwinia* strains[8]. Carvone has been recognized as the active antimicrobial component whiles other important ingredient of the oil, limonene, has no significant antimicrobial activity[1, 5]. Aggarwal and others[9] suggested that more antimicrobial activity of natural oils than their purified components might be due to the synergistic actions of different isomeric forms.

Although information on antimicrobial activity of CEO on food-borne pathogens, spoilage bacteria and also fungi is not scant, little is understood about antibacterial activity of CEO on bacteria isolated from clinical samples. In the present investigation we examined the antibacterial activity of CEO on bacteria available in repository of Epidemiology Laboratory of the Institute and isolated previously from samples of dead (post-mortem) or clinically sick cases.

MATERIALS AND METHODS

Bacterial strains

Three reference strains (*Enterobacter agglomerans*, RAVI-7; *Escherichia coli*, E-382 and *Salmonella enterica* serovar Abortusequi, E-155) and 254 bacterial isolates TABLE 1 belonging to 75 species of 30 genera from samples of morbid or dead animals including buffalo (18), cattle (54), dog (25), elephant (4), goat (5), horse (20), pig (46), spotted deer (6), swamp buffaloes (23), Thamin deer (5),

TABLE 1 : Source of isolation and sensitivity of bacteria to caraway essential oil

Source of isolation	Isolates tested	No. of sensitive isolates (%)	Bacteria sensitive
Buffalo	18	1 (5.6)	*Streptococcus pyogenes* (1)
Cattle	54	5 (9.3)	*Brucella abortus* (1), *Moraxella osloensis* (1), *Pasteurella multocida* (1), *Bacillus cereus* (1), *Erwinia ananas* (1)
Dog	25	3 (12.0)	*Moraxella canis* (2), *Proteus penneri* (1)
Elephant	4	0 (0.0)	
Fish	11	0 (0.0)	
Goat	5	0 (0.0)	
Horse	20	0 (0.0)	
Human	17	2 (11.8)	*Raoultella terrigena* (1), *Dermatophilus congolensis* (1)
Pig	46	3 (6.5)	*Escherichia coli* (2), *Bordetella bronchiseptica* (1)
Poultry birds	3	0 (0.0)	
Reference cultures (*Enterobacter agglomerans* (RAVI-7), *Escheichia coli* (E382), *Salmonella Abortusequi* (E155)	3	0 (0.0)	
Spotted deer	6	0 (0.0)	
Swamp buffalo	23	0 (0.0)	
Swamp deer	5	1 (5.0)	*Pseudomonas aeruginosa*
Thamin deer	5	0 (0.0)	
Tiger	12	0 (0.0)	
Total	257	15 (5.8)	

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TABLE 2 : Sensitivity of bacteria isolated from clinical cases to caraway essential oil

Bacteria tested	Source (number) of isolates tested	Sensitive (%), Source	Bacteria tested	Source (number) of isolates tested	Sensitive (%), Source
Acinetobacter schindleri	Cattle (1)	0 (0.0)	*Moraxella nonliquifaciens*	Cattle (1)	0 (0.0)
Acinetobacter haemolyticus	Cattle (1), Swamp buffalo (1)	0 (0.0)	*Moraxella osloensis*	Cattle (2)	1 (50.0), Cattle
Actinobacillus equeli	Horse (1)	0 (0.0)	*Pasteurella canis*	Cattle (2)	0 (0.0)
Actinomyces pyogenes	Cattle (1)	0 (0.0)	*Pasteurella multocida*	Cattle (2)	1 (50.0), Cattle
Aeromonas caviae	Pig (2)	0 (0.0)	*Plesiomonas shigelloides*	Cattle (5)	0 (0.0)
A. hydrophila	Cattle (1), Swamp buffalo (2), Pig (3)	0 (0.0)	*Proteus mirabilis*	Cattle (2), Horse (2), Tiger (1), Human (1), Dog (3), Fish (1), Poultry birds (2)	0 (0.0)
A. media	Buffalo (3), Cattle (2), Pig (2)	0 (0.0)	*Proteus penneri*	Dog (2)	1 (50.0), Dog
A. salmonicida ssp. salmonicida	Goat (1)	0 (0.0)	*Proteus vulgaris*	Dog (1), Tiger (1)	0 (0.0)
A. schubertii	Swamp buffalo (1)	0 (0.0)	*Pseudomonas aeruginosa*	Buffalo (1), Pig (2), Swamp deer (1), Thamin deer (1)	1 (20.0), Swamp deer
A. sobria	Pig (2)	0 (0.0)	*Pseudomonas fluorescens*	Fish (3)	0 (0.0)
A. veronii	Cattle (2)	0 (0.0)	*Pseudomonas pseudoalcaligenes*	Cattle (1), Fish (2)	0 (0.0)
Agrobacterium tumefaciens	Tiger (1)	0 (0.0)	*Raoultella terrigena*	Cattle (1), Human (2), Thamin deer (1)	1 (25.0), Human
Alkaligenes faecalis	Pig (2), Human (1)	0 (0.0)	*Salmonella enterica* spp. *enterica* ser Abortusequi	Reference (1)	0 (0.0)
Alkaligenes denitrificans	Cattle (1), Swamp buffalo (3)	0 (0.0)	*Salmonella enterica* spp. *enterica* ser Kentucky	Cattle (1), Poultry birds (1)	0 (0.0)
Bacillus alvei	Dog (1)	0 (0.0)	*Salmonella enterica* spp. *enterica* ser Typhimurium	Tiger (1)	0 (0.0)
Bacillus cereus	Buffalo (1), Cattle (2), Horse (1), Spotted deer (2)	1 (16.7), Cattle	*Serratia marcescens*	Pig (1)	0 (0.0)
Bacillus firmus	Dog (2)	0 (0.0)	*Serratia odorifera*	Spotted deer (3)	0 (0.0)
Bacillus stearothermophilus Group I	Dog (1)	0 (0.0)	*Staphylococcus aureus*	Cattle (1), Horse (1)	0 (0.0)
Bordetella bronchiseptica	Dog (1), Pig (1)	1 (50.0), Pig	*Staphylococcus auricularis*	Dog (1), Horse (1)	0 (0.0)
Brahmnella cuniculi	Dog (1)	0 (0.0)	*Staphylococcus capitis ssp. urealyticus*	Dog (2)	0 (0.0)
Brucella abortus	Cattle (2)	1 (50.0), Cattle	*Staphylococcus chromogenes*	Pig (1), Tiger (1)	0 (0.0)
Citrobacter freundii	Dog (1), Fish (3)	0 (0.0)	*Staphylococcus haemolyticus*	Buffalo (1), Horse (1)	0 (0.0)
Dermatophilus congolensis	Human (2)	1 (50.0), Human	*Staphylococcus hyicus*	Human (1)	0 (0.0)

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Bacteria tested	Source (number) of isolates tested	Sensitive (%), Source	Bacteria tested	Source (number) of isolates tested	Sensitive (%), Source
Enterococcus faecalis	Pig (1)	0 (0.0)	*Staphylococcus lentus*	Buffalo (1), Dog (2), Horse (1)	0 (0.0)
Enterococcus raffinosus	Pig (2)	0 (0.0)	*Staphylococcus warneri*	Buffalo (1), Cattle (1)	0 (0.0)
Enterococcus solitarius	Dog (1), Human (1)	0 (0.0)	*Streptococcus adjacens*	Human (1)	0 (0.0)
Errwinia amylovora	Swamp buffalo (4)	0 (0.0)	*Streptococcus bovis*	Pig (5)	0 (0.0)
Erwinia chrysanthemi	Pig (1), Fish (1)	0 (0.0)	*Streptococcus defactivus*	Goat (2)	0 (0.0)
Erwinia ananas	Cattle (1)	1 (100.0), Cattle	*Streptococcus equi ssp. equisimilis*	Buffalo (1), Horse (2)	0 (0.0)
Escherichia coli	Buffalo (1), Cattle (9), Horse (7), Pig (16), Swamp buffalo (10), Goat (2), Tiger (4), Human (4), Dog (4), Reference (1), Elephant (3), Swamp deer (3), Thamin deer (3)	1 (2.9), Pig	*Streptococcus milleri*	Cattle (1), Human (1)	0 (0.0)
Escherichia fergusonii	Human (2), Swamp buffalo (1)	0 (0.0)	*Streptococcus porcinus*	Pig (1)	0 (0.0)
Escherichia vulneris	Cattle (1)	0 (0.0)	*Streptococcus pyogenes*	Buffalo (3), Cattle (1)	1 (25.0), Buffalo
Klebsiella pneumoniae	Buffalo (2), Cattle (2), Pig (2), Tiger (2), Human (2), Elephant (1)	0 (0.0)	*Streptococcus suis*	Pig (1)	0 (0.0)
Klebsiella oxytoca	Cattle (2)	0 (0.0)	*Vibrio mimicus*	Cattle (1)	0 (0.0)
Leminorella ghrimontii	Fish (1)	0 (0.0)	*Xenorhabdus poinarii*	Buffalo (2)	0 (0.0)
Moraxella atlantae	Cattle (3)	0 (0.0)	*Xenorhabdus bovienii*	Buffalo (1)	0 (0.0)
Moraxella canis	Dog (2)	2 (100.0), Dog	Total	257	15 (5.8)

tigers (12), birds (3), fish (11), and human beings (17) were revived from glycerol stocks available in Epidemiology laboratory of the Institute. The strains were tested for purity and identity and stock cultures were made in semisolid nutrient agar[10] for use in the study.

Caraway essential oil (CEO) sensitivity assay

A vial of CEO received as kind gift from Subh Flavours and Fragrance Ltd., New Delhi was stored at ambient temperature, till used for making discs containing 2 mg of the oil in each disc as described earlier[11]. For determining minimum inhibitory concentration (MIC) of sensitive strains agar well method was employed and dimethyl suphoxide (DMSO, Merck Specialities Pvt. Ltd, Mumbai) was used as CEO diluents[11]. For testing sensitivity, bacteria were grown overnight in trypticase soy broth (BD and Co. Sparks, USA) and then inoculated on to Mueller Hinton (MH) agar (BD and Co. Sparks, USA) plates using sterile cotton swabs. For testing *Bordetella*, *Brucella* and *Streptococcus* isolates 5% defibrinated blood was added to MH agar to support the growth of bacteria. Ciprofloxacin 10 µg discs were used as control for which all the three reference strains were sensitive.

RESULTS AND DISCUSSION

Caraway or Shahijeera is reported to be an important herb with multiple therapeutic uses[1-3]. Caraway essential oil (CEO) has been shown to possess potential antimicrobial activity against fungi and bacteria of both pathogenic and spoilage importance[1, 4-9]. However, in the present study on 257 bacteria belonging to 75 species (TABLE 2) of 24 genera of Gram negative bacteria (GNB) and 6 genera of Gram positive bacteria (GPB) only 15 (5.8%) strains (1 GPB

and 14 GNB) were sensitive to CEO. In total 1.9% of 54 GPB and 6.9% of 203 GNB isolates were sensitive to CEO having MIC < 2 mg/ ml. Although comparatively more numbers of GNB isolates were sensitive to CEO than GPB isolates, difference was statistically not very significant (p, 0.16). Sensitivity to CEO in comparatively more numbers of GNBs than GPBs is in contrast to earlier studies reporting more efficacy of CEO against GPBs than GNBs[5]. This difference might be either due to non-inclusion of oxidase positive GNBs or due to less variety of strains included in earlier studies or due to inclusion of selected reference strains of *Salmonella, Escherichia coli, Pseudomonas, Klebsiella pneumoniae* and *B. cereus* etc.[1, 4-9] or due to variation in activity of CEO of different origin as reported earlier[1]. In present study too, none of the three reference strains of GNB were sensitive to CEO.

Of the 254 clinical isolates 184 (72.4%) were sensitive to ciprofloxacin and all three reference strains were also sensitive to ciprofloxacin. Of the 184 strains sensitive to ciprofloxacin 13 were also sensitive to CEO and both the ciprofloxacin resistant but CEO sensitive strains were of *Escherichia coli* isolated from ileum of piglets died of diarrhoea. In the present study CEO could inhibit growth of only 5.8% of the bacteria isolated from clinical sick or dead patients indicating its comparative inefficacy as control antibiotic (ciprofloxacin) was effective against 72.4% bacterial isolates. In earlier studies too[12], ciprofloxacin has been reported to be effective against ~75% of the bacterial isolates from veterinary clinical samples while many of the herbal drugs failed to be equal to affectivity of penicillin in inhibiting growth of bacteria in environment too[13].

Of the 75 oxidase producing and 182 non-oxidase producing strains tested 9 (12%) and 6 (3.3%) were sensitive to CEO, respectively, and difference in sensitivity of the two groups of isolates to CEO was significant (p, 0.007). In present study, oxidase positive strains were comparative more sensitive than oxidase negative strains, how oxidase plays role in sensitivity to CEO is not clear from the study and needs further studies. Among oxidase positive strains too, most of the *Moraxella* strains were sensitive. Sensitivity of *Moraxella* to CEO might be due to in general sensitivity of strains of *Moraxella* to most of the antimicrobials including penicillins[12] but needs more elaborate studies to confirm. On the other hand all the 21 aeromonads were resistant to CEO; resistance among aeromonads to CEO was significantly more common than in other oxidase positive strains (p, 0.095). However, observations of the study cannot figure out cause of CEO resistance in aeromonads which was in contrast to sensitivity of other oxidase positive strains to CEO.

Although isolates tested for sensitivity to CEO were from 15 different sources (TABLE 1), none of the isolate from elephant, fish, goat, horse, poultry birds, reference (*Enterobacter agglomerans* R-7; *Escheichia coli* E-382 and *Salmonella enterica* ser Abortusequi E-155), spotted deer, swamp buffalo, Thamin deer and tiger was sensitive to CEO while 1, 5, 3, 2, 3 and 1 bacteria isolated from clinically sick buffalo, cattle, dog, human, pig and swamp deer, respectively were sensitive to CEO. In general source of isolates (animal) had little effect on the sensitivity of bacteria towards CEO (TABLE 1) except high proportion of isolates from dogs were sensitive than those from swamp buffaloes (p, 0.086) and horse (p, 0.109). Higher proportion of bacterial isolates from humans was sensitive to CEO than those from swamp buffaloes (p, 0.091) and horses (p, 0.115). Similarly, better proportion of bacterial isolates from swamp deer was sensitive to CEO than isolates from swamp buffaloes (p, 0.029), horses (p, 0.041), tiger (p, 0.11) and fish (p, 0.126). The variation in sensitivity of bacteria of different origin to CEO might be due to difference in genetic background of bacteria or exposure of different source animals to similar herbs. The variation in sensitivity of bacterial strains of different origin to an antimicrobial substance is concurrence to earlier observations comparing the sensitivity for other drugs[11-13].

Fifteen CEO sensitive strains belonged to 13 species of bacteria including *Bacillus cereus, Bordetella bronchiseptica, Brucella abortus, Dermatophilus congolensis, Erwinia ananas, Escherichia coli* (two), *Moraxella canis* (two), *Moraxella osloensis, Pasteurella multocida, Proteus penneri, Pseudomonas aeruginosa, Raoultella terrigena* and *Streptococcus pyogenes.* The MIC of CEO for all resistant strains was more than 2.0 mg/ mL while MIC of sensitive strains ranged between

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0.20 mg/ mL to 2mg/ mL, minimum for *M. osloensis* (0.20 mg/ mL) followed by *B. abortus* (0.3 mg/ mL), *B. mallei* (0.40 mg/ mL), *B. bronchiseptica* (0.8 mg/ mL), *R. terrigena* (1.0 mg/ mL), *P. multocida* (1.2 mg/ mL), *E. ananas* (1.8 mg/ mL) and it was 2.0 mg/ mL for rest of the 8 sensitive strains. Sensitivity of *Erwinia* isolate to CEO observed in the study has also been reported earlier[8]. Observations on MIC of CEO for bacteria are in concurrence to earlier studies reporting CEO MIC between 3.3 to 10 mg/ mL[6,7] for most of the food-borne pathogens and some of the bacteria belonging to the same species included in the present study. In the study, though strains of several species of bacteria were sensitive to CEO (MIC < 2mg/ mL) but resistance in the several strains of the same species of the bacteria indicated that bacteria might acquire resistance for CEO as reported earlier for other antimicrobials[12, 14].

CONCLUSION

The study concluded that caraway essential oil possesses antimicrobial activity against only a few clinically important bacteria causing disease or death in animals, birds, fish and humans. The antibacterial activity of CEO was more prominent for some of the strains of high zoonotic importance including *Brucella abortus, Burkholderia mallei* and *Bordetella bronchiseptica* and information might be important in designing antimicrobials for their control and therapy.

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