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Javed Ahamad Khan
1. Department of Natural Resource Management, Bedele Campus, Mattu University, Bedele-P.O. Box-318, Ethiopia
2. Division of Veterinary Public Health, Indian Veterinary Research Institute, Bareilly-243122, India
3. Department of Agricultural Microbiology, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh-202002, India

Ram Swaroop Rathore
Division of Veterinary Public Health, Indian Veterinary Research Institute, Bareilly-243122, India

Iqbal Ahmad
Department of Agricultural Microbiology, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh-202002, India

Rubina Gill
School of Agriculture, Lovely Professional University, Phagwara-144001, India

Fohad Mabood Husain
Department of Food Science and Nutrition, King Saud University, Riyadh-12372, Saudi Arabia

Javeed Akhtar
Department of Chemical Engineering, Adigrat University, P.O.Box-50, Adigrat, Tigray, Ethiopia

Leta Deressa Tolesa
Department of Chemistry, Mattu University, Mettu, P.O. Box-318, Ethiopia

Misbahu Abdu Ayanso
Department of Plant Science, Bedele Campus, Mattu University, Bedele, Ethiopia

Mekonnen Begna
Department of Natural Resource Management, Bedele Campus, Mattu University, Bedele- P.O. Box-318, Ethiopia

Correspondence:
Dr. Javed Ahamad Khan
Assistant Professor, Department of Natural Resource Management, Bedele Campus, Mattu University, Bedele-P.O. Box-318, Ethiopia
Email: jakfor.ra@gmail.com

Evaluation of *in vitro* antibacterial activity of *Carum copticum* extract against drug resistant *Staphylococcus aureus* from clinical and food sources

Javed Ahamad Khan, Ram Swaroop Rathore, Iqbal Ahmad, Rubina Gill, Fohad Mabood Husain, Javeed Akhtar, Leta Deressa Tolesa, Misbahu Abdu Ayanso, Mekonnen Begna

ABSTRACT

Staphylococcus aureus is commensal and human pathogen both. The bacterium is one of the most common causes of food borne infections also in all over the world. *C. copticum* (Ajwain) is an aromatic spice used as a medicinal plant from ancient time for treating different human diseases including infectious diseases. The highest anti-*staphylococcal* activity in terms of inhibition zone was recorded 18.5 mm whereas 14.5 mm as lowest. The MIC range of ethanolic extract was evaluated amid 1.56 mg/ml to 3.12 mg/ml. The *C. copticum* (seed) extract might be useful as anti-staphylococcal agent as well as natural food preservative.

Keywords: *Carum copticum*, Drug resistance, Natural products, Anti-*staphylococcal* Activity; Minimum Inhibitory Concentration (MIC).

INTRODUCTION

Staphylococcus aureus is a commensal bacterium which is marked as important human pathogen and it is a leading cause of bacteraemia and many other severe infections in humans. The bacterium has considered as one of the most common reason of food borne infections in most of the countries of the world. It has estimated that the population incidence of *S. aureus* associated infections ranges from 10 to 30 per 100,000 person-years in the industrialized world [1]. A comprehensive heterogeneity of foods has been remarked with the transmission of *Staphylococcus aureus*, including meat, milk and their products [2].

Antibiotics are key tools for the microbial infection treatment. However, indiscriminate application of antibiotics is displayed as key factor for the advent of multidrug resistant (MDR) strains of important sets of microorganisms including *S. aureus* [3]. In past, among pathogenic *S. aureus* of human origin, the antimicrobial resistance has been familiar and proclaimed from across the globe including India. However, the circumstances are frightening in under developing and developed countries both due to abrupt handling of antibiotics, the incidence of such resistant strains are on surge. Despite the fact that pharma companies discovered plenty of new antimicrobial compounds during the last three decades, resistance towards key antibiotics by bacteria is climbing regularly. Commonly, bacteria sustain the genetic capability to shift and acquire resistance towards these antibiotics which are employed previously as top-notch curative agents. Therefore due to rapid spread of resistant strains of bacteria, the continuous discovery of new antimicrobial agents is highly demanded [4]. Fortunately, natural chemical compound from plants and their products have been used in historic medicine across the globe and subjected to introduce of new antimicrobial compounds and other modern drugs. Since from last three decades, researchers are increasingly focusing their attention towards the herbal products for developing better drugs against drug-resistant bacteria including *Staphylococcus aureus*. Natural antimicrobial compounds extracted from typical and atypical spices were found to own antimicrobial activity and some analyst have probe the antibacterial action of spices against several bacteria like *E. coli*, *Salmonella*, *S. aureus*, *L. monocytogenes*, *Aeromonas spp.* [5] [6] [7]. *Carum copticum* (Ajwain) is an aromatic spice closely resembling thyme in flavour. It is much used as a medicinal plant in ancient time for treating different human diseases like diseases of heart, digestive tract and fever. Further various properties such as antihypertensive, antispasmodic, bronchodilator, hepatoprotective, anti-asthma, antiseptic, antioxidant, carminative, anticholinergic (functional antagonism), histamine(H(1)) inhibitory, and xanthine-like activity also have been described *in vitro* and *in vivo* [8]. However present study was carried out to evaluate the anti-*staphylococcal* activity of *C. copticum*.

MATERIALS AND METHODS

Bacterial cultures

The source of reference/standard cultures of *S. aureus* used in this study is depicted in Table1. All the *S. aureus* cultures were maintained in BHI with 20% glycerol broth and stored at -20°C.

Preparation of *C. copticum* extract

C. copticum (seed) were collected from local shops in Aligarh, India. The extract of *C. copticum* was prepared as per previously described method with slide modifications[9]. Briefly, collected seeds were dried in shade then 100 g of ground seed materials were soaked in 250 ml of 70% ethanol for 5 days. The obtained mixture containing plant material was purified by filtering the mixture through Whatman filter paper no.1. The resulting filtrate was concentrated in a rotatory evaporator at 40^0C temperature under vacuum.

Determination of Antibiotic Resistance

All the *S. aureus* strains were evaluated for antibiotic resistance examined by disc diffusion method as recited previously[10]. A total seven antibiotic discs (Hi Media) that associated to five groups of antimicrobial drugs viz. Penicillin (10 units) and Ampicillin (10 µg) (Penicillins or beta lactam), Chloramphenicol (30 µg) (Phenicols), Streptomycin (10 µg) and Kanamycin (30 µg) (Aminoglycosides), Lincomycin (2 µg) (Lincosamide), Erythromycin (15 µg) (Macrolides) were scrutinized. In concise, 0.1 ml of *S. aureus* suspension (10^8 cfu/ml) was processed using Brain Heart Infusion (BHI) broth (Pronadisa, Spain) and spread onto Muller Hinton (MH) agar (Hi-Media) petri dishes. These petri dishes were kept at 37°C for 18 h for incubation and the sensitivity of antibiotics examined measuring the inhibition zone diameter of bacterial growth around the antibiotic disc.

Assertion of Minimum inhibitory concentration (MIC) of *C. copticum* extract

Minimum inhibitory concentrations of the *C. copticum* extract against various *S. aureus* strains were determined by diluting the extract in Muller Hinton broth as described previously[11]. Briefly inoculum was prepared in MH broth by growing *S. aureus* isolate and incubated at 35°C for 18h. The inoculum concentration was adjusted to 10^4cfu/ml by McFarland's nephelometer tube. The concentrations of 6.25, 3.12, 1.56, 0.78, 0.39 and 0.19 mg/ml of the ethanolic extract were prepared in Muller Hinton broth, separately by using twofold serial dilution and incubated the tubes at 35°C for 18h.The MIC was recognized as the minimum concentration of the *C. copticum* extract dilutions that inhibited visible growth of *S. aureus.* Controls without *C. copticum* extract, without bacterial inoculum or with *C. copticum* extract were also included in the study.

Assertion of Anti-*staphylococcal* activity

In vitro anti-*staphylococcal* activity of ethanolic extract of *C. copticum* (seeds) was evaluated by agar well diffusion method as described previously[12]. For this purpose, bacterial inoculum was prepared by utilizing a loopful fresh bacterial growth of *S. aureus* and suspended in 5 ml of TSB broth. The inoculated TSB broth was then incubated at 35^0C for 18 h. Later, the inoculum concentration was adjusted to 10^4cfu/ml by using McFarland's nephelometer tube. Then 0.1 ml of diluted *S. aureus* inoculum (10^4 cfu/ ml) was spread on MH agar petri dishes. Wells of 6 mm diameter were stabbed into MH agar petri dishes and poured with 100 µl (6.25 mg/ml) of *C. copticum* extract. The solvent blank and chloramphenicol (100 µg/ml) antibiotic to which the *S. aureus* were sensitive also utilized as control. The plates were kept for 18 h at 35°C in incubator. Anti-*staphylococcal* activity was figure out by measuring the inhibition zone around the *S. aureus* growth.

RESULTS

Antibiotic resistance of *S. aureus* strains

The antibacterial resistance profiles of six *S. aureus* strains were analysed against seven antibiotics and represented in Table 2. The antibiotic susceptibility profiles verified drug resistance among 66.6% strains. The highest resistance 33.3% was observed against kanamycin whereas resistance of 16.6 was observed against penicillin, ampicillin, erythromycin, lincomycin and streptomycin. All the strains were found sensitive towards the chloramphenicol. Multidrug resistance (resistant more than one antibiotic) was observed in 33.3% of *S. aureus* strains (BSA-5 & SA-1).

Minimum inhibitory concentration (MIC) and Anti-*staphylococcal* activity of *C. Copticum* extract

The MIC range was observed amid 1.56 mg/ml to 3.12 mg/ml against various strains of *S. aureus*. The MIC of 1.56 mg/ml against four strains (BSA-5, FSA-21, CSA-39, MSA-4) whereas MIC of 3.12 mg/ml was observed against two strains (MTCC-1145, SA-1) by ethanolic extract of *C. copticum* (Table 3). Anti-*staphylococcal* activity of the extract of *C. copticum* obtained by the agar well diffusion method *in vitro* has shown in Table 3. The dry weight of the extract was found 6.25 mg/100 g of powder. Against majority of strains a clear zone of inhibition was observed (Fig. 1). The highest anti-*staphylococcal* activity in terms of inhibition zone was recorded 18.5 mm whereas 14.5 mm as lowest.

Table 1: Detail of standard bacterial cultures used in this study

S. aureus strains	Source of origin	Organization
BSA-5	Food (Beef)	Division of VPH, IVRI, Bareilly
FSA-21	Food (Fish)	Division of VPH, IVRI, Bareilly
CSA-39	Food (Chicken)	Division of VPH, IVRI, Bareilly
MSA-4	Food (Buffalo milk)	Division of VPH, IVRI, Bareilly
MTCC-1145	Clinical (Reference strain)	Division of Veterinary Medicine, IVRI, Bareilly
SA-1	Clinical (Pus)	Department of Ag. Microbiology, AMU, Aligarh.

Table 2: Antibiotic resistance profile of *S. aureus* strains used in this study

***S. aureus* strains**	**Source**	**Antibiotic resistance profile**
BSA-5	Food	K, L
FSA-21	Food	-*
CSA-39	Food	E
MSA-4	Food	-*
MTCC-1145	Clinical	K
SA-1	Clinical	Am, P, S

-* either sensitive/intermediate to antibiotics used.

P- Penicillin, Am- Ampicillin, Cl- Chloramphenicol, E- Erythromycin, K- Kanamycin, L- Lincomycin, S- Streptomycin.

Table 3: Detail of Minimum Inhibitory Concentrations (MICs) and Anti-*Staphylococcal* activity of ethanolic extract of *C. Copticum*

***S. aureus* strain**	**Yield in mg/100gm of powder**	**Minimum inhibitory concentration (mg/ml)**	**Anti-*staphylococcal* activity (radius in mm)**
BSA – 5	6.25	1.56	18.5
FSA – 21	6.25	1.56	15.7
CSA – 39	6.25	1.56	18.0
MSA–4	6.25	1.56	16.0
MTCC-1145	6.25	3.12	14.5
SA-1	6.25	3.12	14.8

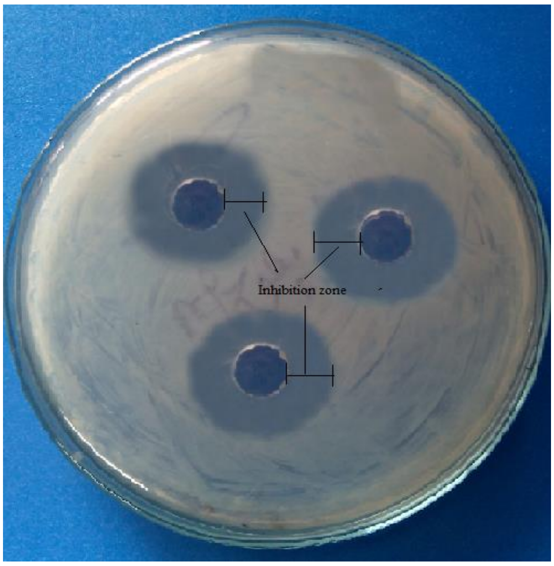

Figure 1: Anti-*staphylococcal* activity of ethanolic extract of *C. copticum*

DISCUSSION

S. aureus is a stern human pathogen brings innumerable bacterial infections. Initially the antibiotic penicillin was potentially efficacious for curing *Staphylococcal* infections. However, due ubiquitous application of the penicillin the surge of penicillin-resistant *S. aureus* (PRSA) was appeared. In 1970s, beta-lactamase-resistant penicillins like methicillin and oxacillin methicillin-resistant *S. aureus* (MRSA) were introduced and later they were identified as important cause of infectious diseases acquired in hospitals and communities throughout the world. Since then the resistance problem is existing among *S. aureus*. Moreover, many recent studies showed that drug resistant *S. aureus* may disseminate along the food chain which is suggesting vital exigency for managing programs to circumvent food transmission. However, the treatment of drug resistant *S. aureus* infections is complicated. It is observed that medicinal plants used in history obtained numerous chemical compounds with known therapeutic properties. They exhibited antidiabetic, antioxidant, antibacterial, anti-inflammatory, antipyretic activities, gastro-protective effects and noticeably more important medicinal properties [13]. The countless efforts have been performed to search plant-based natural antibacterial compounds active against multidrug resistant strains of *S. aureus*. Due to this vital need for various plant-derived anti-*Staphylococcal* antibacterial compounds sustaining effective MIC values have been identified in past two decades by concerned investigators. These examples grant to support and quantify the exploration of natural products importance [14] [15] [16]. Herbs and spices are in general observed as safe and manifested to be efficacious against many disorders. These natural products are also substantially utilized, specifically, in many Asian, African and other countries including India. In past few years, the use of spices/herbs is moderately increasing in developed countries also due to their useful properties. In history, essential oils derived from spices have been used traditionally in preservation of foods against microbial decay[17]. Many of these oils are "generally regarded as safe"(GRAS) and have pleasurable odours and taste. Moreover, essential oils have been used securely in herbal medicine as anti-microbial compounds[8].

In the present study, ethanolic extracts of *C. copticum* seeds exhibited strong anti-*staphylococcal* activity. The *in vitro* anti-*staphylococcal* activities and MICs of crude extract (ethanol) were recorded. The antimicrobial (anti-*staphylococcal*) property may be attributed due to the presence diverse essential oil fractions which sustain active components and influence the some metabolic functions of microbial cellular system. Terpenoids are recognized as chief compounds from spices so are speculated as major contributor for their antimicrobial properties. This has described by some researchers also that the antimicrobial activity of *C. copticum* is due to most frequent terpene compounds present, such as thymol, cymene, terpinolene, α-terpinene, α-phellandrene and β-phellandrene against several microbial strains including *S. aureus*[4] [17]. The anti-*staphylococcal* activity (inhibition zone) was amid 14.5 mm to 18.5 mm whereas the MIC ranged from 1.56 mg/ml to 3.12 mg/ml. The anti-*staphylococcal* activity in terms of inhibition zone was quite similar among food strains (15.7 mm to 18.0 mm) whereas little lower inhibition zone were observed within clinical strains (14.5 mm & 14.8 mm). Similarly growth of *S. aureus* (MTCC-1145, SA-1) in terms of MIC was inhibited by *C. copticum* extract with slight higher concentrations of 3.12 mg/ml with clinical strains (Table 3). It might be due to higher level of resistance of clinical strains[18] .

CONCLUSION

In conclusion, ethanolic extract of *C. copticum* seeds exhibited good anti-*staphylococcal* activity *in vitro* which permits it as potent source of new antimicrobial compounds against *Staphylococcus aureus* associated infections. Further *C. copticum* can be employed for utilizing as anti-*Staphylococcal* agents in meat and milk products as natural preservative depending upon the desired flavour of the products. However, further studies are still required to investigate its application in medicine and food industries because due to few impediment about utilizing this ethanolic extract, such as reduction of antimicrobial activity when extract are included to food stuffs consisting protein, carbohydrate, fat, and the pungent flavour of *C. copticum*. An achievable method is to utilize *C. copticum* extract in amalgamation with other preservatives like acid, salt, sugar, and other chemical preservatives etc.

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Conflict of Interest

None declared.

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ORCID ID

Javed Ahamad Khan: http://orcid.org/0000-0002-2327-8326

Ram Swaroop Rathore: http://orcid.org/0000-0003-3413-9767

Iqbal Ahmad: http://orcid.org/0000-0001-8447-4497

Fohad Mabood Husain: http://orcid.org/0000-0001-6598-4328

Javeed Akhtar: http://orcid.org/0000-0003-2429-1896

Leta Deressa Tolesa: http://orcid.org/0000-0002-4105-895X

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