

Full Length Research Paper

***In vitro* assessment of antimicrobial potency and synergistic effects of three medicinal plants' (*Mentha arvensis*, *Carissa carandas* and *Calendula officinalis*) extract against pathogenic bacteria**

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The increased attention toward replacing chemical antimicrobials with natural remedies has led to increased studies. To prove the antimicrobial characteristics of plant extracts, their effect on infectious bacteria has to be studied in laboratory. In this study, aqueous and ethanolic extract leaves/fruits of three commonly available medicinal plants (*Mentha arvensis*, *Carissa carandas* and *Calendula officinalis*) individually and in combinations were tested for their antimicrobial activity against three different pathogenic bacteria (*Bacillus cereus* (MCCB-0143), *Staphylococcus aureus* (MCCB-0139) and *Escherichia coli* (MCCB-0018)) using agar disc diffusion, agar well diffusion. Among plants extracts, *C. carandas* fruits (Cc) and *M. arvensis* ethanolic extract (MaE) showed strong antimicrobial activity against *E. coli* (MCCB-0018). The ethanolic combination (ethanolic *Calendula*: *Carissa*: *Mentha* 1:2:1) showed strong antimicrobial activity against *B. cereus* and F112 (fruits *Calendula*: *Carissa carandas*: *Mentha arvensis*, 1:2:1) showed significant ($P < 0.05$) antimicrobial activity against *S. aureus* (MCCB-0139). An individual and synergistic activity of ZOI ranging from 0.16-28.0 mm was active against test organism. The highest ZOI 28.0 ± 1.50 was observed in *Carissa* fruit extracts. The study reveals that plant extracts and their combination have significant effect of antibacterial activity. The plant extract seems promising for the development of a new herbal preparation for bacterial infection.

Key words: Antimicrobial activity, *Mentha arvensis*, *Carissa carandas*, *Calendula officinalis*.

INTRODUCTION

The concern towards the use of traditional medicine and medicinal plants nowa days is given much importance in developing countries for the maintenance of good health

(Kone et al., 2007). The scientific search for new drugs from natural products remains a serious task for scientists worldwide. It is a fact that a large segment of

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the population in tropical countries relies on traditional medicine for their health needs. Over 80% of populations in the developing world make use of medicinal plants extracts to provide health (WHO, 2002). The searches for new compounds with antimicrobial activity from plants have been the subject for intense research in recent years (Sofowora, 1996). This is due to the fact that plants are widely used in folk medicine to combat various diseases in human caused by pathogenic organisms (Cruz et al., 2007).

Escherichia coli is the most common uropathogen of all forms of urinary tract infections (UTI) and is responsible for 80% of cases. The commonest urinary pathogen accounting for over 80% of community-acquired infection is due to *E. coli* (Singh et al., 2014). *Staphylococcus aureus* is one of the leading causes of human infections of the skin, soft tissues, bones and joints (Yousef et al., 2013).

The potential of *Bacillus cereus* to cause systemic infections is of serious concern. Apart from gastrointestinal infections, it causes respiratory tract infections, eye infections, CNS infections, cutaneous infections and urinary tract infections. The potential of this bacterium to cause life threatening infections has increased (Pavani, 2014).

Many researchers are aiming to scientifically prove the use of plant extracts as an effective means of controlling infections and body malfunctions (More et al., 2008). It is estimated that plant materials are present in, or have provided the models for 50% Western drugs. Many commercially proven drugs used in modern medicine were initially used in crude form in traditional or folk healing practices, or for other purposes that suggested potentially useful biological activity. The primary benefits of using plant-derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more (Robbers et al., 1996).

Mentha arvensis (Lamiaceae) leaves are rich source of secondary phyto constituents, which impart the therapeutic effects against allergic and inflammatory diseases. On phytochemical analysis, it is found that *M. arvensis* leaves contain tannin, flavones, flavonols, xanones, flavonones and steroids. It shows antibacterial activity against infectious diseases microorganism (Khan and Khatoon, 2008).

Carissa carandas (Apocynaceae) is a species of flowering shrub in the dogbane family, Apocynaceae. It produces berry-sized fruits. *C. carandas* is traditionally used as stomachic, antidiarrheal and anthelmintic; stem is used to strengthen tendons; fruits are used in skin infections and leaves are remedy for fevers, earache and syphilitic pain. It fruits have also been studied for their analgesic, anti inflammatory and lipase activity (Kirtikar et al., 2003).

Calendula officinalis (Asteraceae), commonly known as pot marigold, is an important medicinal plant used in our traditional system of medicine to treat various diseases. It

is a phytotherapeutic plant rich in biologically active metabolites like sesquiterpens, alcohol, saponins, triterpens, flavonoids, hydroxycoumarin, carotenoids, tannin, and volatile oils (0.1-0.2%). These components have antiseptic action, anti-inflammatory, antiedematous, immunomodulatory activity and antimicrobial effects (Ao, 2007).

This study aimed to investigate the different extractions and preparations of these plants for *in vitro* antibacterial activity and scientifically justifying medicinal uses of these plants. The bacteria used in this study are pathogenic which cause diseases like urinary tract infection, skin and inflammatory infections, etc.

The selection of methods and techniques for investigating *in vitro* antibacterial activity of medicinal plants can be a challenging task when faced with the various methods employed in literature. The different requirements of the selected bacteria and the novel uses of the medicinal plants formed the basis for selecting the methods used in this research.

MATERIALS AND METHODS

Sample collection

Plant samples, *M. arvensis* (Leaves), *C. Carandas* (Leaves, fruits) and *C. officinalis* (Leaves) were collected from "Botanical Garden, Banaras Hindu University". These were thoroughly washed, dried enough and crushed by hand. The dried material was crushed in mixer grinder to coarse powder. The dried powder was stored in airtight bottles at 28°C for further extraction.

Aqueous extraction

One gram of dried powder was extracted in 7.0 ml distilled water for 6 h at slow heat. Every 2 h, it was filtered and centrifuged at 5000 *g* for 15 min. The supernatant was collected and concentrated to make the final volume one-fourth of the original volume. It was autoclaved at 121°C and 15 lbs pressure and then stored at 4°C (Ghosh et al., 2008).

Solvent extraction

Two grams of dried powder was extracted in 20 ml of distilled water and kept on a rotary shaker at 190-220 rpm for 24 h. Thereafter, it was filtered and centrifuged at 5000 *g* for 15 min. The supernatant was collected and the solvent was evaporated to volume of one-fourth of the original volume.

Culture media and incubation conditions

The three pathogenic bacterial strains used in this study were selected from our strain collection, which included 1 Gram-negative bacteria *E. coli* (MCCB-0018) and 2 Gram-positive bacteria, *S. aureus* (MCCB-0139), and *B. subtilis* (MCCB-0143). All strains were grown on agar media supplemented with 0.5% NaCl, 1% peptone, tryptone, and yeast extract. All cultures were incubated at 37°C for 24 h. Gentamycin, ampicillin and penicillin were used as standard antibiotics against all pathogenic bacterial strain.

Antimicrobial susceptibility testing

For antimicrobial susceptibility methods described below, the bacterial suspensions were prepared by suspending three to five well-isolated colonies from appropriate agar plates into 3 ml broth (adjusted to pH 5.9) and the turbidity was adjusted equivalent to a 0.5 McFarland standard (No et al., 2002).

Bioassay studies

The disc agar diffusion method (Sardari et al., 1998; Abdullahi et al., 2010) was used for the test.

Disk diffusion assay

For the disk diffusion method, the bacterial suspension prepared above was inoculated onto the entire surface of agar plate (pH 5.9) with a sterile cotton-tipped swab to form an even lawn. Eight sterile paper disks impregnated with 20 µl diluted plant extracts were placed on the surface of each plate using a sterile pair of forceps. The plates were incubated aerobically at 37°C for 24 h. The diameter of inhibition zone was measured after 24 h incubation using a ruler.

Agar well diffusion method

Stock solution of test material: The herbal residues so obtained and stored at 4°C were dissolved in dimethyl sulfoxide (DMSO) to give a concentration of 0.2 g/ml. These were kept at 28°C till further use. During diffusion assay, DMSO was taken as a control. The range of volume of test solution was 10-15 µl. The wells were then sealed with molten Muller-Hinton Agar (MHA) and kept for 10 min. These plates were then swabbed with 0.5 Mc Farland adjusted 16-18 h old culture of the test organisms and incubated at 37°C overnight. The inhibition zones were recorded in the test well as well as the control well. The assay was repeated twice (Klancnik et al., 2010).

Minimum inhibitory concentration (MIC)

Minimal inhibitory concentrations (MIC) are regarded as the lowest concentration of extract that inhibits growth of test organisms. The method of Eloff (1999) was used. Gentamycin and ampicillin were used as positive control in the antibacterial tests. The experiments were performed in triplicate.

Statistical analysis and preparation of data

All the treatment data were statistically evaluated with SPSS/16.00 software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by LSD's test. $P < 0.05$ was considered to indicate statistical significance. All the results were expressed as mean $\pm$ S.E. for the three replicate in each treatment.

RESULTS AND DISCUSSION

Antibiogram

In recent years, much research has been conducted in the field of antimicrobial effects of different plants. In this study it was found that the extract of plant prevented the

growth of *S. aureus*, *B. cereus* and *E. coli*.

Antibiotics belonging to different classes, penicillins, ampicillin and gentamicin were evaluated to profile the resistant pattern of the test microorganisms. The entire test microorganisms were resistant to ampicillin, gentamicin and penicillin.

S. aureus MCCB-0139 was sensitive to ampicillin only. Although less potent towards gram positives, the MIC ranges of these drugs are within ranges that make them useful for the treatment of patients with clinically important infections caused by *S. aureus*, in particular MRSA (Blondeau, 1999).

Disk diffusion methods

One way analysis of variance was used to determine whether levels of significance with plant extract treated in *S. aureus* MCCB-0139 bacterial strain were different among control, antibiotic Penicillin. The analysis showed significant difference among the treatment (F16, 34=1.7 $P < 0.05$). The treatment T3 (MaE), T5 (CcF), T6 (CcE), T12 (F112), T14 (E121), and T11 (F121) showed significant result with other treatment; treatment T6 (CcE) zone of inhibition (12 mm) showed greatest significance in this treatment (Table 1), which indicates (CcE) ethanolic *C. caranda* fruit showed very efficient antimicrobial result against *S. aureus* MCCB-0139. The combination of plant extract T12 (F112) (ethanolic extract of *C. officinalis*: *C. carandas*: *M. arvensis* 1:1:2) zone of inhibition (68 mm) showed greatest significant effect against *S. aureus* MCCB-0139; whereas in *B. cereus*, the analysis showed significant difference among the treatment (F 16.34=165.894, $P < 0.05$). The treatment T5 (CcF), T6(CcE), T7 (CcA), T9 (A112), T14 (E121), T15(E112) showed significant result with other treatments; somewhat treatment T6(CcE) and T5 (CcF) were greatly significant in this treatment, indicating (CcE) ethanolic *C. carandas* and *C. carandas* fruit (CcF) zone of inhibition (17 mm) showed very efficient antimicrobial result against *B. cereus* MCCB-0143. The combination of plant extract T15 (E121) (ethanolic extract of *C. officinalis*: *C. carandas*: *M. arvensis* 1:2:1) zone of inhibition (17 mm) was significantly against *B. cereus* MCCB-0143 (Table 1).

Analysis of variance was used to determine whether levels of significant with plant extract treated in *E. coli* MCCB-001 eight bacterial strain were different among control, antibiotic gentamicin. The analysis showed significant difference among the treatment (F16, 34=78.17 $P < 0.05$). The treatment T4 (MaA), T5 (CcF), T6 (CcE), T8 (A121), T9(A112), T11(F121), T14(E121), T15(E112) showed significant result with other treatment; somewhat treatment T6 (CcE) showed greatest significant in this treatment, indicating ethanolic *C. carandas* zone of inhibition (25 mm) showed very efficient antimicrobial result against *E. coli* MCCB-0018. The combination of plant extract T8 (A121) (aqueous extract of *C. officinalis*:

C. carandas: *M. arvensis* 1:2:1) zone of inhibition (18 mm) and T11 (F121) (Fruits *C. officinalis*: *Carissa*: *M. arvensis* (1:1:2) zone of inhibition (16 mm) were significant against *E. coli* MCCB-0018 (Figure 5). These findings present that *C. carandas* and *M. arvensis* showed antimicrobial activity, as indicated by zone of inhibition either separately or in combination.

The present study has shown that *M. arvensis* is potentially a rich source of antibacterial agent. This demonstrates its importance in traditional remedies. *M. arvensis* leaves extracts tested inhibited the growth of all pathogens and were very effective as compared to standard antibiotic penicillin. The ethanolic extract is highly effective against all pathogens because more organic compounds contain tannin, flavones, flavonols, xanones, flavonols, flavonones and steroids. It was leached in this solvent extract to detect antimicrobial activity and clearly demonstrated that ethanol is a better solvent as compared to aqueous extract. *M. arvensis* has the potential to generate herbal metabolites. This could serve as selective agents for anti-microbial property (WHO, 2004). The potential for developing antimicrobials from plants appears rewarding as it leads to the development of new drugs which is needed today. Further research is necessary to find the active compounds within and their full spectrum of efficacy. *C. carandas* is a good antimicrobial activity; it has a specific type of flavonoid, proanthocyanidins (PAC); in cranberries it provides urinary tract benefits by interfering with the ability of pathogenic *E. coli* to cause infections in the urinary tract (Vidlar et al., 2010). Cranberries are thought to provide health benefits due to their flavonoid and phytonutrient content s. These naturally occurring compounds have antioxidant and antimicrobial benefits that are evident in the oral cavity, gastrointestinal (GI) tract (Zhang et al., 2005). Bacteriuria is common when bowel is used as the material for urinary diversion because the epithelium lacks inhibitory action against bacterial adherence. The intestine normally exists in symbiosis with bacteria, with no inflammatory reaction. Therefore, urine in an intestinal neobladder is less bacteriostatic than urine in a native bladder. The adhesion mechanism of *E. coli* to the cells of the intestinal wall appears to be the same as those involved in adherence to the urothelium. Howell et al. (1998) showed that cranberry inhibits the adherence of *E. coli* to bladder epithelial cells. Thus, there was a rationale for treating bacteriuria with *C. carandas*. The ointment base of *C. officinalis* ointment (10%) has successfully cleared the condition. *C. officinalis* extract heals wounds as well as internal and external ulcers (Preethi and Kuttan, 2009). It is an antiseptic and in addition improves blood flow to the affected area. As an antifungal agent, it can be used to treat athlete's foot, ringworm, and candida infection because it has good stability, penetrability and is also easy to apply. A visual inspection method was used for assessing the antibacterial activity of plant extracts in

studies using the respective solvents as a re dissolving agent (Vidal-Ollivier et al., 2001). Overall, the three plants and their combination show significant antimicrobial activity against *S. aureus* than other plants.

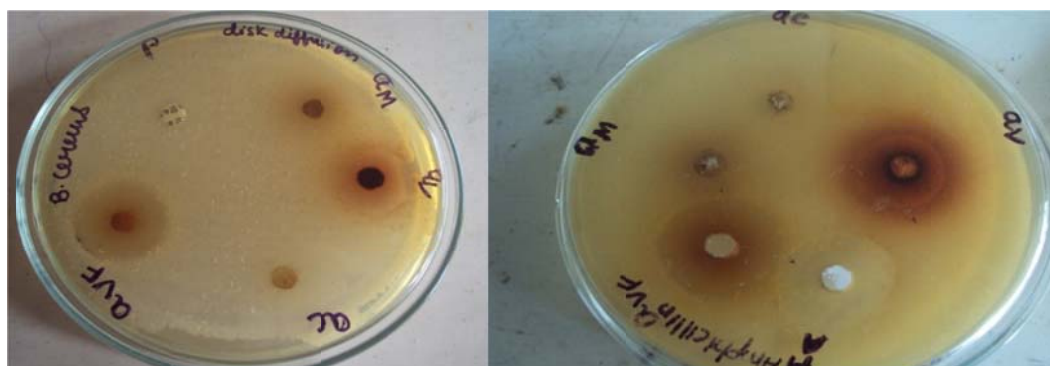
Agar well diffusion

Antimicrobial activity of plant extract was evaluated based on the diameters of clear inhibition zone surrounding the well. If there is no inhibition zone, it is assumed that there is no antimicrobial activity (Figure 1). Figure 4 shows representative agar well diffusion plates with different bacteria after 24 h incubation. The diameter of inhibition zone of *S. aureus* MCCB-0139 is larger than that of *E. coli* MCCB-0018, *B. cereus* MCCB-0143, indicating *S. aureus* is more susceptible to plant extract preparation than *E. coli* MCCB-0018, *B. cereus* MCCB-0143. Figure 5 shows the antimicrobial activity of plant extract of *C. carandas*, *C. officinalis*, *M. arvensis* with different combination against Gram-negative *E. coli* strains, and Gram-positive *S. aureus*, *B. cereus* MCCB-0143 bacterial strains by agar well diffusion method. With regard to diameters of the inhibition zones, plant extract with various combinations all demonstrated effective inhibition on the growth of these bacteria. Among these three bacterial strains, *E. coli* MCCB-0018 strains were significantly more susceptible when treated with *C. carandas* fruits while other stains were more resistant ($P < 0.05$). The mean of average size of inhibition zones varied from 0.01 to 2.8 cm against *E. coli* MCCB-0018, 0.13 to 1.6 cm against *B. aureus*, and 0.4 to 3.6 cm against Gram-positive bacteria *S. aureus* (Figure 2). The present study revealed potential antibacterial activity of the plant extracts obtained from the medicinal plant. The validation of potential antifungal activity has been validated against known organisms, such as *E. coli* MCCB-0018, *S. aureus* MCCB-0139, *B. cereus* MCCB-0143. The potential of the plant extract was tested against some standard antibiotics of different classes like penicillin, ampicillin and gentamicin.

The three medicinal plants selected for investigation in this study form part of the traditional medicinal plants used in India. These plants are indigenously used for treating skin conditions by topical applications onto the affected area. The growing demand and popularity of medicinal plants in rural as well as urban communities (Matsiliza and Barker, 2001) has placed many medicinal plants under the threat of extinction. Medicinal plants are used for treatment of acute to chronic conditions. The growing threat and spread of antibiotic resistance by a wide range of common pathogens has led to increased investigations into traditional medicinal plants as alternatives. Antibiotic resistances are not selective in that antibiotic resistance with the same consequences may affect people living in urban and rural communities around the world. Antibiotics that once readily cured a

Table 1. Antimicrobial activity of plant extracts with individual and synergistic effects against *S. aureus*, *B. cereus* and *E. coli* determined by disc diffusion method (data are expressed as mean $\pm$ SE, n=3).

Treatment	Code	Detail of combination tested	Zone of inhibition (mm)		
			<i>S. aureus</i>	<i>B. cereus</i>	<i>E. coli</i>
T1	CoE	<i>Calendula officinalis</i> ethanolic	6.3 $\pm$ 0.04	7.3 $\pm$ 0.04	6.5 $\pm$ 0.29
T2	CoA	<i>Calendula officinalis</i> aqueous	3.5 $\pm$ 0.03	0 $\pm$ 0.00	5.6 $\pm$ 0.08
T3	MaE	<i>Mentha arvensis</i> ethanolic	10 $\pm$ 0.09	7.3 $\pm$ 0.04	1.9 $\pm$ 0.08
T4	MaA	<i>Mentha arvensis</i> aqueous	7 $\pm$ 0.57	5.6 $\pm$ 0.09	14 $\pm$ 0.12
T5	CcF	<i>Carissa carandas</i> fruits	9 $\pm$ 0.05	17 $\pm$ 0.07	15 $\pm$ 0.12
T6	CcE	<i>Carissa carandas</i> ethanolic	12 $\pm$ 0.04	17 $\pm$ 0.12	25 $\pm$ 0.57
T7	CcA	<i>Carissa carandas</i> aqueous	1.8 $\pm$ 0.04	14 $\pm$ 0.09	5.6 $\pm$ 0.66
T8	A121	Aqueous <i>Calendula</i> : <i>Carissa</i> : <i>Mentha</i> (1:2:1)	15 $\pm$ 0.05	9.3 $\pm$ 0.04	18 $\pm$ 0.88
T9	A112	Aqueous <i>Calendula</i> : <i>Carissa</i> : <i>Mentha</i> (1:1:2)	13 $\pm$ 0.07	12 $\pm$ 0.10	12 $\pm$ 0.88
T10	A211	Aqueous <i>Calendula</i> : <i>Carissa</i> : <i>Mentha</i> (2:1:1)	4.3 $\pm$ 0.12	6 $\pm$ 0.05	4.3 $\pm$ 0.66
T11	F121	Fruits <i>Calendula</i> : <i>Carissa</i> : <i>Mentha</i> (1:2:1)	18 $\pm$ 0.02	8.5 $\pm$ 0.05	16 $\pm$ 0.88
T12	F112	Fruits <i>Calendula</i> : <i>Carissa</i> : <i>Mentha</i> (1:1:2)	68 $\pm$ 5.1	7.2 $\pm$ 0.01	7.3 $\pm$ 0.88
T13	F211	Fruits <i>Calendula</i> : <i>Carissa</i> : <i>Mentha</i> (2:1:1)	4.3 $\pm$ 0.03	5.2 $\pm$ 0.03	2.6 $\pm$ 0.33
T14	E121	Ethanolic <i>Calendula</i> : <i>Carissa</i> : <i>Mentha</i> (1:2:1)	21 $\pm$ 0.08	15 $\pm$ 0.05	10 $\pm$ 0.00
T15	E112	Ethanolic <i>Calendula</i> : <i>Carissa</i> : <i>Mentha</i> (1:1:2)	20 $\pm$ 0.05	17 $\pm$ 0.02	14 $\pm$ 0.05
T16	E211	Ethanolic <i>Calendula</i> : <i>Carissa</i> : <i>Mentha</i> (2:1:1)	4.3 $\pm$ 0.02	5.9 $\pm$ 0.05	0.33 $\pm$ 0.33
T17	ANT	Penicillin/Gentamycin	35 $\pm$ 0.02	34 $\pm$ 0.04	37 $\pm$ 0.066

**Figure 1.** Antibacterial activity of aqueous and ethanolic extracts of *Mentha arvensis*, *Calendula officinalis*, *Carissa carandas* with their different combination showing zone of inhibition by Agar well diffusion methods. Abbreviations are given in Table 1.

wide range of infections are becoming less useful mainly due to the misuse of antibiotics and the development of antibiotic resistance (Nostro et al., 2000). The antibacterial activity of the plant extracts against the different clinical strains is shown in Table 1. Establishing the antibacterial activity of the plant extracts also contributed on a whole to the scientific investigation done on medicinal plants. The selection and standardization of an appropriate plant extraction procedure is essential as it may influence the results on medicinal plants (Nostro et al., 2000; George et al., 2001). Ethnobotanical information revealed that all the plants selected in this study are traditionally

used fresh for medicinal purposes (Grierson and Afolayan, 1999).

Staphylococcus aureus

One way analysis of variance was used to determine whether levels of significance with plant extract treated in *S. aureus* MCCB-0139 bacterial strain were different among control, antibiotic Penicillin. The analysis showed significant difference among the treatment (F16, 34=65.109 P<0.05). The treatment T6 (CcE), T8 (A121),

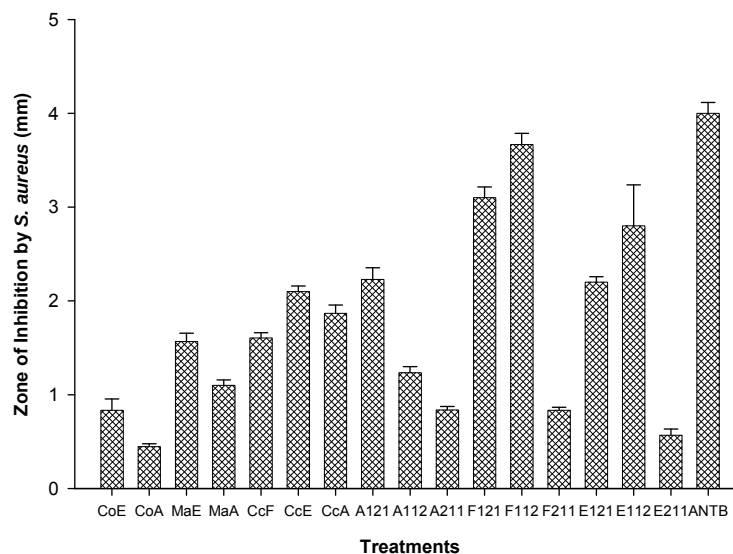


Figure 2. Evaluation of antimicrobial potency and synergistic effects of plant extract by agar well diffusion method against *Staphylococcus aureus* (data are expressed as mean $\pm$ SE, n=3). Abbreviations are given in Table 1.

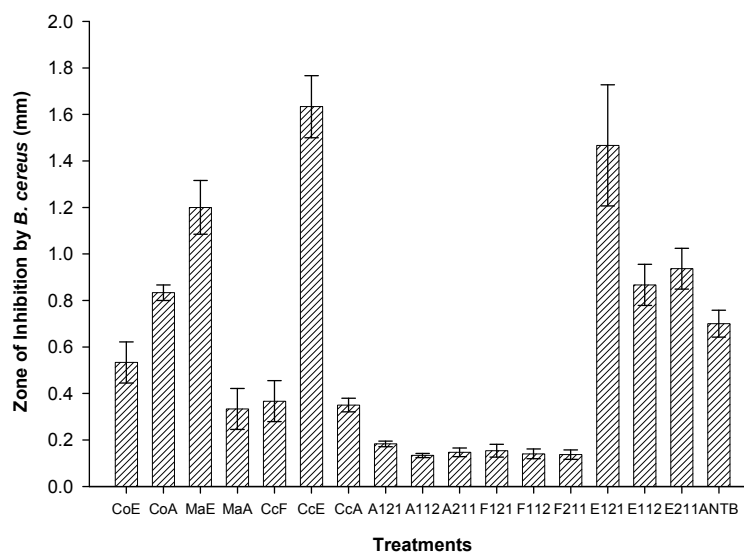


Figure 3. Evaluation of antimicrobial potency and synergistic effects of plant extract by agar well diffusion method against *Bacillus cereus* (data are expressed as mean $\pm$ SE, n=3). Abbreviations are given in Table 1.

T11(F121), T12(F112), T14(E121) and T15(E112) showed significant result with other treatment; somewhat treatment T12 had greatest significance in this treatment, indicating *M. arvensis* fruit showed very efficient antimicrobial result against *S. aureus* MCCB-0139 (Figure 2).

B. cereus

Analysis of variance was used to determine whether levels

of significance with plant extract treated in *B. cereus* MCCB-0143 bacterial strain were different among control, antibiotic penicillin. The analysis showed significant difference among the treatment (F 16,34= 28.135, $P < 0.05$; Figure 3). The treatment T3(MaE), T6(CcE), T14(E121), T15(E112), T16 (E211) showed significant result with other treatment; somewhat treatment T6 (CcE) showed greatest significance in this treatment, which indicates *C. carandas* fruit showed very efficient antimicrobial result against *B. cereus* MCCB-0143 (Figure 2). The combination of plant extract T14 (E121) (ethanolic

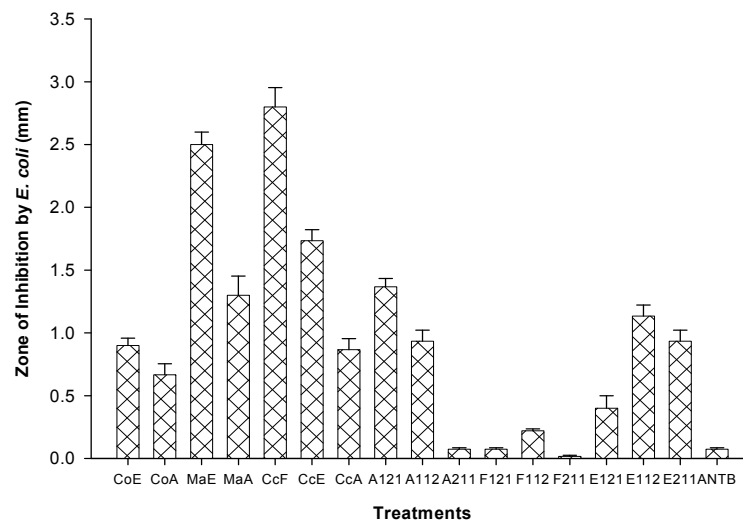


Figure 4. Evaluation of antimicrobial potency and synergistic effects of plant extract by agar well diffusion method against *Escherichia coli* (data are expressed as mean $\pm$ SE, n=3). Abbreviations are given in Table 1.

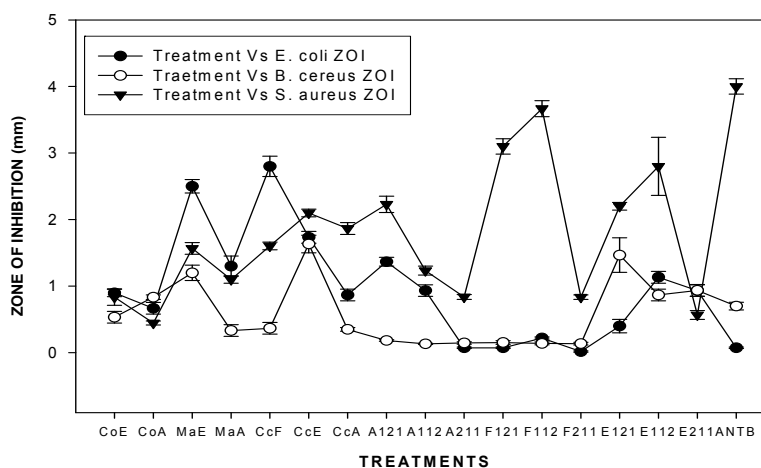


Figure 5. Evaluation of three antimicrobial potency and synergistic effects of three plant extract by disk diffusion method against *S. aureus*, *B. cereus* and *E. coli* (data are expressed as mean $\pm$ SE, n=3). Abbreviations are given in Table 1.

extract of *C. officinalis*: *C. carandas*: *M. arvensis* 1:2:1) and T12 (F112) (Fruits *C. officinalis*: *C. carandas*: *M. arvensis* 1:1:2) showed greatest significance against *B. cereus* MCCB-0143. These findings present that *C. carandas*, *M. arvensis* showed antimicrobial activity, as indicated by zone of inhibition separately or in combination (Figure 2).

E. coli

One way analysis of variance was used to determine whether levels of significance with plant extracts treated in *E. coli* MCCB-0018 bacterial strain differed among

control, antibiotic Gentamycin. The analysis showed significant difference among the treatments ($F_{16, 34} = 94.773$, $P < 0.05$). The treatment T3 (MaE), T5 (CcF), T6 (CcE), T8 (A121) T15 (E112) showed significant results with other treatments; somewhat treatment T5 showed greatest significance in this treatment (Figure 4), indicating *C. carandas* fruit showed very efficient antimicrobial results against *E. coli* MCCB-0018 (Table 1). The combination of plant extracts E112 (ethanolic extract of *C. officinalis*: *C. carandas* : *M. arvensis*, 1:1:2) showed greatest significance against *E. coli* MCCB-0018. These findings present that *C. carandas*, *M. arvensis* showed antimicrobial activity, as indicated by zone of inhibition separately or in combination.

Conclusion

This present research findings proved that this study is helpful for developing new drugs from plant extract for managing bacterial diseases and associated complications. The study reveals that plant extracts and their combination have significant effect on antibacterial activity. The plant extract seems promising for the development of a new medicine for bacterial infection.

Conflict of interests

The authors did not declare any conflict of interest.

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REFERENCES

- Abdullahi MI, Iliya I, Haruna AK, Sule MI, Musa AM, Abdullahi MS (2010). A Preliminary phytochemical and antimicrobial investigation of leaf extracts of *Ochna schweinfurthiana* (Ochnaceae) Afr. J. Pharm. Pharmacol. 4(2):083-086.
- Ao C (2007). Comparative anatomy of bisexual and female florets, embryology in *Calendula officinalis* (Asteraceae), a naturalized horticultural plant. Sci. Hortic. 114 (3):214-219.
- Blondeau J (1999). Expanded activity and utility of the new fluoroquinolones: a review. Clin. Ther. 21:3-40.
- Cruz MCS, Santos PO, Barbosa Jr AM, Melo DLFM, Alviano, CS (2007). plant extract as an effective means of infection and body malfunction, Antoniolli A.R., Alviano DS, Trindade RC.: J. Ethnopharmacol. 111.
- Eloff JN (1999). It is possible to use herbarium specimens to screen for anti-bacterial components in some plants. J. Ethnopharmacol. 67(3):355-360.
- George J, Laing MD, Drewes SE (2001). Phytochemical research in South Africa. S. Afr. J. Sci. 97 (3/4):93-06.
- Ghosh A, Das BK, Roy A, Mandal B and Chandra G (2008). Antibacterial activity of some medicinal plant extracts. J. Nat. Med. 62:259-262.
- Grierson DS, Afolayan AJ (1999). An ethnobotanical study of plants for the treatment of wounds in the Eastern Cape, South Africa. J. Ethnopharmacol. 67:327-332.
- Howell AB, Vorsa N, Der Marderosian A, Foo LY (1998). Inhibition of adherence of *P. fimbriatus* *Escherichia coli* to uroepithelial-cell surfaces by proanthocyanidin extract from cranberry. N Engl. J. Med. 339:1085-1086.
- Khan SW, Khatoun S (2008). Ethnobotanical studies on some useful herbs of Haramosh and Bugrote Valleys in Gilgit, Northern areas of Pakistan. Pak. J. Bot. 40(1):43-58.
- Kirtikar KR, Basu BD (2003) Indian Medicinal Plants, Lalit Mohan Basu, Allahabad. 2: 1546-1549.
- Klancnik A, Piskernik S, Jersek B, Mozina SS (2010). Evaluation of diffusion and dilution methods to determine the antibacterial activity of plant extracts. J. Microbiol. Methods 81:121-126.
- Kone WM, Atinehou KKK, Scou-N Douba Dossom (2007). Evaluation of 17 medicinal plants from Ivory Coast for their in vitro activity against *Streptococcus pneumoniae*. Afr. J. Trad. Complement. Altern. Med. 4:17-22.
- Matsiliza B, Barker NP (2001). A preliminary survey of plants used in traditional medicine in the Grahamstown area. S. Afr. J. Bot. 67(2):177-182.
- More G, Tshikalange TE, Lall N, Botha F, Meyer JJM (2008). Antimicrobial activity of medicinal plants against oral microorganisms J. Ethnopharmacol. 119:473-477.
- No HK, Park NY, Lee SH, Meyers SP (2002). Antibacterial activity of chitosans and chitosan oligomers with different molecular weights." Int. J. Food Microbiol. 74:65-72.
- Nostro A, Germano MP, D Angelo V., Marino A, Cannatelli MA (2000). Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Lett. Microbiol. 30 (1):379-384.
- Pavani G (2014). *Bacillus cereus* as a nongastrointestinal pathogen. Int. J. Res. Med. Sci. 2(1):28-30.
- Preethi KC, Kuttan R (2009). Wound healing activity of flower extract of *C. officinalis*. J. Basic Clin. Physiol. Pharmacol. 20(1):73-79.
- Robbers JM, Speedie and Tyler V (1996). Pharmacognosy and pharmacobiotechnology. Williams and Wilkins, Baltimore. pp. 1-14.
- Sardari SA, Gholameraza RG, Daneshmand M (1998). Phytopharmaceuticals. Part 1: antifungal Activity of Selected Iranian and Canadian plants. Pharm. Biol. 36:180-188.
- Singh B, Srivastava RK, Tilak, R, Katiyar D, Chauhan RS (2014). Urinary Tract infection and its risk factors in Women: an Appraisal. J. Pure Appl. Microbiol. 8 (5):4155-4162.
- Sofowora A (1996). Research on Medicinal Plants and Traditional Medicine in Africa. J. Altern. Complement. Med. 2 (3):365 – 372.
- Vidal-Ollivier E, Elias R, Faure F, Babadjanian A, Crespin F, Balansard G, and Boudon G (2001). Flavonol glycosides from *Calendula officinalis* flowers. Planta Med. 55:73-74.
- Vidlar A, Vostalova J, Ulrichova J, Student V, Stejskal D, Reichenbach R, Vrbkova J, Ruzicka F, Simanek V (2010). The effectiveness of dried cranberries (*Vaccinium macrocarpon*) in men with lower urinary tract symptoms. Br. J. Nutr. 104(8):1181-1189.
- WHO (2004). *Menthae Piperitae*, Monographs on Selected Medicinal Plants - Vol. 2 Geneva Switzerland, Department of Essential Drugs and Other Medicines, World Health Organization, pp. 188-205.
- World Health Organization (2002). Traditional medicine. Growing needs and potential, WHO policy Perspectives on Medicines, pp. 1n6, WHO, Geneva.
- Yousef M, Abouzeed AE, Fraje Z and Mohamed O A (2013). Antibacterial In-Vitro Activities of Selected Medicinal Plants against Methicillin Resistant *Staphylococcus Aureus* from Libyan Environment. J. Environ. Anal. Toxicol. 3:7.
- Zhang L, Ma J, Pan K, Go VL, Chen, J, You, W (2005). Efficacy of cranberry juice on *helicobacter pylori* infection: a double-blind, randomized placebo-controlled trial. *Helicobacter* 10(2):139-145.