Evaluation of Potential Antibacterial Activity of Aqueous Extracts Clove (Syzygium Aromaticum) Against Clinical and Foodborne Bacterial Strains

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Abstract: The medicinal and preservative uses of clove (syzygium aromaticum) have been claimed for centuries and it was valued by the people of ancient times for its beneficial health effects. This study was conducted to evaluate the antibacterial activity of aqueous extract of clove against clinical and foodborne isolates. In vitro antibacterial activity of different concentrations (40, 35, 30, 25, 20, 15, 10 and 5%) ranged from 400 mg/ml to 50 mg/ml of aqueous extract of clove was investigated against five clinical isolates including Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi and Streptococcus pyogenes and one foodborne isolate Salmonella enterica ATCC 14028. Agar well diffusion assay and filter paper disk diffusion assay were used to determine the growth inhibition. MICs and MBCs/MLCs were evaluated by broth dilution method. Antibiotic susceptibility testing was performed by Kirby-Bauer Disk Diffusion Susceptibility Test. The aqueous extract of clove exhibited strong inhibition against S. enterica ATCC 14028, E. coli, K. pneumoniae, P. aeruginosa, S. typhi And S. pyogenes. The maximum diameters of zones of inhibition that were measured ranged between 23-20 mm/100 µl in well diffusion assay and 12-11 mm/10 µl in filter paper disk diffusion assay for 40% (400 mg/ml) concentration of aqueous extract. The minimum concentration of extract that checked for growth inhibition was 50 mg/ml and was inhibitory and effective in well diffusion assay. The MBC of clove extract was found to be 40 mg/ml for P. aeruginosa, 70 mg/ml for S. typhi, 80 mg/ml for S. enterica ATCC 14028 and E. coli and 90 mg/ml for S. pyogenes. The MICs were also recorded. In conclusion, since the aqueous extract of clove has bacteriostatic and bactericidal activity against all test isolates that were resistant against various antibiotics tested, so its effective use can be made in therapy for infections caused by these organisms as in recent years multidrug resistance has emerged among pathogenic bacteria against antibiotic therapy. Moreover, as spice extract showed remarkable activity against S. enterica atcc 14028 and E. coli which are pathogenic and important food spoilage organisms so it may also provide an alternative to conventional antibacterial food additives.

Key words: Syzygium aromaticum • MBC • MLC • Clove Extract

INTRODUCTION

Discovering healing powers in plants is not a new idea and had been applied by the people of ancient times to meet their health needs [1]. Plants exhibit the tremendous ability to synthesize aromatic substances, most of which include phenols or their oxygen substituted derivatives [2]. In most of the cases, these substances aid plants in defense mechanism against attack of microorganisms and insects etc. some substances such as terpenoids, are responsible for aroma and flavor. Some herbs and spices used traditionally for seasonings of foods have also provided useful medicinal compounds [3].

The use of plant derivative as antimicrobials has been virtually non-existent due to the discovery of antibiotics in 1950s and it is reported that every year two or three antibiotics are being launched, on average [4]. In recent decades the pace of using herbal medicines is increasing as scientist have realized that the effective life span of antibiotics is limited due to multidrug resistance among pathogenic microorganism [5]. Therefore plant sources as
natural antimicrobials are being investigated. Moreover, people have become aware of problems that arise due to the misuse or over prescription of traditional antibiotics, in result the use of plant extracts and other alternative forms of medical therapy is getting great popularity from the late 1990s, literature describes [6]. Worldwide, infectious diseases are the major cause of mortality accounting for about one-half of all deaths in tropical countries. It will not be surprising to know the statistics in developing countries but mortality rate due to infectious diseases is rapidly increasing in developed countries also such as in USA due to difficulty in the treatment of MDR organisms [7]. Historically plants have been considered a safe and inspirational source for novel drug compounds as botanical medicines always have made great contributions to human health [8]. Several pharmaceutical industries are working for manufacturing of natural botanical drugs through isolating so called active substances from plant extract [9]. According to an estimation plant materials have contributed for about 50% western drugs. In last 3-4 decades an extensive research has been done to discover the hidden antimicrobial properties of herbs and spices and their components to make their use in medicines and food preservation. However, they have been used in ancient times for these purposes. Beneficial health effects and preservative powers of clove buds and of clove oil are well known and literature shows that clove has antibacterial activities [10], antiulcer activities [11], antioxidant activity [12] and in nanoparticles synthesis [13]. Keeping in view these beneficial activities of clove (S. aromaticum), an endeavor was made to check antimicrobial activity against model gram positive and gram negative clinical and foodborne isolates and assess its efficacy.

**MATERIALS AND METHODS**

**Microorganisms:** Total six bacterial isolates were included in this study. Out of six isolates five were clinical isolates which were collected from Darul Sehat Hospital, Karachi including Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi and Streptococcus pyogenes and one foodborne isolate Salmonella enterica ATCC 14028 was also included in the present study.

Purity of cultures was checked by making Gram's stain and by performing biochemical testing. MacConkey agar (Oxoid) was used as differential medium for Gram negative isolates. All isolates were sub-cultured and maintained on nutrient agar (Oxoid) slants.

**Preparation Of Maximum Recovery Diluent (MRD):** MRD was prepared by dissolving 1 g Peptone (Oxoid,) and 8.5 g NaCl (Scharlau) in 1 litre of distilled water and was autoclaved at 121°C for 15 min. It was used as diluent for the preparation of inoculum for all assays that were performed for the determination of antibacterial activity of clove extract, \textit{in vitro}.

**Aqueous Extract Preparation:** Good quality dry clove buds (Syzygium aromaticum) were purchased from the spice store. In order to obtain the spice extract 40 g of dry clove buds were crushed by using a domestic blender (Model: MJ-176NR, National, Japan) at medium speed and then transferred into 100 mL sterile distilled water in a screw capped glass bottle. The homogenate was allowed to macerate overnight for about 10-11 h. The homogenate was then heated and after cooling, it was first sieved through a fine mesh cloth and then it was sterilized by using 0.20 Micron corning® membrane filter. This extract was considered as the stock solution of 40% w/v concentration.

Different concentrations including 35, 30, 25, 20, 15, 10 and 5% concentrations from stock solution of extract were prepared by diluting it with required volumes of sterile distilled water for determination of antibacterial activity.

**Preparation of Inoculum:** Pure culture of test organism was inoculated onto broth medium (MacConkey broth, Oxoid) for Gram negative isolates while Tryptic soya broth (TSB, Oxoid) for Gram positive isolates and incubated at 37°C for 18-24 h. The turbidity of this broth suspension was adjusted to a 0.5 McFarland standard to get the initial count of 10^5-10^6 CFU/mL and was used within 15-20 min of preparation for antimicrobial activity assays and to evaluate MICs and MBCs/MLCs.

**Antimicrobial Activity Assays for Extract:** \textit{In vitro} antibacterial activity of different concentrations (40, 35, 30, 25 20, 15, 10 and 5%) ranged from 400 mg/mL to 50 mg/mL of aqueous extract of clove was investigated against five Gram negative and Gram positive isolates. Agar well diffusion assay and filter paper disk diffusion assay were used to determine the growth inhibition.

**Agar Well Diffusion Assay:** To perform agar well diffusion assay 18 mL of sterile Muller Hinton Agar (Oxoid) was poured into a sterile petri dish containing 1 mL of broth suspension (10^5-10^4 CFU/mL) of the test organism and mixed. Wells were prepared by using alcohol dipped sterile borer of 8mm in diameter and 100 µL
of different concentrations (40, 35, 30, 25, 20, 15, 10 and 5% w/v) of spice extract were added in different wells in the plate and left at room temperature for about 30 min. Sterile distilled water was used for negative control.

All plates were incubated at 37°C for 24±2 h. The diameter of the clear zone around each well was measured and expressed in mm as its antimicrobial activity. Each test was run in triplicate with three replications (n=3).

**Filter Paper Disk Diffusion Assay:** Filter paper disks of 6 mm in diameter were prepared by using Whatman grade No 4 filter paper and were autoclaved for sterilization. A sterile swab was dipped into the inoculum (10⁶-10⁸ CFU/mL) and swabbed on surface of Muller Hinton Agar (Oxoid). 10 µL of different concentrations (40, 35, 30, 25, 20, 15, 10 and 5% w/v) of spice extract were transferred on different sterile filter paper disks and were placed on the surface of the agar with the help of sterile forceps. Sterile distilled water was used as negative control? After 30 min the plates were incubated at 37ºC for 24±2 h. The diameter of the clear zone around the disks was measured in mm and expressed as its antimicrobical activity. Each test was done in triplicate with three replications (n=9).

**Determination of MBCs/MLCs**

**Broth Dilution Method:** Minimum Bactericidal Concentration (MBC)/ Minimum Lethal Concentration (MLC) for cultures were determined by using broth dilution method in Nutrient Broth (Oxoid). Each tube was inoculated with 10 µL of culture suspension (10⁶-10⁸ CFU/mL) of test organism, including inoculated extract-free broth tube to monitor the adequacy of the broth to support the growth of the organism. All tubes were incubated at 37ºC for 24±2 h. Each test was run in triplicate with three replications (n=9).

MBC/MLC was evaluated by sub-culturing the contents of the tube onto agar plates and after incubation for 24±2 h and 48±2 h no growth of the test organism at particular concentration was considered as MBC.

**Antibiotic Susceptibility Testing:** Antibiotic susceptibility testing was conducted by using the Kirby-Bauer disk diffusion susceptibility method [14]. The antibiotics (Oxoid) used were levofloxacin (5 µg), tetracycline (30 µg), oxacillin (1µg), gentamicin (10 µg), streptomycin (10 µg), amoxicillin (10 µg) and chloramphenicol (30 µg). Each test was done in triplicate (n=3).

**Data Analysis:** Data obtained from all experiments was analysed. Statistical analysis was carried out by using MS Excel 2010 and standard deviation, standard error, linear regression and Pearson’s correlation were evaluated.

**RESULTS**

A total of six isolates including five Gram negative and one Gram positive isolate were tested in vitro for antibacterial activity of different concentrations of aqueous extract of clove (S. aromaticum) in the present investigation. The aqueous extract of clove exhibited an excellent antibacterial activity against all clinical isolates and foodborne isolate at different concentrations ranged from 400 mg/mL to 50 mg/mL. Antibiotic susceptibility testing was also performed for all test organisms to determine the susceptibility or resistance to different antibiotics.

**Antibiotic Susceptibility Testing:** In antibiotic susceptibility testing all clinical isolates and foodborne isolate were resistant to oxacillin (Table 1). *P. aeruginosa* was the most resistant clinical isolate to various antibiotics and was found to be resistant against levofloxacin, tetracycline, amoxicillin, streptomycin and oxacillin (no zones of inhibitions were recorded). In addition it was found to be intermediate to chloramphenicol and susceptible to gentamicin. *K. pneumoniae* was the second most resistant clinical isolate which showed resistant to amoxicillin, streptomycin, oxacillin and gentamicin (no zones of inhibitions were recorded) and was also resistant to tetracycline (zone size was 10 mm). However it was sensitive to levofloxacin and chloramphenicol. *E. coli* was found to be resistant to amoxicillin and oxacillin. *S. enterica* ATCC 14028 and *S. pyogenes* were resistant against streptomycin and oxacillin but were susceptible to all other antibiotics tested. *P. aeruginosa* was the only isolate that showed resistant against levofloxacin (no zone of inhibition was recorded) whereas all other test isolates were susceptible to this antibiotic.

**Well Diffusion Assay:** The results of well diffusion assay showed that clove extract exhibits strong inhibitory effects against all test organisms (Fig. 1). The maximum diameter of zones of inhibition that were measured
Fig. 1: Antibacterial Activity of clove extract in well diffusion assay. [A] *S. enterica* ATCC 14028, [B] *E. coli*, [C] *P. aeruginosa* and [D] *S. typhi*.

### Table 1: Diameters of zone of inhibition of antibiotics against test isolates

<table>
<thead>
<tr>
<th>Organisms</th>
<th>LEV</th>
<th>C</th>
<th>TE</th>
<th>AML</th>
<th>CN</th>
<th>OX</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. enterica</em> ATCC 14028</td>
<td>38</td>
<td>32</td>
<td>20</td>
<td>22</td>
<td>21</td>
<td>NR*</td>
<td>12*</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>30</td>
<td>23</td>
<td>22</td>
<td>9*</td>
<td>23</td>
<td>NR*</td>
<td>21</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>23</td>
<td>21</td>
<td>10*</td>
<td>0*</td>
<td>0*</td>
<td>NR*</td>
<td>0*</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>0*</td>
<td>NR**</td>
<td>0*</td>
<td>0*</td>
<td>15</td>
<td>NR*</td>
<td>NR*</td>
</tr>
<tr>
<td><em>S. pyogenes</em></td>
<td>31</td>
<td>28</td>
<td>24</td>
<td>20</td>
<td>21</td>
<td>0*</td>
<td>10*</td>
</tr>
</tbody>
</table>

LEV (levofloxacin), C (chloramphenicol), TE (tetracycline), AML (amoxicillin), CN (gentamicin), OX (oxacillin) and S (streptomycin), NR (Not Required)

*Resistant, **Intermediate (According to Clinical Laboratory Standards Institute Performance Standard document). All values in data are the averages of each test that was done in triplicate (n=3).

### Table 2: Antibacterial Activity Of Different Concentrations Of Clove Extract In Well Diffusion Assay With Pearson's Correlation

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Diameter of zone of inhibition* (mm/100 µL of spice extract)±Std.DEV</th>
<th>Pearson's Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40%</td>
<td>35%</td>
</tr>
<tr>
<td><em>S. enterica</em> ATCC 14028</td>
<td>22.666 ±0.577</td>
<td>22.000</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>21.5±0.5</td>
<td>20.666 ±0.577</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>21.5±0.5</td>
<td>20.5±0.5</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>21.833 ±0.288</td>
<td>21±0</td>
</tr>
<tr>
<td><em>S. typhi</em></td>
<td>19.666 ±0.577</td>
<td>19.333 ±0.577</td>
</tr>
<tr>
<td><em>S. pyogenes</em></td>
<td>21.333 ±0.288</td>
<td>21±0</td>
</tr>
</tbody>
</table>

All values of zone of inhibition which shown in data are the average of all experiments that were done in triplicates with three replications (n=9). *Well diameter (8mm) is also included. 0 = No zone of inhibition.

Pearson’s correlation of +1 indicates that there is a perfect positive linear relationship between two variables.

The antibacterial activity of clove extract ranged between 23-20 mm/100 µL of spice extract (Table 2). The minimum concentration of clove extract that were tested for growth inhibition was 50 mg/mL (5%) and was found to be inhibitory against all test isolates.

The highest inhibition was recorded against foodborne isolate *S. enterica* ATCC 14028. It was the most sensitive isolate in well diffusion assay and gave the maximum zone of inhibition i.e. 23 mm at 40% concentration and was also strongly inhibited at 5% concentration of clove extract with zone size 18 mm which was comparatively larger than zones that obtained against other isolates at this concentration. Clove extract was very effective against *E. coli* and *K. pneumoniae*. Maximum zone sizes that obtained were same for both organisms i.e. 23 mm at 40% concentration. *P. aeruginosa* that was resistant against levofloxacin, tetracycline, streptomycin, amoxicillin and chloramphenicol in antibiotic susceptibility testing was
strongly inhibited by the clove extract and maximum zone size was 22 mm at 40% concentration. Even it was found to be sensitive to minimum concentration of clove extract i.e. 5% (zone size was 14 mm). P. aeruginosa was the only clinical isolate that was highly resistant against levofloxacin (5 µg), no zone of inhibition obtained in antibiotic susceptibility testing.

*S. typhi* was the only clinical isolate which was not inhibited at 5% concentration of clove extract but showed good susceptibility at 40% concentration with zone size 20 mm which was comparatively smaller than zones noticed for other isolates. In comparing with foodborne isolate *S. enterica* ATCC 14028 that showed the highest inhibition, less growth inhibition was observed against *S. typhi*. *S. pyogenes* clinical isolate as a Gram positive bacterial isolates was included in the present study and clove extract showed an excellent antibacterial activity against it. Maximum zone size was 21 mm at 40% concentration and even growth inhibition was recorded at 5% concentration (zone size 11 mm); however no inhibition was recorded at 10% and 5% concentrations.

In case of Gram positive isolate clove extract showed good inhibitory effect against *S. pyogenes* even in very small volume, as the zone size was 12 mm/10 µL at 40% concentration which was comparatively larger than zones that other isolates produced at same concentration. Moreover good inhibition was noticed for *S. pyogenes* at 15% concentration (zone size was 11 mm); however no inhibition was recorded at 10% and 5% concentrations. The values of $R^2$ were found to be significant for all test organisms.

**Table 4: Minimal Bactericidal Concentrations (MBCs) and Minimal Inhibitory Concentrations (MICs) of clove extract against test isolates in broth dilution assay**

<table>
<thead>
<tr>
<th>Isolates</th>
<th>MBC (mg/ml)</th>
<th>MIC (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. enterica</em> ATCC 14028</td>
<td>80</td>
<td>70</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>80</td>
<td>70</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>80</td>
<td>30</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td><em>S. pyogenes</em></td>
<td>90</td>
<td>80</td>
</tr>
</tbody>
</table>

**Discussion**

Results obtained from the present investigation show that aqueous extract of clove (*S. aromaticum*) has bactericidal and bacteriostatic potentials. Values of MICs which were recorded for this study ranged between 30 mg/mL to 80 mg/mL. Eugenol, the active component present in clove has been tested for antimycoplasmal activity *in vitro* and has found to be effective against *M. hominis* clinical isolate [15]. However different extraction methods have been applied for extraction of active components of clove which have a profound effect in the evaluation of its antimicrobial activity. Mandal et al. used the ethanolic extract of clove (*S. aromaticum*) and showed the maximum zone of inhibition 11 mm/10 µL at 40% concentration of clove extract, but showed no inhibition at 15, 10 and 5% concentrations. *S. typhi* was the only Gram negative clinical isolate which showed susceptibility at 5% concentration and the zone size was 9 mm.

**MICs and MBCs/MLCs:** Clove (*S. aromaticum*) extract showed bacteriostatic and bactericidal effects. The results of MICs and MBCs/MLCs of clove extract were recorded as in Table 4.

The MICs of clove extract was found to be 30 mg/mL for *P aeruginosa*, 60 mg/mL for *S. typhi*, 70mg/mL for *S. enterica* ATCC 14028 and *E. coli* and 80 mg/mL for *S. pyogenes*.

**Filter Paper Disk Diffusion Assay:** All isolates were tested for growth inhibition by filter paper disk diffusion assay. A very small volume of clove (*S. aromaticum*) extract i.e. 10 µL was used to check its effectiveness and it was found to be effective against all isolates from 40% concentration to 20% concentration (Table 3). *S. enterica* ATCC 14028, *E. coli*, *K. pneumoniae* and *P. aeruginosa* showed the maximum zone of inhibition 11 mm/10 µL at 40% concentration of clove extract, but showed no inhibition at 15, 10 and 5% concentrations. *S. typhi* was the only Gram negative clinical isolate which showed susceptibility at 5% concentration and the zone size was 9 mm.

In case of Gram positive isolate clove extract showed good inhibitory effect against *S. pyogenes* even in very small volume, as the zone size was 12 mm/10 µL at 40% concentration which was comparatively larger than zones that other isolates produced at same concentration. Moreover good inhibition was noticed for *S. pyogenes* at 15% concentration (zone size was 11 mm); however no inhibition was recorded at 10% and 5% concentrations. The values of $R^2$ were found to be significant for all test organisms.

**Table 3: Antibacterial Activity of Different Concentrations of Clove Extract in Filter Paper Disk Diffusion Assay with Pearson’s Correlation**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Pearson's Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter of zone of inhibition (mm)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40%</td>
</tr>
<tr>
<td><em>S. enterica</em> ATCC 14028</td>
<td>10.833 ±0.288</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>10.833 ±0.288</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>11±0</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>11±0</td>
</tr>
<tr>
<td><em>S. typhi</em></td>
<td>11±0</td>
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</tr>
</tbody>
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All values of zone of inhibition which shown in data are the average values of all experiments that were done in triplicates with three replications (n=9). * Disk diameter (6 mm) is also included. 0 = No zone of inhibition. Pearson’s correlation of +1 indicates that there is a perfect positive linear relationship between two variables.
checked its effectiveness against different isolates of methicillin resistant *S. aureus* (MRSA) but did not include other genera [16]. Whereas, in the present study a very simple, conventional and cost effective method was used for the preparation of spice extract. Distilled water was used as solvent instead of organic solvents such as methanol, ethanol and acetone for the preparation of extract which was screened for antibacterial activity against different bacterial isolated from five genera.

Clove buds extract by using organic solvents has found to be effective in inhibiting quorum sensing-controlled virulence factor production in *P. aeruginosa* PAO1 [17]. The volatile oil of clove was tested against plant pathogens, food poisoning and spoilage bacteria and presented good inhibitory effects [18].

López *et al.* [19] evaluated the antimicrobial activity of clove essential oil against foodborne isolates and reported that *P. aeruginosa* was the least inhibited isolate but in the present study *P. aeruginosa* clinical isolate which was found to be highly resistant to various antibiotics, was the highly inhibited isolate by clove extract (MIC 30mg/mL).

In Nzeako’s and his colleagues investigation water extract of clove (*S. aromaticum*) produced inhibition zones between 10 mm for *E. coli* and 15.7 mm for *P. aeruginosa* [20], whereas in present investigation zone size was 21.5 mm for *E. coli* and 21.833 mm for *P. aeruginosa* by using aqueous extract of clove, so the present study shows the significant and encouraging results.

Since there is a rapid increase in multidrug resistance among pathogenic microorganisms, therefore the plant source for the development of natural antimicrobials are being investigated. In addition, people have now become much aware of the health hazards due to the misuse or over prescription of traditional antibiotics so, consumer’s interest has now transferred towards the use of herbal medicines. According to a report there was 37% increase observed in the sales of botanical medicines over year 1995 [21] and approximately 80% of the world population prefer to use botanical preparations to fulfill their health needs and herbs and spices have proved safe and effective with less side effects [22]. Moreover the application of natural antimicrobials as preservative and food additives to inhibit the growth of food spoilage bacteria and fungi and to increase the shelf life of product has considered better due to the consumer preference, satisfaction and comfort, since various health hazards are associated with the use of artificial food preservatives and additives which have been known to cause health problems. Some of the foodborne outbreaks may take place at large scale. An outbreak of salmonellosis occurred in the USA in 1994 that affected about 224,000 persons. In addition, *S. enterica* serotype Newport highly multiresistant strain has emerged in the USA in 1999. Salmonellosis has considered the most occurring bacterial foodborne disease that accounts for more than 325,000 cases per year in OECD countries, according to the WHO report, so that the trend to discover new alternatives to control foodborne infections is being explored [23]. In the present study foodborne isolate *S. enterica* ATCC 14028 was also tested and clove extract has shown strong inhibitory effects against it as discussed earlier in results. Eugenol which is the active component and constitutes about 72-90% of the essential oil of clove buds is poorly soluble in water but soluble in organic solvents, while in the present study distilled water was used for the preparation of extract, so further work is required to evaluate the effectiveness of organic extract of clove that may be more effective and may give more encouraging results.

**CONCLUSION**

The present investigation has demonstrated that clove (*S. aromaticum*) could be used as an antibacterial agent. Results indicates that the aqueous extract of clove exhibits bacteriostatic and bactericidal activity against all test isolates that were resistant to various antibiotics, so its effective use can be made in therapy for infections caused by these organisms as in recent years multidrug resistance has emerged among pathogenic bacteria against antibiotic therapy. Moreover, as spice extract showed remarkable activity against *S. enterica* ATCC 14028 and *E. coli* which are pathogenic and important foodborne pathogens so it may also provide an alternative to conventional antibacterial food additives. In addition, there are less chances of developing resistant to natural antimicrobials when used in medicines and food preservation.

**REFERENCES**