ANTIMICROBIAL EFFECT OF SEED EXTRACTS, LEAVES AND CRUDE OIL OF CARDAMOM (ELETTARIA CARDAMOMUM) AGAINST DIFFERENT TYPES OF BACTERIA IN HILLA CITY, IRAQ

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ABSTRACT

Objective: Cardamom is commonly known as queen of spices for the versatile use in culinary practice. This study carried out to determine whether seeds, leaves aqueous extract and crude oil of cardamom (Elettaria Cardamomum Maton) has inhibitory activity on some pathogenic bacteria isolated from different clinical samples. Methods: In vitro antimicrobial activity of Elettaria cardamomum fruit extract, leaves and oil were studied against Staphylococcus aureus, S. epidermidis, Streptococcus pneumonia, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumonia, Enterobacter spp. Acinetobacter, E. coli, Serretia spp. and Salmonella typhi. The inhibitory effect of extracts were compared with standard antibiotic ciprofloxacin. Results: The largest mean zone of inhibition was obtained with the oil of fruits (35mm) and fruit aqueous extract and their leaves extract (30mm) this study depicts that the oil, leaves and fruit extract of Elettaria cardamomum can be used as potential source of novel antimicrobial agents used for different types of bacteria in different infections. On the basis of the experimental results and discussion, it can be postulated that the aqueous seed, leaves extracts and crude oil of
cardamom possesses the potent antimicrobial properties. These extracts have an active
to a Salmonella typhi Gram positive and Gram negative bacteria. Conclusions: Most of
cardamom’s extracts show high antibacterial activity against both gram positive and gram
negative bacteria, therefore cardamom can provide protection to a certain extent against our
natural enemies like bacterial pathogens.

Keywords: Elettaria cardamomum, zone of inhibition, different bacteria.

INTRODUCTION

Plant extracts have been used for a wide variety of purposes for many thousands of years.
Renewed interest in natural preventives. Many researches have documented the antifungal
and antibacterial effects of plants. Herbs and spices are generally considered safe and
proved to be effective against certain ailments. The plant of cardamom (Elettaria cardamomum) of the zingiberaceae family is one of the worlds very ancient and
expensive spices mainly grown in Sir Lanka and south India. The seeds of their ripe fruits are
used medicinally as aspice and also as flavoring agent in curries, coffee and cakes,
particularly in the Arab countries.

Recently, it is a common phenomenon that microorganisms are developing their resistance to
many commercial antibiotics that is a major cause of failure to treat various infectious
diseases. Now day's considerable attention has been focused on identifying naturally
occurring active compounds, capable of inhibiting and controlling some infectious bacterial
diseases. Cardamom is commonly known as queen of spices for the versatile use in
culinary practice. Cardamom is a perennial shrub with thick, fleshy, lateral roots which can
grow to a high of eight feet.

Cardamom oil is used in food perfumery and liquor pharmaceutical industries as a flavor and
a carminative. In medicine, it is used as a power full aromatic, antiseptic, stimulant
carminative, stomachic, expectorant, anti-spasmodic and diuretic. Ciprofloxacin is
asecond generation flourquinolone antibiotics. It spectrum of bacterial pathogens responsible
for respiratory, urinary tract, gastrointestinal and abdominal infection including gram
negative and gram positive bacteria. This study carried out to determine whether seeds,
leaves aqueous extract and crude oil of cardamom (Elettaria Cardamomum Maton) has
inhibitory activity on some pathogenic bacteria isolated from different clinical samples.
MATERIAL AND METHODS

Plant collection
Preparation of aquatic extracts
Elettaria cardamomum fruits, leaves and crude oil were obtained from the local market of Hilla city – Iraq (February, 2014). The samples fruit and leaves were carefully washed under running tap water followed by sterile distilled water and then air dried for two days, pounded using a mixer grinder and stored in airtight bottles. Preparation of aquatic extracts of Elettaria cardamomum fruits, and leaves [Aqueous extract was soaked 50 gram of Elettaria cardamomum fruits, and leaves by 100 ml distilled water, and allowed to stand for 72 hr, and sterilized by filtration (using Millipore 0.45 filter paper)]. This extract was considered as the 50% concentration of the extract. Oil of cardamom were collected from a retail food store. The extracts stored in sterile bottles and kept in freezer at 4°C until further use for screening of antimicrobial activity.

Bacterial Isolates
Different fifteen clinical microbial isolates (Gram positive, Gram negative (listed at table-1) were isolated and identified by using conventional biochemical tests and Api system (Biomeraux, France) and cultivated in pure culture, at microbiological laboratory/college of Medicine / Babylon University.

Table 1: Bacterial isolates used in this study

<table>
<thead>
<tr>
<th>Gram negative bacteria</th>
<th>Gram positive bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella typhi</td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>Staphylococcus epidermidis</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>Streptococcus pneumonia</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td></td>
</tr>
<tr>
<td>Enterobacter spp.</td>
<td></td>
</tr>
<tr>
<td>Acinetobacter</td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td></td>
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<tr>
<td>Serratiaspp</td>
<td></td>
</tr>
</tbody>
</table>

In vitro Antibacterial activity testing using Agar well diffusion assay
Loop full growths from bacterial isolates were inoculated into nutrient broth incubated at 37 °C for 18 hours. The bacterial suspensions were diluted with normal saline. Adjust the turbidity and compare with standard tube (McFarland number 0.5) to yield a uniform suspension containing 1.5×10⁸ CFU / ml. Cotton swab was dipped and streak into adjustment suspension the entire Mueller-Hinton agar (for all tested bacteria) surface of plates and the
plates were left for one 5-15 minutes at room temperature to dry. Media were cut into four wells (5mm diameter) by cork borer and add 20µ of the cardamom extracts solutions and oil of cardamom (The plates were performed in triplicates). All plate of the tested organisms was then allowed to incubate at 37°C for overnight. After 24 h of incubation, each extract was noted for zone of inhibition for all isolates. The diameters of the zone of inhibitions were measured by measuring scale in millimeter (mm).

**Antibacterial activity assay**
The antibacterial activity was determined by agar disc diffusion [Forbes, 2007]. Agar plates were inoculated with 0.1 ml broth culture of tested organisms and was spreader with sterile an L-shaped rod glass spreader. The antibiotics disks of ciprofloxacinwere add in the center of agar plate. (The plates were performed in triplicates). All plate of the tested organisms was then allowed to incubate at 37°C for overnight. After 24 h of incubation, each extract was noted for zone of inhibition for all isolates. The diameters of the zone of inhibitions were measured by measuring scale in millimeter (mm).

**Statistical analysis**
Bonferroni test recommended by ([12]) was used for statistical analysis (P ≤ 0.05) to show if there is any significant differences between results of cardamom extract.

**Test microorganisms**
Eleven gram positive and gram negative bacteria were used in this study to determine the antimicrobial activity of Elettariacardamomum. All bacterial strains were maintained on freshly prepared blood agar. Isolated were identified to the specieslevel based on the stander biochemical and microbiological methods ([10]).

**Antimicrobial assay**
The antimicrobial activity was tested using agar well diffusion method. Agar plates were swabbed with 100ml of respected broth culture (1.5x10⁶ CFU/ml), standardized by 0.5 MacFarland) and were kept at room temperature for 15min for absorption to take place. Wells of 6mm size were made with sterile borer in inoculated agar plates and loaded with 100ml of plant extracts. Prior to incubation at 37°C for 24hrs. The petri dishes were kept at room temperature for 15 min. in order to promote diffusion of the extracts into the agar ([13], [14]). All the tests were made in triplicate and the mean diameter of inhibition zones in millimeter.
RESULT AND DISCUSSION

The results of the antimicrobial activity assays indicated that cardamom (seeds, leaves and oil) had inhibiting activity on *Staphylococcus aureus*, *S. epidermidis*, *Streptococcus pneumonia*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumonia*, *Enterobacter spp.*, *Acinetobacter*, *E.coli*, *Serretia spp.* and *Salmonella typhi*. (Fig. 1, 2, and 3).

Statistical analysis showed no significant differences between effect of aquatic extract of leaf cardamom and oil of cardamom extracton bacterial isolates, there were significant differences between aquatic extract of seed cardamom and oil of cardamom extracton bacterial isolates, there were significant differences between aquatic extract of leaf cardamom and aquatic extract of seed cardamom extract on bacterial isolates, on bacterial isolates, at level (P ≤ 0.05). The beneficial health effects of extracts from much type of plants that are used as seasoning agents in foods and beverages have been claimed for countries.

In this study, the purpose was to examine the inhibitory effects of cardamom (*Elettaria Cardamomum Maton*) seed extract, leaves extracts and crude oil. On some pathogenic bacteria causing different illnesses in humans with agar well diffusion method as in vitro. In this study, extracts of cardamom displayed a variable degree of antimicrobial activity on different microorganisms. *Streptococcus pneumoniae* and *Enterobacter spp.* were found to be more sensitive strains than other to crude oil (35mm), while *Serratia spp.* and *Salmonella typhi* present more sensitive to leaves extract (30mm), and *E.coli* present the more sensitive strain for seeds extract (30mm). On the other hand *Staphylococcus aureus*, *S. epidermidis*, *Pseudomonas aeruginosa* and *Proteus mirabilis* were found to be resistant to seed extract.

Antimicrobial characteristic of the herbs are due to various chemical compounds including volatile oils, alkaloids, tannins and lipids that are presented in their tissues, cardamom seed yield 4% of volatile oil containing a high proportion of terpinyl acetate and cincole and small quantities of other monoterpenes, including, alcohols and esters[^15^]. Similar to our findings,[^16^] reported that the extract of *Elettaria Cardamomum* seed displayed a variable degree of antimicrobial activity on different microorganism. *Staphylococcus aureus* was found to be more sensitive strain than the others.

Some investigators noted that sensitivity of microorganisms to chemotherapeutics differs according to type of strains. Antimicrobial characteristics of herbs are due to various
chemical compound including volatile oils, alkaloids, tannins and lipids that are present in their tissue\(^{[17]}\). The chemical composition of Elettaria Cardamomum varies considerably with variety, region and age of the product. The content of volatile oils in the seeds is strongly dependent on storage conditions\(^{[18]}\). Furthermore, (Figure 4) show the effect of seed extracts, leaves and crude oil of cardamom (Elettaria cardamomum) against different types of bacteria. Crude oil of cardamom was more effective against different types of bacteria than other cardamom extracts (leaves and seed extracts). The antibacterial activity of Crude oil of cardamom contributed to the active component of this extract.

This result agrees with the results of \(^{[19]}\) who found out that the content of essential oil of Cardamom in the seeds is strongly dependent on storage conditions, but may be as high as 8%. In the oil were found α terpineol 45%, myrcene 27%, limonene 8%, menthone 6%, β-phellandrene 3%, 1,8-cineol 2%, sabinene 2% and heptane 2%. Other sources report 1,8-cineol (20 to 50%), α-terpenylacetate (30%), sabinene, limonene (2 to 14%), and borneol. In the seeds of round cardamom from Jawa (A. kepulaga), the content of essential oil is lower (2 to 4%), and the oil contains mainly 1,8 cineol (up to 70%) plus β-pinene (16%); furthermore, α-pinene, α-terpineol and humulene were found.

Recently, several authors reported that cardamom has antibacterial, antifungal, anti-cancer, antioxidant and also gastro prote effect\(^{[16, 20, 21, 22, and 23]}\). On the other hand, Antibacterial activity of cardamom (Elettaria cardamomum) extract was compared a ciprofloxacin, Figure (5) summarized the antibacterial isolates from different site of infection were sensitive to antibacterial ciprofloxacin, and the effect of this antibacterial was less than the effect. Today, most pathogenic organisms are becoming resistant to antibiotics. To overcome this alarming problem, the discovery of novel active compounds against new targets is a matter of urgency. Most of the spices extracted either in water or in organic solvents have biologically active compounds, which can be used in the synthesis of potent drugs. Thus spices, which are normal ingredients of our routine food preparations, can provide protection to a certain extent against our natural enemies like bacterial pathogens.

The present study also reported that cardamom has potent antimicrobial activity against some human pathogenic Gram positive and Gram negative bacteria according to the zone of inhibition results showed that the aqueous extracts of seeds, leaves and crude oil of cardamom were observed against Salmonella typhi this result similar to that of \(^{[24]}\). Moreover, relatively higher activity was seen at high concentration of oil of cardamom against
Salmonella typhi\textsuperscript{(25)}. Moreover, in the present study, bacterial species including Gram positive and Gram negative bacteria exhibited different degree of sensitivity to the test extract that may occur due to the differences of the chemical composition and structure of cell wall of both types of microorganisms\textsuperscript{(26)}.

Figure (1) effect of seed extracts, of cardamom (Elettariacardamomum) against different types of bacteria.

Figure (2) effect of leaves extracts and of cardamom (Elettariacardamomum) against different types of bacteria.
Figure (3) effect of crude oil of cardamom (Elettaria cardamomum) against different types of bacteria.

Figure (4) effect of seed extracts, leaves and crude oil of cardamom (Elettaria cardamomum) against different types of bacteria.

Figure (5) effect of ciprofloxacin against different types of bacteria.
CONCLUSION
On the basis of the experimental results and discussion, it can be postulated that the aqueous seed, leaves extracts and crude oil of cardamom possesses the potent antimicrobial properties. These extracts have an active against *Salmonella typhi* Gram positive and Gram negative bacteria.

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REFERENCES
10- Forbes BA, Sahm DF and Weissfeld AS. (Bailey and Scotts' Diagnostic microbiology)


13- Khokrae 2008

14- Rios 1988


25- Singh G, Kiran S, Marimuthu P, Isidorov V and Vinogorova V. (Antioxidant and antimicrobial activities of essential oil and various oleoresins of Elettaria Cardamomum
(seeds and various oleoresins of Elettaria Cardamomum (seeds and pods)). JSciFood Agric. 2009; 88:280-289.