COMPARATIVE IN VITRO ANTIMICROBIAL ACTIVITY OF ELETTARIA CARDAMOMUM FRUIT AND MENTHA SPICATA LEAVES

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ABSTRACT

The study aim was to evaluate the antibacterial activities of E. Cardamomum and Mentha spicata N. hexane, methanolic and aqueous extracts. Antimicrobial property of plant extracts were evaluated using disc diffusion technique against two Gram positive bacteria (Staphylococcus aureus and Bacillus subtilis), three Gram negative bacteria (Escherichia coli - Pseudomonas aeruginosa and Salmonella typhi) and one fungi (Candida albicans). The results showed that all the test organisms including C. albicans were resistant to the methanolic and aqueous extracts. Except Pseudomonas aeruginosa, the rest of test organisms were sensitive to n-Hexane extract with C.albicans being the most sensitive. The methanolic extract of Mentha spicata was the most active inhibiting all the test organisms except Pseudomonas aeruginosa which is again resistant to this extract but C. albicans being the most sensitive. It was also found that E. coli was sensitive to both n-Hexane and aqueous extracts.

KEYWORDS: Elettaria Cardamomum, Mentha spicata, antimicrobial activity, N.hexane, methanol, water.
INTRODUCTION
The bacterial diseases are becoming serious threat in developing countries, where peoples are not aware of their primary healthcare. Due to the lack of proper treatment, indiscriminate use of antibiotics and also ignorance are the major problems to control such bacterial diseases. Nowadays, it is a common phenomenon that microorganisms are developing their resistance to many commercial antibiotics that is the major cause of failure to treat various infectious diseases (Davies, 1994). Recently, considerable attention has been focused on identifying naturally occurring active compounds, capable of inhibiting and controlling some infectious bacterial diseases.

Cardamom is the dried fruit of the tall perennial herbaceous plant, *Elettaria Cardamomum* Maton, and belonging to the family Zingiberaceae, and locally known as Habahan. This herb is cultivated commercially in India, Sri Lanka, Guatemala and Tanzania. The fruit are tri-ocular, ovoid, oblong or greenish-brown capsules containing about 15-20 reddish brown seeds. The cardamom seeds have a warm, slightly pungent and highly aromatic flavor (Anonymous, 1977).

The extract from cardamom (*Elettaria Cardamomum* (L.) Maton) seeds produced by low temperature extraction with tetrafluoroethane characterized with higher content of 3 terpinyl acetate (36.8%), 1,8-cineole (29.2%), linalyl acetate (5.2%), sabinene (3.9%) and linalool (3.1%) with characteristic odour and taste (Gochev et al., 2012).

The chemical composition of cardamom varies considerably with variety, region and age of the product. The content of volatile oils in the seeds is strongly dependant on storage conditions (Lawrence, 1979). It has been used in India as a spice flavoring of tea, coffee, cakes, bread, and flavoring sweet dishes and drinks. Another use of Cardamom is in traditional Chinese and Indian medicine as a digestive aid, and for the treatment of intestinal gas. It has also been added on massage oils and lotions as well as soaps, detergents, and perfumes for its soothing properties (Ravindran, 2002). Cardamom oil is used in food, perfumery, and liquor a pharmaceutical industries as a flavor and a carminative. In medicine, it is used as a powerful aromatic, antiseptic, stimulant, carminative, stomachic, expectorant, anti-spasmodic and diuretic (Korikontimath, 1999).

*Mentha spicata* L (Spearmint) is a herbaceous, perennial plant growing 30–100 cm tall, and a wide-spreading fleshy underground rhizome. The leaves are 5–9 cm long and 1.5–3 cm
broad, with a serrated margin and produces flowers in slender spikes. Spearmint is often cultivated for its aromatic and carminative oil, referred to as oil of spearmint. The most abundant compound in spearmint oil is R-(-)-carvone, which gives spearmint its distinctive smell. Spearmint oil also contains significant amounts of limonene, dihydrocarvone, and 1, 8-cineol (Hussain et al., 2010)

In folk medicine, mentha can be used for cough, common cold, loss of appetite, sinusitis, fever, bronchitis, vomiting, indigestion, nausea, intestinal colic and (Starburch, 2001). It is also a safe and effective therapeutic option for the treatment of chemotherapy-induced nausea and emesis in patients (Najaran et al., 2013). It can have a calming effect when used for insomnia or massages. Essential oil of Spearmint was found to have some antimicrobial activity (Hussain et al., 2010). Mentha is widely used as a source of flavoring agents for the essential oils, and has been used as a valuable source of the potent antioxidant and in cosmetic industries (Shetty 2001).

Some of the most important bioactive phytochemical constituents are Tannin, Alkaloids, Saponins, Flavonoids, Steroids, Anthraquinones, Coumarins and Sterols and Terpenes (Ullah et al., 2011).

Because of the common use of Elettaria Cardamomum fruit and Mentha spicata leaves in the treatment of various ailments, we aimed to evaluate their antimicrobial activity against two Gram positive bacteria (Staphylococcus aureus and Bacillus subtilis), three Gram negative bacteria (Escherichia coli - Pseudomonas aeruginosa and Salmonella typhi) and one fungi (Candida albicans).

MATERIALS AND METHODS

Preparation of extracts

The cardamom (Eletteria Cardamomum) and Mentha spicata were obtained from Omdurman local market for spices. Cardamoms fruits and Mentha leaves were shade dried and ground using an electric grinder and were kept in plastic bags in the laboratory until extraction procedure. Using soxhlet apparatus, extraction was performed using different analytical grade solvent namely methanol, N.hexane and water.
Extraction of Plant Material
Sixty grams of plant material were extracted with 250 ml of methanol for 6 hours. The extract was filtered and concentrated. The steps above were repeated by using N-hexane. Water extract was done by steam distillation. One gram of each extract was dissolved in 1 ml of the same solvent to prepare 100% concentration.

Test microorganisms
The test microorganisms were kindly provided by the scientist of the National Health Institute, Khartoum. And they included.

Bacteria
Escherichia coli - ATCC/27853, Pseudomonas aeruginosa- ATCC/2785, Salmonella typhi-NCTC/25936, Bacillus subtilis- NCTC/8236 and Saphylococcus aureus- ATCC/25923
NCTC National Collection Type Culture, London, UK.
ATCC American Type Culture Collection, Manassas, AV, USA.

Fungi
Candida albicans

Culture Media
Bacteria were maintained on Nutrient Agar (Merck) and C. albicans on Sabouraud Dextrose Agar (Merck).

Antimicrobial activity test
In the present study, the disc diffusion technique as described by Hanafy and Hatem (1991) was applied. The N. hexane extracts of cardamom, were transferred into sterile bottles containing filter paper (Whatman No:1 ; 6 mm diameter). Bottles were then placed into a water bath (50°C) for complete removal of Hexane with periodical shakings to allow an even distribution of the extract between discs. All strains used in the study were inoculated to Tryptic Soya Broth (TSB) and incubated at 35±0.1°C for 24 h and were allowed to grow until they reach 108-109 cfu/ml. Then, 0.1 ml of inoculua from the prepared culture was transferred to Mueller-Hinton Agar (MHA) medium. The inoculua were spread to surface of plates with a sterile swab and the inoculated plates were dried at room temperature. Paper discs embedded within a plant extract were placed on previously inoculated plates and were incubated at 35±0.1oC for 48 h. After incubation the zones of growth inhibition around disks
were measured in mm (8). Antibacterial activity studies were carried out for each test strains in triplicates and average measurement were calculated.

RESULTS

The results of antimicrobial activity of *Elettaria Cardamomum* is shown in Table1. All the test organisms including *C. albicans* were resistant to the methanolic and aqueous extracts. Except *Pseudomonas aeruginosa*, the rest of test organisms were sensitive to n-Hexane extract with *C. albicans* being the most sensitive test organism with an inhibition zone of 18.5mm.

The results showing the antimicrobial activity of *Mentha spicata* extracts are shown in Fig. 1. It is clear that the methanolic extract was the most active inhibiting all the test organisms except *Pseudomonas aeruginosa* which is again resistant to this extract but *C. albicans* being the most sensitive with an inhibition zone of 15.5mm. It was also found that *E. coli* was sensitive to both n-Hexane and aqueous extracts with inhibition zones of 2.5 and 3.5mm respectively. The n-hexane extract also inhibited *Staph. aureus* with an inhibition zone of 19.5mm.

Table 1: Inhibition of microorganism by different *E. Cardamomum* extracts (100%)

<table>
<thead>
<tr>
<th>Microorganism</th>
<th><em>Elettaria Cardamomum</em> extract (100%)</th>
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<tbody>
<tr>
<td></td>
<td>N.hexane</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>12</td>
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<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>-</td>
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<tr>
<td><em>Klebsiella pneumonia</em></td>
<td>14</td>
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<tr>
<td><em>Salmonella tphi</em></td>
<td>15</td>
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<tr>
<td><em>Bacillus subtilis</em></td>
<td>14.5</td>
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<tr>
<td><em>Staphylococcus aureus</em></td>
<td>14.5</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>18.5</td>
</tr>
</tbody>
</table>
DISCUSSION

Naturally derived compounds from plants may have applications in controlling pathogens in foods (Bowles et al., 1998). The challenge is to isolate, stabilize and incorporate natural antimicrobials into foods without adversely affecting sensory, nutritional and safety characteristics (Beuchat and Montville, 1989). This has to be achieved without significantly increasing production, processing and marketing costs. Some compounds of essential oil have been found to inhibit peptidoglycan synthesis (Ogunlana, 1987), damage microbial membrane structures (Cox, 2000), and modify bacterial membrane surface hydrophobicity. In this study, extract of cardamom seed and spearmint showed a variable degree of antimicrobial activity on different microorganisms. The inhibitory effect detected in the present study may be due to the presence of various chemical compounds including volatile oils, alkaloids, tannins and lipids that are presented in their tissue. A study performed by Hêro and Akrayi, (2012) showed that the methanolic extract of Iraqi Cardamoms was better than ethanolic and aqueous extracts when tested against Staphylococcus aureus and Proteus mirabilis. On the other hand, Cowan (1999) found that the ethanolic extract was the most effective as antimicrobial whereas the same extract was found not effective against Enterococci strains (Sharma et al., 2013).

There is evidence in the literature that the essential oils of some Lamiaceae plants possess a moderate to good antibacterial activities (Goren et al., 2002) (Mimica-Dukić et al., 2003). Cantore et al. (2004) reported that M. spicata oil possessed better antibacterial activity than
the standard drugs streptomycin + penicillin. Sivropoulou et al., (1995) stated that the antimicrobial activity of *M. spicata* oil might be attributed to high contents of carvone and cis-carveol. Bader et al. (2003) stated that carvone exhibited better antimicrobial activity than the entire oil spresent in spearmint. The high concentrations of carvone and pulegone can be used as explanation for traditional uses of the two *Mentha* species for treating microbe related illnesses.

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**REFERENCE**


