Acetone extracts of indigenous plants and culinary spices used in India revealed activity against clinical isolates of methicillin resistant Staphylococcus aureus (MRSA)

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**KEYWORDS**

- MRSA
- Culinary spices
- Indigenous plants
- Antibacterial and MIC

**ABSTRACT**

Acetone extracts of Indigenous plants and culinary spices used in India were tested for antibacterial activity against clinical isolates of methicillin resistant Staphylococcus aureus (MRSA) by agar well diffusion assay and minimum inhibitory concentrations (MIC) were determined. Anacardium occidentale revealed highest inhibitory activity against all the tested strains of MRSA followed by Gloriosa superba and Mangifera indica. The zones of inhibition and MIC of Anacardium occidentale were in the range 20.0 ± 1.0 to 26.0 ± 1.25 mm and 9.54 to 38.15 ng/ml, respectively. Among the spices tested Nigella sativa showed strongest antibacterial activity with zones of inhibition in the range of 17.0 ± 1.0mm to 23.0 ± 1.25mm and MIC values in the range of 4.88 to 78.13 µg/ml, followed by Cinnamomum zeylanicum, Illicium verum and Pimpinella anisum. The other plants and spices included in the investigation did not show significant activity against MRSA. The results obtained for the plants and spices used in the present study have given new hope for the novel plant derived chemotherapeutic agents to confront the problem of nosocomial infections in particular, those involving MRSA.

**1. Introduction**

Phytotherapy is gaining momentum these days, owing to the adverse effects of the synthetic drug molecules and development of resistance by the microbes to these drugs. The problem of antimicrobial drug resistance is not new, but it has increased during the last decade, creating a serious threat to the treatment of infectious diseases (Conly, 2002). Some important human pathogens that have recently been reported to have acquired antibiotic resistance are; Mycobacterium tuberculosis, Staphylococcus aureus, Neisseria gonorrhoeae, Haemophilus influenzae, and Pseudomonas aeruginosa (WHO, 2002).

Staphylococcus aureus has been recognized as one of the most important gram positive bacterial pathogens contributing for infections and epidemics throughout the world. Resistance to Staphylococcus was reported in 1961, when first methicillin resistant Staphylococcus aureus (MRSA) was isolated in Europe, followed by Australia in 1966 and Unites States of America in 1968. MRSA continues to be a major cause of serious infection to man, both in hospitals and in the community. In the early 1980’s MRSA reports consisted of isolated cases but later in 1982 epidemic MRSA strains (EMRSA) were described as multi-resistant strains with special capacity to colonize patients and staff and cause widespread outbreaks of infections. These epidemic MRSA strains have subsequently spread to various parts of the world.

Resistance to antimicrobials is a natural biological phenomenon that can be amplified or accelerated by a variety of factors, including human practices. The use of an antimicrobial for any infection, real or feared, in any dose and over any time period, forces microbes to either adapt or die, in a phenomenon known as “selective pressure”. The microbes, which adapt and survive, carry genes for resistance, which can be passed on when bacteria replicates. The resistance mechanisms in microorganisms are based on four strategies. 1) Inactivation of the drug, 2) Prevention of the drug to reach its target, 3) Reduction of target’s susceptibility, or 4) Acquisition of new, insensitive target. Resistance can spread either vertically, by dissemination of resistant clones, e.g. in case of MRSA or horizontally, by inter- and intra-species-specific gene transfer, such as by i) Transduction, ii) Conjugation, and iii) Transformation (Brigitte, 2002).

The selective pressure exerted by widespread antimicrobial use is considered to be a major factor in the emergence of resistance. In some countries, antimicrobial drugs are available over the counter and in others, their abuse in prophylactic and empirical therapy and the indiscriminate use of broad-spectrum
antimicrobial drugs in the community have been major contributors. Resistance factors can spread rapidly, not only locally but also, with greater movement of people around the world, globally. Microorganisms and their resistance factors may also be transferred from country to country in animals and commercially produced fruits and vegetables.

Hospitals are a critical component of the antimicrobial resistance problem worldwide. The combination of highly susceptible patients, intensive and prolonged antimicrobial use, and cross-infection has resulted in nosocomial infections with highly resistant bacterial pathogens. Resistant hospital-acquired infections are expensive to control and extremely difficult to eradicate (WHO, 2002). The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, action must be taken to reduce this problem, for example, to control the use of antibiotic, develop research to better understand the genetic mechanism of resistance, and to continue studies to develop new drugs, either synthetic or natural (Nascimento, et al., 2000).

The use of medicinal plants all over the world predates the introduction of antibiotics. Herbal medicine has been widely used and formed an integral part of primary health care in China (Liu, 1987), Ethiopia (Desta, 1993), Argentina (Anesini and Perez, 1993) and Papua, New Guinea (Nick, 1995). Traditional medical practitioners in Southwest, Nigeria, use a variety of herbal preparations to treat different kinds of microbial diseases including MRSA associated diseases. Hence an attempt was made to screen selected indigenous plants and culinary spices for their antibacterial activity against clinical isolates of MRSA. So that new leads from the novel plant derived natural products could be developed for the treatment of infectious diseases.

Materials and Methods

Fresh indigenous plant material of Achyranthes aspera L. (whole plant), Amaranthus gangeticus Linn (leaf), Anacardium occidentale L. (cashew nut shell), Calotropis gigantea R. Br. (Leaf), Gloriosa superba L. (tubers), Mangifera indica L. (seed kernel), and Sesanba grandiflora L. Pers (Leaf) were collected from different locations of Krishna District, Andhra Pradesh, India. Whereas culinary spices: Cinnamomum zeylanicum Bl. (bark), Cuminum cyminum L.(seed), Illicium verum (hook fruit), Menthe piperata (leaf), Myristica fragrans, Houtt.(seed), Nigella sativa Linn. (seed), Pimpinella anisum L (seed) and Terminalia chebula Retz. (seed), were brought from Kaleswara Rao Market Vijayawada, Krishna District, Andhra Pradesh, India. Their botanical identities were determined and authenticated by A. Rama Krishna MSc., MPhil. Sr. Lecture, Head of the Department of Botany and Principal of Vemulapalli Kodanda Ramah College, Gannavaram, Krishna District, Andhra Pradesh, India. Samples were deposited in the department herbarium.

EXTRACTION PROCEDURE

Shade dried plant parts were reduced to fine powder and 10g of powder was taken into a 250 ml conical flask and 100 ml of acetone was added. After thorough mixing the flask was kept on a rotary shaker at 190 – 220 r/min for 48 hours and it was filtered through whatman filter paper No1. (WHATMAN Ltd., England). The filtrate was evaporated until dry in a water bath at 80°C temperature. The crude extract was prepared by dissolving known amount of the dry extract in dimethyl sulfoxide (DMSO; Merck Specialties Private Limited, Mumbai, India), to have a stock solution of 10 mg/ml concentration and stored at 4°C.

Bacterial culture

The MRSA strains used in this study were clinical isolates from blood, urine, pus, ear swabs, eye swabs and nasal swabs of patients in the diabetic care centers and intensive care units of various corporate hospitals in East Godavari, West Godavari and Krishna districts of Andhra Pradesh, India. The swabs and body fluids of patient’s samples were inoculated onto blood agar plates. The inoculated plates were incubated at 37°C for 18-24 h. After inoculation on blood agar, the swabs were placed in brain heart infusion (BHI) with 7.5% sodium chloride, which were also incubated at 37°C for 18-24 h. Inoculated BHI broth was sub cultured onto blood agar plates. From these blood agar plates, the colonies which were opaque, circular, pigmented with β hemolysis were identified as S. aureus by the Grams staining, catalase and coagulate (slide and tube) tests (Cadness, 1943). A total of 153 coagulate positive S. aureus strains were isolated and identified from 478 clinical samples. Antibiotic sensitivity pattern was determined by standard antibiotic disc diffusion technique using, oxacillin (1 µg), gentamicin (10 µg), erythromycin (15 µg), co-trimoxazole (25µg), vancomycin (30 µg) discs (Hi-media, India) with quality control strain of S. aureus (ATCC 25923) as per clinical and laboratory standards institute (CLSI) former National Committee for Clinical Laboratory Standards (NCCLS) guidelines. Bacterial suspension matching 0.5 Mc Farland turbidity standards were inoculated on Muller-Hinton agar containing 4%NaCl and 6 µg/ml oxacillin (Cipla pharmaceutical Pvt. Ltd, India). Isolates showing visible growth after 24h incubation at 33-35°C were identified as MRSA. For further confirmation of MRSA phenotypically, the isolates were inoculated on MeReSa Hi-chrome agar supplemented with cefoxitin and incubated at 370C for 24 h. Isolates producing bluish green colour colonies on MeReSa agar were considered as positive for MRSA. Oxford strains of S. aureus (ATCC 25923) sensitive to methicillin and S. aureus (ATCC 33591) resistant to methicillin were used as control organisms. In the oxacillin resistant coagulate positive S. aureus isolates, PCR for the amplification of the mecA gene was performed using specific primers F 5’TGGCATATGTTGTAACACATTCG- 3’ and R 5’-CTGGAACTTGTTGAGCAGAG- 3’. PCR was carried out in 25 µl volume with 10x PCR buffer, 20 pM primers, 1.5 mM MgCl2, 10 mM DNTPs, 1.25U Taq DNA Polymerase and 2 µl of template DNA. The cyclic conditions were initial denaturation 3 min at 920C, followed by 30 cycles of 1 min at 92°C, 1 min at 56°C, and 1 min at 72, with a final 3
min elongation step at 72°C. PCR product of 310 bp was visualized on 1% gels stained with ethidium bromide. Finally 82 MRSA were identified. Out of 82 MRSA, 5 isolates were selected for screening the plant extracts for antibacterial activity and determination of MIC. The type strains were procured from ATCC (USA) through Himedia Ltd, Mumbai, India.

Methods

Agar well diffusion method

The agar well diffusion assay described by Perez et al. (1990) was adopted. Briefly, 0.1 ml of diluted inoculums (2 x 108 CFU/ml) of test organism was spread on Muller-Hinton agar plates. Wells of 6 mm diameter were punched into the agar and filled with 10µl of plant extract of 10 mg/ml concentration and solvent blank (DMSO) separately. The plates were incubated at 37°C, overnight. Ten µl of oxacillin at 5 µg/ml concentration was used as positive control. Zone of inhibition of MRSA growth around each well was measured in mm. Each test was carried out in triplicate.

Determination of minimum inhibitory concentration (MIC)

The MIC of individual extract was determined using serial microplate dilution method of Eloff (1998) with few modifications. Acetone extracts of selected indigenous plants and spices were dissolved in DMSO to give a stock concentration of 10 mg/ml, while the antibiotic oxacillin used as a positive control was dissolved in ultrapure water to give a stock concentration of 5 mg/ml. Two fold serial dilution of test extracts (100 µl) in sterile normal saline was prepared in 96-well microtitre plate and 50µl overnight fresh bacterial culture suspensions were adjusted to 0.5 McFarland standard turbidity unit were added to each well (Bassole, et.al., 2003). The plates were incubated overnight at 37°C and bacterial growth was detected by adding 20 µl of p-iodonitrotetrazolium violet (INT) to each well. After incubation at 37°C for 30 min, INT is reduced to a red formazan by biologically active organisms, in this case the dividing bacteria. The concentration of extract was taken as the MIC when there is absence of red colour in the well due to inhibition of bacterial growth. Solvent controls and standard antibiotic oxacillin were included in each experiment that was done in triplicate.

RESULTS

Antibacterial activity of acetone extract of seven selected indigenous plants by agar well diffusion and their minimum inhibitory concentrations by microplate dilution method were presented in Table 1 and 2, respectively. Among the seven indigenous plants, Anacardium occidentale revealed strongest activity against the clinical isolates of MRSA including ATCC 33591 with zones of inhibition and MIC values in the range 20.0 ± 1.0 to 26.0 ± 1.25 mm and 0.009 to 0.038 µg/ml, respectively (Fig. 1). However, Mangifera indica and Gloriosa superba showed maximum zones of inhibition of 18.0 ± 1.25 and 19.0 ± 1.0 mm with MIC range standing at 2.44 to 78.13 and 4.88 to 39.06 µg/ml, respectively. Achyranthes aspera, Amanthas gangeticus, Sesbania grandiflora and Calotropis gigantea revealed no significant antibacterial activity.

Antibacterial activity of acetone extract of eight selected culinary spices by agar well diffusion and their minimum inhibitory concentrations by microplate dilution method were presented in Table 3 and 4, respectively. Among the spices, Nigella sativa revealed strongest activity against the clinical isolates of MRSA including ATCC 33591 with zones of inhibition and MIC values in the range 17.0 ± 1.0 to 23.0 ± 1.25mm and 4.88 to 78.13 µg/ml, respectively. The maximum zones of inhibition observed for Cinnamomum zeylanicum, Illicium verum and Pimpinella anisum against tested MRSA organisms were 17.0 ± 1.0, 16.0 ± 1.0 and 14.0 ± 0.5 mm respectively. Their respective MIC values were 19.53 to 78.13, 39.06 to 312.5 and 39.06 to 312.5 µg/ml. Cumminum cyminum, Mentha piperata, Myristica fragrans and Terminalia chebula did not show significant activity against clinical isolates of MRSA.

Table 1 Antimicrobial activity of acetone extracts of selected indigenous plants

<table>
<thead>
<tr>
<th>Name of the plant</th>
<th>Zone of inhibition in mm (Mean±SD)</th>
<th>ATCC3591 ATCC3591</th>
<th>Isolate 1</th>
<th>Isolate 2</th>
<th>Isolate 3</th>
<th>Isolate 4</th>
<th>Isolate 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Achyranthes aspera</td>
<td>0.0 ± 0.25</td>
<td>0.0 ± 0.25</td>
<td>0.0 ± 0.18</td>
<td>0.0 ± 0.12</td>
<td>0.0 ± 0.25</td>
<td>0.0 ± 0.18</td>
<td></td>
</tr>
<tr>
<td>Anacardium occidentale</td>
<td>2.0 ± 0.5</td>
<td>1.0 ± 0.25</td>
<td>1.0 ± 0.30</td>
<td>1.0 ± 0.30</td>
<td>1.0 ± 0.30</td>
<td>1.0 ± 0.30</td>
<td></td>
</tr>
<tr>
<td>Amanthas gangeticus</td>
<td>2.0 ± 0.25</td>
<td>1.0 ± 0.25</td>
<td>1.0 ± 0.25</td>
<td>1.0 ± 0.25</td>
<td>1.0 ± 0.25</td>
<td>1.0 ± 0.25</td>
<td></td>
</tr>
<tr>
<td>Calotropis gigantea</td>
<td>0.0 ± 0.25</td>
<td>0.0 ± 0.25</td>
<td>0.0 ± 0.10</td>
<td>0.0 ± 0.10</td>
<td>0.0 ± 0.10</td>
<td>0.0 ± 0.10</td>
<td></td>
</tr>
<tr>
<td>Gloriosa superba</td>
<td>19.0 ± 1.0</td>
<td>16.0 ± 1.25</td>
<td>17.0 ± 1.25</td>
<td>19.0 ± 1.25</td>
<td>19.0 ± 1.25</td>
<td>17.0 ± 1.25</td>
<td></td>
</tr>
<tr>
<td>Mangifera indica</td>
<td>15.0 ± 1.0</td>
<td>12.0 ± 0.5</td>
<td>11.0 ± 1.0</td>
<td>10.0 ± 1.0</td>
<td>14.0 ± 1.0</td>
<td>12.0 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>Sesbania grandiflora</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>DMSO</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>Oxacillin</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td></td>
</tr>
</tbody>
</table>

Values are Mean±SD of 3 replicates

Table 2 Minimum inhibitory concentration of acetone extract of selected indigenous plants (µg/ml)

<table>
<thead>
<tr>
<th>Name of the plant</th>
<th>Minimum inhibitory concentration (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Achyranthes aspera</td>
<td>2500, 1250, 2500, 625, 2500, 1250</td>
</tr>
<tr>
<td>Anacardium occidentale</td>
<td>0.019, 0.009, 0.019, 0.009, 0.009, 0.038</td>
</tr>
<tr>
<td>Amanthas gangeticus</td>
<td>2500, 2500, 1250, 2500, 1250</td>
</tr>
<tr>
<td>Calotropis gigantea</td>
<td>5000, 2500, 1250, 5000, 5000</td>
</tr>
<tr>
<td>Gloriosa superba</td>
<td>19.53, 39.06, 9.77, 39.06, 9.77, 4.88</td>
</tr>
<tr>
<td>Mangifera indica</td>
<td>78.13, 19.53, 78.13, 19.53, 78.13</td>
</tr>
<tr>
<td>Sesbania grandiflora</td>
<td>625, 1250, 312.5, 312.5, 1250, 625</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>2500, 2500, 2500, 1250, 2500, 2500</td>
</tr>
</tbody>
</table>

Values are mean of 3 replicates
Table 3 Antimicrobial activity of acetone extracts of selected culinary spices

<table>
<thead>
<tr>
<th>Name of the plant</th>
<th>Zone of inhibition in mm* (Mean±sd)</th>
<th>Isolate 1</th>
<th>Isolate 2</th>
<th>Isolate 3</th>
<th>Isolate 4</th>
<th>Isolate 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Chromemusum</td>
<td>17.0 ± 0.5</td>
<td>15.0 ± 1.5</td>
<td>14.0 ± 1.5</td>
<td>17.0 ± 1.0</td>
<td>12.0 ± 1.5</td>
<td>15.0 ± 0.5</td>
</tr>
<tr>
<td>*Cuminum cyminum</td>
<td>0.0 ± 0.0</td>
<td>0.7 ± 1.5</td>
<td>0.6 ± 1.5</td>
<td>0.9 ± 1.5</td>
<td>0.9 ± 1.5</td>
<td>0.8 ± 0.5</td>
</tr>
<tr>
<td>*Hiliconum</td>
<td>12.0 ± 1.5</td>
<td>12.0 ± 1.5</td>
<td>14.0 ± 1.5</td>
<td>12.0 ± 1.5</td>
<td>13.0 ± 1.5</td>
<td>16.0 ± 1.0</td>
</tr>
<tr>
<td>*Mentha Piperita</td>
<td>0.7 ± 0.3</td>
<td>0.7 ± 0.3</td>
<td>0.7 ± 0.3</td>
<td>0.7 ± 0.3</td>
<td>0.7 ± 0.3</td>
<td>0.8 ± 0.5</td>
</tr>
<tr>
<td>*Myristica fragrans</td>
<td>0.1 ± 0.5</td>
<td>0.3 ± 0.5</td>
<td>0.3 ± 0.5</td>
<td>0.3 ± 0.5</td>
<td>0.3 ± 0.5</td>
<td>0.3 ± 0.5</td>
</tr>
<tr>
<td>*Nigella sativa</td>
<td>21.0 ± 1.25</td>
<td>19.0 ± 1.42</td>
<td>22.0 ± 1.5</td>
<td>18.0 ± 1.0</td>
<td>17.0 ± 1.0</td>
<td>23.0 ± 1.25</td>
</tr>
<tr>
<td>*Pimpinella anuslii</td>
<td>14.0 ± 0.5</td>
<td>12.0 ± 1.0</td>
<td>13.0 ± 1.5</td>
<td>16.0 ± 0.5</td>
<td>14.0 ± 1.0</td>
<td>14.0 ± 1.0</td>
</tr>
<tr>
<td>*Terminalia chebula</td>
<td>0.6 ± 1.0</td>
<td>0.7 ± 1.0</td>
<td>0.8 ± 1.0</td>
<td>0.7 ± 1.0</td>
<td>0.7 ± 1.0</td>
<td>0.8 ± 1.0</td>
</tr>
<tr>
<td>*Oxacillin</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>*DMSO</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
</tbody>
</table>

Values are Mean±sd of 3 replicates

*Zone of inhibition includes 6 mm well diameter

Discussion

MRSA has gained much attention in the last decade, as a major cause of nosocomial infections. β-lactam antibiotic are the preferred drugs against Staphylococcus aureus infections, which act on Pencillin Binding Proteins (PBPP). All the Staphylococcus aureus strains have four PBP (PBP1 to PBP4), but MRSA express a special PBP (PBP2a) from mecA gene. PBP2a takes over the biosynthetic function of normal PBP3 in the presences of inhibitory concentration of β-lactams because PBP2 has a decreased binding affinity to β-lactams (Bachi and Rohrer, 2002). This has resulted in the development of multi-drug resistance against β-lactam and other antibiotics. Moreover, with the increased incidence of vancomycin-resistant MRSA (Hiramatsu, et al., 1997), the choice of drugs to be used against MRSA is shrinking day by day as susceptibility of MRSA to drugs is decreasing by target site alteration, enzyme modification and permeability changes (Brumfitt and Hamilton 1989). Although strategies have been proposed in an attempt to control the spread, the searches for new ways to treat MRSA infections stimulate the investigation of natural compounds as an alternative treatment of these infections.

Multiple drug resistance in human and animal pathogenic microorganisms have been reported in recent years from all over the world, particularly in developing countries, due to indiscriminate use of commercial antibiotics in the treatment of infectious diseases. Though, the resistance development by microbes cannot be stopped, appropriate action will reduce the mortality and health care costs by using antibiotic resistance inhibitors of plant origin (Service 1995). Moreover, traditional remedies utilizing plants still occupy a central place among rural communities of developing countries for curing various diseases in the absence of an efficient primary health care system (Ali, et al., 2001). Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in...
different countries and are a source of many potent drugs (Ahmad and Beg, 2001). The World Health Organization (WHO) is promoting and facilitating the effective use of herbal medicine in developing countries for health programs. The potential of higher plants as a source of new drugs is still largely unexplored. Hence, last decade witnessed an increase in the investigations on plants as a source of new biomolecules for human disease management (Grierson and Afolayan, 1999).

In the present study Anacardium occidentale, L. (cashew nut shell) revealed an excellent inhibitory activity against all the tested strains of MRSA with MIC in the order of 9.54 to 38.15 ng/ml which can be attributed to amphipathic anacardic acid which enters into the membrane lipid bilayers where it disrupts various enzymes, especially components of energy converting systems such as electron transport chains (ETCs) and ATPases due its surfactant nature (Gedam 1986). It also inhibits lipid synthesis of bacterial cells by inhibiting glyceral-3-phosphate dehydrogenase (Murata et al., 1997). Chelation might also play a role in the antimicrobial activity of anacardic acid as it shows high selectivity toward Fe2+ and Cu2+ and there by reducing their bioavailability for bacteria (Nagabhushana et al., 1991). Further, more anacardic acid is known to exert β-lactamase inhibitory activity (Coates et al., 1994).

The MIC range of G. superba against MRSA obtained in the present study was much lower than that reported by Hemaiswarya et al., (2009) where crude petroleum ether, methanol and aqueous extracts of the root tubers of G. superba exhibited a broad spectrum of antibacterial activity against both the Gram-negative and Gram-positive bacteria like Escherichia coli, Proteus vulgaris, Salmonella typhi, Bacillus subtilis, and Staphylococcus aureus with MIC of extracts in the concentration range of 1 mg/ml, this difference in MIC range might be due to differences in the solvents used for the extraction. Probably acetone has extracted the compound responsible for the antimicrobial activity better than the solvents used by Hemaiswarya et al., (2009).

Among the eight culinary spices tested, Nigella sativa, Linn. (seed) revealed strongest inhibitory activity against all strains of MRSA. The zones of inhibition obtained were in agreement with those of Mashhadian et al., (2005) who used Nigella sativa seed extract against Staphylococcus aureus and reported an average of zone of inhibition of 20 mm, this is almost similar to the range obtained in the present study (17 to 23 mm) (Fig. 2). The antimicrobial activity of Cinnamomum zeylanicum, Bl. (Bark) obtained in the present study could be attributed to the presence of eugenol and cinnamaldehyde (Farag, et al., 1989). These are the two phenolic components of cinnamonum, which render them effective against the tested micro-organisms. Activity shown by Illicium verum (hook fruit) was supported by a similar report from Khesorn Manthachit (2002) stating that crude ethanolic extract of the fruits of Illicium verum showed antimicrobial activity against gram positive cocci Staphylococcus aureus ATCC 25923, and gram negative Escherichia coli ATCC2592, Pseudomonas aeruginosa ATCC27853, Candida albicans ATCC, A. M. T. Antimychophytes. The antimicrobial activity of hook fruit extract might be due to presence of anethole (Lis-Balchin, et al. 1997). Pimpinella anisum (seed) also showed good antibacterial activity against all MRSA strains used. Methanol, acetone and petroleum ether extract of Pimpinella anisum, L fruit (aniseed) is active against gram positive and gram negative pathogenic bacteria like Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, and Klebsiella pneumonia and Candida albicans (Akhtar et al., 2008; Ates and Erdogru 2003). Cumminum cyminum (seed), Mentha piperata (whole plant) and Terminalia chebula (seed) and Myristica fragrans (seed) showed very poor antibacterial activity against all clinical isolates of MRSA but Narasimhan and Dhahe (2006) reported that trimyristin, an active compound obtained from seed of Myristica fragrans, exhibited good antibacterial properties against Gram-positive and Gram negative bacteria, the lack of activity in the present study might be due to presence of trimyristin in low concentrations in the fruit or failure of the solvent in the present study to extract this active compound.

In conclusion, the results suggest that Anacardium occidentale (cashew nut shell), Gloriosa superba (tubers) and Cinnamomum zeylanicum (bark), Illicium verum (fruit), Pimpinella anisum, L fruit (aniseed) and Nigella sativa (seed) can be explored for new leads to counter the problem of MRSA infections pending further studies.

References


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