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Antibacterial activity of *Calotropis procera* and *Ficus sycomorus* extracts on some pathogenic microorganisms

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In *vitro* antibacterial potential of chloroform, absolute ethanol, methanol, ethanol (70%) and aqueous extracts of *Calotropis procera* and *Ficus sycomorus* leaves and latex were evaluated against five Gram-negative bacteria (*Neisseria lactamica* ATCC 23970, *Salmonella typhi* ATCC 19430, *Shigella flexneri* ATCC 12022, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922) and two Gram-positive bacteria (methicillin-resistant *Staphylococcus aureus* MRSA ATCC 43300, *S. aureus* CONS ATCC 29213). The antibacterial activities were expressed as zone of inhibition; minimum inhibitory concentrations (MIC) and also the survival curve was determined as kinetic studies. Interestingly, among all the tested extracts, aqueous and ethanol (either absolute or 70%) of *C. procera* and *F. sycomorus* leaves and latex were the best solvents for elute polar antibacterial substances and showed bacteriocidal effect against most Gram-positive and negative bacteria. Also, latex extracts were more pronounced than leaf extracts on human pathogenic bacteria. The most resistant bacterium was *E. faecalis* against both plant extracts. On the other hand, *S. aureus* MRSA was the most sensitive bacteria especially with ethanol 70% extract of leaves and latex for both plants. The results of MIC for these extracts show more or less values higher than the chloramphenicol. Our conclusion confirms that, susceptibility of Gram-positive bacteria to the aqueous or ethanolic extracts of leaves for both plants was more than those of Gram-negative bacteria. The activities of 70% ethanol extracts recorded highest activity against Gram-negative bacteria than those of other extracts. The results therefore established a good support for the use of *C. procera* and/or *F. sycomorus* in traditional medicine against Gram-positive and negative pathogenic bacteria.

**Key words:** *Calotropis procera, Ficus sycomorus*, plant latex, leaf extract, pathogenic bacteria.

INTRODUCTION

Some bacterial infections cause high rate of mortality in human population and aquaculture organisms (Kandhasamy and Arunachalam, 2008). For example, *Enterococcus faecalis* is the causative agent of...
inflammatory bowel disease (Balish and Warner, 2002). *Escherichia coli* and *Staphylococcus aureus* cause diseases like mastitis and abortion, while *Salmonella* sp. causes diarrhea and typhoid fever (Jawetz et al., 1995). *Shigella flexneri* strains are most frequently linked with endemic outbreaks of shigellosis in the developing world. *S. flexneri* invades the colonic and rectal epithelium of its host and causes severe tissue damage ranging from watery diarrhea to severe dysentery characterized by fever, abdominal cramping and bloody mucoid stool (Jennison and Verma, 2004).

On the other hand, *Neisseria lactamica* is not associated with infection in normally healthy people, and lives as a harmless commensal (Snyder and Saunders, 2006). Unlike *Neisseria meningitidis* and *Neisseria gonorrhoeae*, the rare cases of disease caused by *N. lactamica* are largely due to some compromise in the patient, and it is not normally pathogenic (Denning and Gill, 1991).

Control of infectious diseases is seriously threatened by the continuous increase in the number of microorganisms that are resistance to the chemical antimicrobial drugs; although new antibiotics are being steadily synthesized (Jazani et al., 2011). Due to the development of antibiotic resistance by various known and unknown reasons, in pathogenic and opportunistic microorganisms, the scientific community is constantly trying to develop new drugs and drug targets at present. In the last two decades, few new antibiotics, developed by the pharmaceutical companies, did not show the enhanced activity against the multidrug resistance bacteria (Sosa et al., 2003). Hence, the interest in plants, as a potential and promising source of pharmaceutical agents, has been dramatically increased (Kareem et al., 2008; Nenaah and Essam, 2011; Salem et al., 2011).

For example, dried latex and chloroform extract of *Calotropis procera* roots has been reported to possess anti-inflammatory activity (Kumar and Basu, 1994). Aqueous extract of *C. procera* latex has been found to exhibit antibacterial activity against carcinogenic bacteria (Kalpesh et al., 2012). The alcoholic extract from different parts of *C. procera* possess antimicrobial and spermicidal activity (Kamath and Rana, 2002). The anti-inflammatory property of the latex of *C. procera* was studied in vivo. A single dose of the aqueous suspension of the dried latex was effective to a significant level against an acute inflammatory response (Kumar and Basu, 1994). The decoction of the aerial part of *C. procera* is commonly used in Saudi Arabian traditional medicine for the treatment of various diseases including fever, joint pain, muscular spasm and constipation. The ethanol extract of the plant was tested on laboratory animals for its antipyretic, analgesic, anti-inflammatory, antibacterial, purgative and muscle relaxant activities (Mueen et al., 2004).

*Ficus sycomorus*, another medicinal plant belonging to the family Moraceae, was reported for its inhibitory effect on bacterial growth (Ahmadu et al., 2007; Kubmarawa et al., 2007; Hassan et al., 2006a). The phytochemical analysis of *F. sycomorus* revealed the presence of tannins, anthraquiones, flavonoids, saponins, steroids and alkaloids (Adeshina et al., 2009). The presence of flavonoids in all the *Ficus sycomorus* extracts could probably be responsible for the observed antibacterial activity (Salem et al., 2013). Hence, the present study aimed to screen and evaluate the efficiency of different solvent extracts of *Calotropis procera* and *Ficus sycomorus* latex and leaves as antibacterial agents against some common pathogenic bacteria.

**MATERIALS AND METHODS**

**Sample collection and preparation of plant extracts**

Leaves and latex of *C. procera* and *F. sycomorus* were collected from South Valley University campus at Qena city, Egypt. Plant latex was collected by cutting the green stems and receiving the white milky latex in sterile bottles. Latex was centrifuged using a Biofuge™ Primo R (Germany) cooling centrifuge at 1500 rpm for 30 min at 4°C. The supernatant was discarded and the pellet was evaporated till dryness on a water bath at 100°C. Crude latex was stored at -20°C until being used (Singhal and Kumar, 2009). Healthy leaves of *C. procera* and *F. sycomorus* were collected, washed thoroughly with tap water followed by distilled water and air dried on a paper towel for 4 days. Dry leaves were ground in a tissue grinder (IKA® A10, Germany) to fine powder. Ten grams of each dried sample (leaves and latex of *C. procera* and *F. sycomorus*) were dissolved in 100 ml of five different solvents (water, methanol, absolute ethanol, 70% ethanol and chloroform) under stirring condition (150 rpm) for five days at room temperature. All solutions were filtered through Whatman #1 sterile filter paper. For aqueous extracts, the filtrates were stored at 4°C while for other extracts, the filtrates were dried at room temperature then the residues were dissolved in its original solvents to give 50 mg ml⁻¹. The extracts were stored at 4°C until being used against the tested bacteria (modified from Verastegui et al., 1996).

**Bacterial strains and culturing conditions**

The used bacterial strains were kindly provided by Luxor International Hospital, Luxor, Egypt. Bacterial cultures were maintained on Muller- Hinton (Muller and Hinton, 1941) agar slants and subcultured on Muller- Hinton broth then incubated at 37°C for 18 h before carrying out the test. Seven strains were used including five Gram negative bacteria including *N. lactamica* (ATCC23970), *S. typhi* (ATCC19430), *S. flexneri* (ATCC12022), *E. coli* (ATCC25922) and two Gram positive bacteria including methicillin- resistant *S. aureus* (MRSA, ATCC43300) and *S. aureus* (CONS, ATCC29213).

**Antibacterial activities and minimum inhibitory concentrations (MICs)**

Antibacterial activity was determined against the above bacterial strains using the disk diffusion method as described previously (modified from, Bauer et al., 1966). Whatman # 1 filter paper disks of 6 mm diameter were sterilized by autoclaving for 15 min at 121°C. The sterile disks were impregnated with different concentra-
tions of the used extracts (50, 40, 30 and 20 mg ml⁻¹) for MIC determination. Agar plates were inoculated with 0.1 ml of the tested microorganisms broth cultures using spread plate method. The bacterial inocula were adjusted to approximately 1.5 x 10⁸ CFU ml⁻¹. The impregnated disks, in extracts and control solutions, were placed on the inoculated medium and the plates were incubated at 37°C for 24 h. Methanol, absolute ethanol, 70% ethanol and chloroform were used as negative controls while chloromphenicol (25 mg/ ml) was used as a positive control. Diameter of the growth inhibition halos (clearing zones) around each treated disk was measured in millimeters. Finally, the minimum inhibitory concentration (MIC) was defined as the lowest concentration of plant extracts that inhibit the growth of each strain, with the highest clearing zone value. All tests were carried out in triplicate.

Kinetic study of the extracts

Kinetic studies for the most active plant extracts on the tested microorganisms were carried out according to Kareem et al. (2008). An overnight broth culture of each strain (5 ml) was mixed with 25 ml fresh Muller- Hinton broth followed by the addition of 1 ml of the tested extract (10 mg ml⁻¹). The mixture was thoroughly shaken on a mechanical shaker. The optical density at 427 nm was determined at 30 min intervals for 5 h as described earlier by Kareem et al. (2008), using a "SPECTRONIC® GENESYS ™  2PC" Spectrophotometer, Spectronic Instruments, USA.

RESULTS

Minimum inhibitory concentrations (MICs) and activity of the extracts

There were variations in diameters of inhibition zones caused by the tested plant extracts. In general, extracts of C. procera showed higher antibacterial activity compared to F. sycomorus extracts. The negative control, methanol, absolute ethanol, 70% ethanol and chloroform did not inhibit growth of the tested pathogens (data not shown). Among Gram +ve bacteria, S. aureus MRSA was the most sensitive to both leaves and latex extracts of C. procera; aqueous and 70% ethanol leaves extracts of F. sycomorus, and absolute ethanol extract of F. sycomorus latex (Tables 1 and 2). Compared to chloramphenicol (inhibition zone 14 mm), the higher antibacterial activity for the S. aureus MRSA was recorded for absolute ethanol extract of F. sycomorus latex and aqueous extract of leaves with 17 and 13.2 mm, respectively and an MIC of 50 mg ml⁻¹ for both. For C. procera, methanol and 70% ethanol extracts of leaves and latex recorded the highest antibacterial activity (14.5 and 13 mm, respectively) as shown in Tables 1 and 2 with MIC value of 50 mg ml⁻¹ for both.

S. aureus CONS was very susceptible to the most tested C. procera extracts with exception of methanol, absolute ethanol and chloroform extracts of latex. Among these extracts, that showed higher activity compared to chloramphenicol (inhibition zone of 13 mm), were the 70% ethanol extract of leaves and latex (17 and 15 mm, respectively with MIC of 20 mg ml⁻¹) and absolute ethanol extract of leaves (15 mm with MIC of 50 mg ml⁻¹). Only ethanol 70% extract of F. sycomorus leaves recorded activity against the same organism (18 mm, MIC of 50 mg ml⁻¹).

Among all the pathogenic Gram -ve bacteria, only E. faecalis was resistant to both plant extracts (Table 1). On the other hand, S. typhi, S. flexenri and E. coli were sensitive to some of C. procera extracts such as absolute ethanol extract of leaves and ethanol 70% extract of latex and leaves on S. typhi (16; 15 and 13 mm with MIC of 20 and 50 mg ml⁻¹, respectively) and E. coli (11; 12 and 9 mm with MIC of 20; 50 and 40 mg ml⁻¹, respectively). Also, aqueous extract of C. procera leaves and chloroform extract of latex recorded antibacterial activity against E. coli (10 and 9 mm with MIC of 30 and 20 mg ml⁻¹, respectively). Only absolute ethanol extract of C. procera leaves recorded an inhibition zone higher than chloramphenicol on S. flexenri (24 mm with MIC of 50 mg ml⁻¹).

In general, the entire tested Gram -ve bacteria were resistant to F. sycomorus extracts. The exceptions were the aqueous extracts of latex on N. lactamica (zones of inhibition 13 mm and MIC of 20 mg ml⁻¹) as well as, absolute ethanol and 70% ethanol latex extract on E. coli (zone of inhibition 20 mm and MIC of 50 and 30 mg ml⁻¹ respectively) (Tables 1 and 2). The commensal N. lactamica was very sensitive to C. procera extracts such as absolute ethanol and ethanol 70% (zones of inhibition 27 and 25 mm and MIC of 30 and 50 mg ml⁻¹, respectively).

Kinetic study of the extracts

The kinetic studies for both plant extracts were done only for the most active extracts against the most sensitive bacteria to confirm the activity of the extract as either bacteriostatic or bactericidal. The effect of 70% ethanol and aqueous extracts of C. procera latex and leaves on the growth dynamics of E. faecalis, compared to normal growth curve, showed that the extracts exhibited same characteristics. Although the inhibitory effect of C. procera aqueous extract of leaves was more pronounced than ethanol 70% extract of latex, compared to control, both extracts exhibited bacteriostatic effects (Figure 1A).

There were variations between the effect of C. procera leaves and latex extracts on the growth dynamics of S. typhi, compared to normal growth curve. The inhibitory effect of aqueous and 70% ethanol extracts of C. procera latex, absolute ethanol and 70% extract of leaves were more pronounced than water extract of leaves. The effect of the former extracts was bactericidal on S. typhi (Figure 1B). On the other hand, only 70% ethanol extract of F. sycomorus leaves had bactericidal effect on the same organism compared to control (Figure 2A). The bactericidal effect was obvious on E. coli for the extracts of 70% ethanol, chloroform (for C. procera latex), aqueous
Table 1. Inhibition halo diameter of leaves and latex extracts of *C. Procera* and *F. sycomorus* against some pathogenic bacteria.

<table>
<thead>
<tr>
<th>Plant extract</th>
<th>Inhibition halo diameter (mm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>S. aureus</em> MRSA (ATCC 43300)</td>
</tr>
<tr>
<td></td>
<td>Latex</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>14 ± 0.3</td>
</tr>
<tr>
<td>(25 mg/ml)</td>
<td></td>
</tr>
<tr>
<td><em>C. procera</em> extracts</td>
<td></td>
</tr>
<tr>
<td>Aqueous</td>
<td>11±0.2</td>
</tr>
<tr>
<td>Methanol</td>
<td>9±0.3</td>
</tr>
<tr>
<td>Ethanol</td>
<td>11±0.0</td>
</tr>
<tr>
<td>Ethanol 70%</td>
<td>13±0.1</td>
</tr>
<tr>
<td>Cloroform</td>
<td>10±0.0</td>
</tr>
<tr>
<td><em>F. sycomorus</em> extracts</td>
<td></td>
</tr>
<tr>
<td>Aqueous</td>
<td>-ve</td>
</tr>
<tr>
<td>Methanol</td>
<td>-ve</td>
</tr>
<tr>
<td>Ethanol</td>
<td>17±0</td>
</tr>
<tr>
<td>Ethanol 70%</td>
<td>-ve</td>
</tr>
</tbody>
</table>

*Mean ± SD, n=3; ≠ showed bacteriocidal effect; -ve = negative effect.

DISCUSSION

Drug resistance of human pathogenic bacteria has been reported all over the world and the situation is alarming in developing as well as developed countries due to indiscriminate use of antibiotics. Plants are important source of potentially useful structures for the development of novel chemotherapeutic agents and the first step towards this goal is the *in vitro* antibacterial assay (Valero and Salmerón, 2003). According to the findings of the present study, the aqueous and ethanolic extracts (either absolute or 70%) and the latex of *C. procera* showed considerable antibacterial activities against most tested microorganisms (Tables 1 and 2). In all cases, and regardless of the microorganism tested, the used solvent was a determinant factor for antimicrobial agents extraction. Some findings was recorded by Nennah and Essam (2011) considering the antibacterial activities of aqueous and organic extracts of *C. procera* against human pathogenic bacteria and 70% ethanol extracts of leaves (Figure 1C). While, for the same organism, 70% ethanol latex extract of *F. sycomorus* exhibited bacteriostatic effect (Figure 2B).

For *S. aureus* MRSA, only aqueous and chloroform latex extracts of *C. procera* exhibited bactericidal effect, while the other tested were bacteriostatic (Figure 1D). Furthermore, only *F. sycomorus* aqueous extract of leaves was bactericidal after 60 min compared to control (Figure 2C).
Table 2. MIC’s value of leaves and latex extracts of *C. Procera* and *F. sycomorus* against some pathogenic bacteria.

<table>
<thead>
<tr>
<th>Plant extract</th>
<th>Minimum Inhibitory concentrations MIC (mg ml(^{-1}))^*</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>S. aureus</em> MRSA (ATCC 43300)</td>
<td><em>S. aureus</em> Cons (ATCC 29213)</td>
</tr>
<tr>
<td><strong>C. procera extracts</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latex</td>
<td>Leaves</td>
<td>Latex</td>
</tr>
<tr>
<td>Aqueous</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Methanol</td>
<td>20</td>
<td>50</td>
</tr>
<tr>
<td>Ethanol</td>
<td>20</td>
<td>50</td>
</tr>
<tr>
<td>Ethanol 70%</td>
<td>50</td>
<td>30</td>
</tr>
<tr>
<td>Cloroform</td>
<td>20</td>
<td>-ve</td>
</tr>
<tr>
<td><strong>F. sycomorus extracts</strong></td>
<td></td>
<td></td>
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<tr>
<td>Latex</td>
<td>Leaves</td>
<td>Latex</td>
</tr>
<tr>
<td>Ethanol</td>
<td>50</td>
<td>20</td>
</tr>
<tr>
<td>Ethanol 70%</td>
<td>-ve</td>
<td>20</td>
</tr>
</tbody>
</table>

-ve = negative effect.

bacteria. Also, in a study conducted by Kawo et al. (2009) a weak antibacterial properties of ethanolic extracts of *C. procera* leaves and latex against *E. coli*, *S. aureus*, *Salmonella* sp. and *Pseudomonas* sp. was recorded by using paper-disc diffusion and broth dilution techniques. The results obtained revealed that ethanol was the best extractive solvent for a fraction with antibacterial activity. Also, ethanol was reported for its efficiency for extracting the antimicrobial active substances from *Calotropis* compared to other solvents (Kareem et al., 2008). In our study, aqueous and ethanol (either absolute or 70%) extracts were the popular solvents that elute polar substances, and latex extracts are more active than leaf extracts on human pathogenic bacteria. This may indicate that the used solvent is an important factor for the isolation of selective bioactive compounds (Salem et al., 2011). Some workers showed that methanol extraction yielded higher antimicrobial activity than hexane and ethyl acetate (Manilal et al., 2009; Rangaiah et al., 2010). In our results, aqueous and ethanol extracts of latex and leaves exhibited much more bioactivity than other extracts. The highest antibacterial activity (inhibition zone of 27 mm) was recorded for the ethanolic extract of *C. procera* leaves against *N. lactamica* (Table 1). On the contrary, methanol extracts of leaves and latex showed no bioactivity against all the tested organisms (Tables 1 and 2). The greater resistance of Gram -ve bacteria to latex extract, may be due to the differences in the cell wall structure between Gram +ve and -ve bacteria. The Gram -ve bacterial outer membrane acts as a barrier to many substances, including antibiotics (Burt, 2004). Chemically, the latex of *C. procera* is composed of various classes of phytochemical compounds. These were extensively proved in various studies which include proteolytic enzymes, cardenolides, alkaloids, cardioactive glycoside like calactin, calotropain, proceroside, syriogenine, calotoxin and uscharin, as well as tannins, flavonoids and procerain, a stable cysteine protease (Mossa et al., 1991; Dubey and
A. Enterococcus faecalis (ATCC 29212)

B. Salmonella typhi (ATCC 19430)

Figure 1. Time-kill kinetic analysis of C. procera extracts against Enterococcus faecalis, Salmonella typhi, E. coli and Staphylococcus aureus MRSA.
MRSA was confirmed as bactericidal effect (Figure 1B, C and D). This may be indicative for the presence of broad spectrum antibiotics (Larhsini et al., 1999).

Aqueous extract of *F. sycomorus* was reported earlier for its inhibitory effect on bacterial growth (Shankar et al., 2004). It contains pharmacologically active substances including tannins, saponins, reducing sugars, alkaloids and flavones aglycones without any haematological, hepatic or renal toxicities (Kubmarawa et al. 2007).

In our results, the antibacterial activity of *F. sycomorus* extracts (absolute ethanol latex extract; aqueous and 70% ethanol leaves extracts) was reported against both Gram +ve bacteria and only *S. typhi*, had a bactericidal effect (Tables 1 and 2; Figure 2A, C). These constituents could be responsible for the antibacterial activities of *F. sycomorus* against the two Gram +ve bacteria tested.

The low bioactivity observed for crude latex extracts, of both *F. sycomorus* and *C. procera*, against some Gram -ve bacteria (such as *S. flexenri* and *E. faecalis*) resulted from the previous effect, dilution of active constituents or from antagonism among extract constituents (Tables 1 and 2).

The same results were reported earlier for *Caltrops gigantea* latex against some pathogenic Gram negative bacteria.
A. *Salmonella typhi* (ATCC 19430)

Figure 2. Time-kill kinetic analysis of *F. sycomorus* extracts against *Salmonella typhi*, *E. coli* and *Staphylococcus aureus* MRSA.

B. *E. coli* (ATCC 25922)

MIC's are considered the "gold standards" for determining the susceptibility of microorganisms to antimicrobials. MIC's are used in diagnostic laboratories to confirm unusual resistance, to give a definite answer when a borderline result is obtained by other methods of
testing, or when disc diffusion methods are not applicable. In general, the lowest MIC's (20 mg ml⁻¹) were recorded for latex extracts while the highest MIC's (50 mg ml⁻¹) recorded for leaves extracts for both plants (Table 2). According to Rangaiah et al. (2010) and Patra et al. (2008), this indicates the presence of active constituents in the plant extracts that can be used in pharmaceutical industry. This was also attributed to the presence of high concentrations of polysaccharides in some plants including C. procera and F. sycomorus that have antimicrobial properties (Akhtar et al., 1992; Yamashita et al., 2001; Doughari, 2006; Hassan et al., 2006b).

The results of our preliminary screening assays justify the use of 70% ethanol latex and leaves extracts of C. procera in the ethnomedicine field. However, it is important to note that the crude extract of C. procera latex need to be further purified through antibacterial activity-guided fractionation to isolate and identify the compounds responsible for this activity. In conclusion, the remarkable bactericidal effects of C. procera latex extract and F. sycomorus leaves extracts suggest that these extracts can be a useful source for the development of novel antibacterial formulations.

Conflict of Interests
The author(s) have not declared any conflict of interests.

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