**In vitro ethnotherapeutic potential of the acetone extract of the bark of *Ziziphus mucronata* Willd. subsp. *mucronata* Willd.: Antimicrobial and toxicity evaluations**

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The antimicrobial and the cytotoxicity activities of the crude acetone bark extract of *Ziziphus mucronata* were determined by agar diffusion, macrobroth dilution assays and brine shrimp lethality assay, respectively, to ascertain its relevance in ethnomedicine. The results show that both Gram negative and Gram positive bacteria tested were equally inhibited. At the highest concentration (10000 µg/ml) of the extract, the bacterial inhibition zones ranged between 16 and 21 ± 1.0 mm, the fungal inhibition zones ranged between 13 and 19 ± 1.0 mm while *Fusarium sporotrichioides*, *Candida glabrata* and *Trichophyton mucoides* were not affected. The minimum inhibitory concentrations (MICs) values of the extract were between 78.1 and 312.5 µg/ml for the bacteria while the minimum bactericidal concentrations (MBCs) were between 156.3 and 2500 µg/ml. For the fungi, the MICs were between 1250 and 10000 µg/ml, the minimum fungicidal concentrations (MFCs) were between 5000 and 20000 µg/ml and the LC$_{50}$ of the brine shrimp lethality assay was 90.27 µg/ml indicating a low level of toxicity. The degree of the pharmacological activity of *Z. mucronata* shows the significant medicinal potential of this plant in the treatment of microbial infections and justifies its use in complementary and alternative medicines in South Africa.

Key words: *Ziziphus mucronata*, antibacterial, cytotoxicity, fungicidal, ethanol extract, medicinal potential.

**INTRODUCTION**

Globally, pathogen resistance to antibiotics, an under-appreciated threat to public health in nations around the globe (Zhang et al., 2006), is a rapidly growing problem, leading to an urgent need for novel antimicrobial agents (Kumar and Schweizer 2005; Edgar et al., 2012). With this increased incidence of resistance to antibiotics and appearance of new infectious agents, many products have been evaluated directly for antimicrobial activity as well as their resistance modifying ability (Gibbons, 2004; Coutinho et al., 2009). These evaluations involved using new compounds, which are not based on the existing synthetic antimicrobial agents (Shah, 2005) but rather on natural products from plants considered interesting alternatives for treatment (Lu et al., 2007; Mbwanmo et al., 2007). Plant-based traditional knowledge is a recognized tool playing a major role in the treatment of human traumas and diseases worldwide (Principe, 1991).

On all continents, people have used hundreds to thousands of indigenous plants for treatment of ailments since prehistoric times (Levetin and McMahon, 2002). In search for new sources of drugs and nutraceuticals (Ghosh, 2003; Sharma and Mujundar, 2003), there is a growing interest in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity (Al-Bayati and Al-Mola, 2008). While numerous
drugs have entered the international pharmacopoeia through ethnobotany and traditional medicine (Ergene et al., 2006), contemporary pharmacopoeias contain at least 25% of drugs derived from plants and many synthetic analogues built on prototype compounds isolated from plants (De Silva, 2005) because they contain active substances effective against human pathogens and relent or cure diseases (Hadi and Bremner, 2001). However, to establish the potential therapeutic effects and ascertain the safety of the phytopharmaceuticals, many medicinal plants have been investigated in vitro for their antimicrobial and cytotoxicity activities pending their confirmation in vivo tests.

Ziziphus mucronata Willd. subsp. mucronata Willd. (Buffalo thorn) (Family: Rhamnaceae) widely distributed in South Africa (Foden and Potter, 2005; Raimondo et al., 2009), often found in thorny vegetation in both temperate and tropical climates, open scrubland, woodland, forest margins and riverine vegetation, is usually a shrub or small to medium sized tree up to 9 m tall. It has a trunk frequently crooked and branches spreading and drooping well above ground or near the base. While the thorns of the plant are in pairs, reddish brown, one straight and one bent, the flowers are small, yellow, inconspicuous, bisexual, in tight axillary clusters and often produce copious nectar (Bekele-Tesemma et al., 1993; Bein et al., 1996).

Traditionally, a poultice of the powdered and baked roots is used to relieve any pain. Boils, glandular swellings and other skin infections are treated with leaf paste. Leaf paste combined with an infusion of the roots is used to treat tuberculosis gland swellings, measles, dysentery, lumbago and chest complaints. Roots are used to treat snakebite. The bark containing 12 to 15% tannin is used as an emetic and steam bath to purify the complexion. Bark decoction is used for rheumatism and stomach troubles.

Bark infusion is used to treat coughs (Venter and Venter, 1996; van Wyk and Gericke, 2000). Though this plant has been implicated traditionally in the treatment of many infections, its pharmacological importance has not been scientifically documented. Therefore, this study was aimed at screening the acetone extract of the bark of Z. mucronata against bacteria and fungi of clinical importance and reporting the toxicity of the extract using brine shrimp lethality assay to ascertain its relevance in ethnomedicine.

MATERIALS AND METHODS

Collection of plant material

The stem bark materials of Z. mucronata were collected from the plant growing within the University of Fort Hare campus in Alice, South Africa. The plant was authenticated in the Department of Botany and a voucher specimen (OLA/2010/2M/01) was prepared and deposited in the Griffen Herbarium of the University.

Extract preparation

The bark sample was air-dried at room temperature, pulverized in a mill (Christy Lab Mill, Christy and Norris Ltd; Process Engineers, Chelmsford, England) and stored in a sterile air-tight container for further use. The extract of the bark was prepared in accordance to the description of Basri and Fan (2005). About 100 g of the pulverized sample was seeped in 500 ml of acetone for 72 h with shaking (Stuart Scientific Orbital Shaker, Staffordshire, UK). The plant material was extracted for two other consecutive times. The extracts were combined, filtered through Whatman No. 1 filter paper and concentrated under reduced pressure at 40°C using a rotary evaporator (Laborotara 4000 - efficient, Heldolph, Germany). The resulting crude extract was stored at -20°C until assayed. Stock solutions and serial dilutions of extracts and fractions were prepared in dimethylsulphoxide (DMSO) (Ambrozin et al., 2004).

Test organisms

The bacterial isolates used in this study included Micrococcus luteus, Bacillus subtilis KZN, Enterococcus faecalis KZN, Staphylococcus aureus OK1, S. aureus OK5, Klebsiella pneumoniae KZN, Shigella sonnei (ATCC 29930), B. pumilus (ATCC 14884), Enterobacter cloacae (ATCC 13047), K. pneumoniae (ATCC 10031), Pseudomonas aeruginosa (ATCC 19582) and Acinetobacter calcoaceticus anitratis CSIR. The fungal isolates included Absidia corymbifera, Candida krusi, Candida albicans, Cryptococcus neoforms, Candida rugosa, Candida glabrata (ATCC 2001), Aspergillus niger, Aspergillus terreus, Aspergillus flavus, Penicillium notatum and Trichophyton mucoides (ATCC 201382). These organisms were obtained from the Department of Biochemistry and Microbiology, University of Fort Hare, Alice, South Africa. The bacteria were maintained on nutrient agar while the fungal isolates were maintained on potato dextrose agar and broth which were used for the antifungal assays.

Preparation of inocula

For the bacterial inoculums preparation, the inoculum of each test bacterial strains was prepared using the colony suspension method (EUCAST, 2000). Colonies picked from 24 h old cultures grown on nutrient agar were used to make suspensions of the test organisms in saline solution to give an optical density of approximately 0.1 at 600nm. The suspension was then diluted 1:100 by transferring 0.1 ml of the bacterial suspension to 9.9 ml of sterile nutrient broth before use. The density of bacterial suspension for susceptibility test was finally determined by comparison with 0.5 McFarland standard of Barium sulphate solution (Cheesebrough, 2002).

The fungal inoculums were prepared by dropping 1 cm² of seven day old spore producing cultures in sterile distilled water and vortexed for 30 s to release the fungal spores. The spore density of each fungus was adjusted with spectrophotometer (A595 nm) to obtain a final concentration of approximately 10⁵ spores/ml. For the Candida spp., the inocula were prepared by adding 1 ml of overnight Candida cultures to 9 ml of potato dextrose broth to yield 10⁵ colony forming units (CFU) per ml of the inoculum.

Antimicrobial assay by agar diffusion method (Inhibition zones)

The susceptibility screening of the test bacteria to the acetone extract of the bark of Z. mucronata and chloramphenicol, used as control, was done in accordance with the methods described by Irobi et al. (1996) and Akinpelu et al. (2008). For the antibacterial assay, the inoculum of each test bacterial strains was standardized.
at 5 × 10⁶ cfu/ml using McFarland Nephe-lometer standard (NCCLS, 1993). Sterile Mueller Hinton agar plates were seeded with each adjusted test bacterial strain and allowed to stand at 37°C for 30 min. Wells were then bored into the agar medium with heat sterilized 6 mm cork borer and were filled with 100 µL of different concentrations (2500 µg/ml, 5000 µg/ml and 10000 µg/ml) of the extract and chloramphenicol (7.8125 µg/ml, 15.625 µg/ml and 31.25 µg/ml). For the antifungal assay, 100 µL of the fungal solutions was dispensed on potato dextrose agar plates and spread evenly with a sterile glass rod and allowed to stand for 1 h on the laboratory bench. Wells were then bored into the agar medium with a heat sterilized 6 mm cork borer. The wells were filled with 100 µL of different concentrations (5000, 10000 and 20000 µg/ml) of the extract. In both bacterial and fungal cultures, the antimicrobial solutions were not allowed to spill onto the surface of the agar while the culture plates were allowed to stand on the laboratory bench for 30 min to allow proper diffusion of the solutions before the bacterial culture plates were incubated aerobically at 37°C for 24 h and the fungal cultures incubated at 27°C for 3 to 7 days. Wells in blank Mueller Hinton and potato dextrose agar plates containing 5% DMSO representing the final DMSO concentration in the test plates without the extract served as positive controls. The tests were done in replicate for each of the bacterial and fungal isolates. After 24 h of incubation, the plates were examined if there is any zone of inhibition (Bauer et al., 1966). The diameter of the zones of inhibition of the extract and the antibiotic was measured and interpreted using the CLSI zone diameter interpretative standards (CLSI, 2008).

**Macrobroth dilution for minimum inhibitory concentration (MIC)**

MIC defined as the lowest concentration which resulted in maintenance or reduction of inculmuns viability (Carson et al., 1995) was determined by serial tube dilution technique (Khan et al., 2007) for the microbial isolates. For antibacterial assay, different concentrations of the extract (9.766 to 5000 µg/ml) and chloramphenicol (0.9766 to 500 µg/ml), used as positive control, were separately prepared by serial dilutions in Mueller Hinton broth. The tubes were inoculated with 100 µL of each of the bacterial strains. Blank Mueller Hinton broth was used as negative control. For the antifungal assay, macrobroth dilution assay was performed as described from Fleming et al. (1993). Different concentrations (78.125 to 40000) µg/ml of the crude extract was prepared in potato dextrose broth by serial dilutions. Each broth concentration was inoculated with 100 µL inoculums of approximately 10⁵ spores/ml of the prepared fungal spores’ solution. Two control tubes were included: one with spores and broth but no plant extract and one with broth and plant extract but no spores. The fungal containing tubes were incubated at 27°C for 3 to 5 days. The MICs were determined as described by Dabur et al. (2004).

**Determination of minimum bactericidal and fungicidal concentrations (MBC/MFC)**

The MBC and MFC assays were carried out as described by Cheesbrough (2006). Here, extract-free nutrient and sabouraud dextrose agar plates were inoculated with one loopful of culture taken from each of the first five broth cultures that showed no growth and the first growth-containing tube in the MIC tubes. The MBC and MFC assay plates were respectively incubated at 37°C for 24 h and at 25°C for 3 to 5 days. After the incubation periods, the lowest concentrations of the extract that did not produce bacterial and fungal growth on the solid media were respectively regarded MBC and MFC values for this extract (Irkin and Korukluoglu, 2007). This observation was matched with the MIC test tube that did not show evidence of bacterial growth after 48 h or spore germination for the fungi after five days of incubation.

**RESULTS**

The preliminary antimicrobial screening of *Z. mucronata* shows that the crude acetone extracts of the stem bark exhibited a significant *in vitro* antibacterial activity against Gram negative and Gram positive bacteria and different fungal isolates tested. The zones of inhibition obtained from the crude extract and the chloramphenicol against the selected bacteria was indicated in Table 1. The extract exhibited a concentration dependent activity against the different bacterial strains. At the highest concentration (10000 µg/ml) of the extract, the zones of inhibition ranged between 16 and 21 ± 1.0 mm. At the lowest concentration (2500 µg/ml), the zones of inhibition ranged between 12 and 16 ± 1.0 mm. While *E. faecalis* KZN, *S. aureus* OK₁ and *S. aureus* OK₃ were not susceptible to the different concentrations of chloramphenicol, used as positive control, other bacterial strains had zones of inhibitions ranging between 20 and 27 ± 1.0 mm at the highest concentration (31.25 µg/ml) used. Table 2 summarizes the degree of the antibacterial activity of this crude extract as determined by the macrobroth dilution assay. The minimum inhibitory concentrations (MIC) values of the extract ranged between 78.1 and 312.5 µg/ml while the minimum bactericidal (MBC) values ranged between 156.3 and 2500 µg/ml. The MIC values of chloramphenicol ranged between 1.953 and 31.25 µg/ml for all the tested bacterial strains. While the activity of the extract was less than that of the chloramphenicol, the extract was not selective in its activity against the different tested groups of bacteria.

**Brine shrimp (Artemia salina larvae) lethality test**

The brine shrimp lethality test using the larvae of brine shrimp *A. salina* L, was carried out using the standard procedure (Meyer et al., 1982; McLaughlin and Rogers, 1998). Different concentrations (0.9766 to 500 µg/ml) of the extract were prepared from the stock solution by serial tube dilution technique in different vials. Ten nauplii were transferred into each vial using Pasteur pipettes and were not given food because hatched brine shrimp can survive for up to 48 h without food as they still feed on their yolk-sac. The control vials were prepared using DMSO combined with sea water only and the experiment was replicated three times. After 24 h of incubation, the vials were examined, the numbers of survivors in each vial were counted and percentages of deaths were calculated. Larvae were considered dead if they did not exhibit any observable movement during several seconds of observation. The extract is regarded as being mildly toxic if LC₅₀ > 30<100 µg/ml and non-toxic if its LC₅₀ is greater than 100 µg/ml in the brine shrimp lethality assay (Moshi et al., 2010). The mean mortality percentage and LC₅₀ (lethal concentration for 50% of the population) were determined using statistical analysis and the graph of Logarithm of concentration against percent lethality (Gupta et al., 1996).
Table 1. *In vitro* antibacterial activity of crude acetone extract of the bark of *Ziziphus mucronata*.

<table>
<thead>
<tr>
<th>Tested bacterial isolate</th>
<th>Inhibition zones (± 1.0 mm) from chloramphenicol</th>
<th>Inhibition zones (± 1.0 mm) from <em>Z. mucronata</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>31.25 (µg/ml)</td>
<td>15.63 (µg/ml)</td>
</tr>
<tr>
<td><em>M. luteus</em></td>
<td>26</td>
<td>22</td>
</tr>
<tr>
<td><em>B. subtilis KZN</em></td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td><em>E. faecalis KZN</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>S. aureus OK₁</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>S. aureus OK₃</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>K. pneumoniae KZN</em></td>
<td>22</td>
<td>18</td>
</tr>
<tr>
<td><em>S. sonnei (ATCC 29930)</em></td>
<td>20</td>
<td>16</td>
</tr>
<tr>
<td><em>B. pumilus (ATCC 14884)</em></td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td><em>E. cloacae (ATCC 13047)</em></td>
<td>27</td>
<td>21</td>
</tr>
<tr>
<td><em>K. pneumoniae (ATCC 10031)</em></td>
<td>21</td>
<td>16</td>
</tr>
<tr>
<td><em>P. aeruginosa (ATCC 19582)</em></td>
<td>22</td>
<td>17</td>
</tr>
<tr>
<td>Acinetobacter calcoaceticus anitratus CSIR</td>
<td>23</td>
<td>20</td>
</tr>
</tbody>
</table>

Table 2. Inhibitory and bactericidal activities of acetone extract of the bark of *Ziziphus mucronata*.

<table>
<thead>
<tr>
<th>Tested bacterial isolate</th>
<th>Activity of chloramphenicol</th>
<th>Activity of <em>Z. mucronata</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC µg/ml</td>
<td>MBC µg/ml</td>
</tr>
<tr>
<td><em>M. luteus</em></td>
<td>1.953</td>
<td>312.5</td>
</tr>
<tr>
<td><em>B. subtilis KZN</em></td>
<td>3.906</td>
<td>312.5</td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td>31.25</td>
<td>312.5</td>
</tr>
<tr>
<td><em>S. aureus OK₁</em></td>
<td>7.8125</td>
<td>78.1</td>
</tr>
<tr>
<td><em>S. aureus OK₃</em></td>
<td>7.8125</td>
<td>156.3</td>
</tr>
<tr>
<td><em>K. pneumoniae KZN</em></td>
<td>7.8125</td>
<td>312.5</td>
</tr>
<tr>
<td><em>S. sonnei (ATCC 29930)</em></td>
<td>7.8125</td>
<td>78.1</td>
</tr>
<tr>
<td><em>B. pumilus (ATCC 14884)</em></td>
<td>7.8125</td>
<td>312.5</td>
</tr>
<tr>
<td><em>E. cloacae (ATCC 13047)</em></td>
<td>1.953</td>
<td>312.5</td>
</tr>
<tr>
<td><em>K. pneumoniae (ATCC 10031)</em></td>
<td>1.953</td>
<td>156.3</td>
</tr>
<tr>
<td><em>P. aeruginosa (ATCC 19582)</em></td>
<td>3.906</td>
<td>312.5</td>
</tr>
<tr>
<td>Acinetobacter calcoaceticus anitratus CSIR</td>
<td>7.8125</td>
<td>312.5</td>
</tr>
</tbody>
</table>

having MIC values equally ranging between 78.1 and 312.5 µg/ml. On the contrary, the extract inhibited nine out of 12 fungal isolates tested. While *F. sporotrichoides*, *C. glabrata* and *T. mucoides* did not have zones of inhibition resulting from the different concentrations tested, other fungal isolates had zones of inhibitions that ranged between 13 and 19 ± 1.0 mm at the highest concentration (20 000 µg/ml). *Candida* species had the least zones of inhibition equaled to 13 ± 1.0 mm and *A. corymbifera* had the biggest zone of inhibition (19 ± 1.0 mm) being the
Table 3. *In vitro* antifungal activity of acetone extract of *Ziziphus mucronata*.

<table>
<thead>
<tr>
<th>Tested fungal isolate</th>
<th>Inhibition zones (± 1.0 mm) from <em>Z. mucronata</em></th>
<th>MIC (µg/ml)</th>
<th>MFC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20000 (µg/ml)</td>
<td>10000 (µg/ml)</td>
<td>5000 (µg/ml)</td>
</tr>
<tr>
<td><em>Absidia corymbifera</em></td>
<td>19</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>15</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td><em>Aspergillus terreus</em></td>
<td>15</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td><em>Aspergillus flavus</em></td>
<td>14</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td><em>Candida krusei</em></td>
<td>13</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>13</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td><em>Candida glabrata</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Candida rugosa</em></td>
<td>13</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td><em>Cryptococcus neoformans</em></td>
<td>17</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td><em>Fusarium sporotrichioides</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Penicillium notatum</em></td>
<td>14</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td><em>Trichophyton mucoides</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 1. Graph of logarithm of concentrations versus percentage mortality of brine shrimps interacted with crude acetone extract of the bark of *Z. mucronata*.

Most inhibited isolate by the various concentrations of the extract tested. Against these fungal isolates, the MICs of the extract were between 1250 and 10000 µg/ml while the minimum fungicidal concentrations (MFCs) were between 5000 and 20000 µg/ml (Table 3). The most inhibited fungal isolate, *A. corymbifera*, had the least MIC value of 1250 µg/ml. While *C. albicans*, *C. neoformans*, *C. rugosa*, *A. terreus* and *P. notatum* had MICs of 5000 µg/ml, the MICs of other fungal strains were 10000 µg/ml. The differences in the susceptibility of tested bacterial and fungal isolates as indicated by the antimicrobial assays could be due to the differences in their structural morphology and physiological characteristics. These characteristics could be attributed to the variations in the susceptibility of the fungal isolates to the extract as shown by the different assay methods.

From the *in vitro* cytotoxicity assay, treating brine shrimps (*Artemia salina* larvae) with acetone extract of the bark of *Z. mucronata* showed different mortality rate of brine shrimp which increased proportionately with the increasing concentration of the extract (Figure 1). The mean percentage mortality was plotted against the logarithm of concentrations and the concentration killing fifty percent of the larvae (LC$_{50}$) was determined. From the graph, the lethality concentration of the crude extract at which 50% (LC$_{50}$) mortality of brine shrimp nauplii
occurred was 90.27 µg/ml. This showed that the extract exhibited a mild cytotoxic activity against the brine shrimp.

DISCUSSION

In attempts to harness the antimicrobial substances in medicinal plants and to forestall the menace of microbial resistance and its associated complicacies, many alcoholic plant extracts have been investigated for antimicrobial activities and the effectiveness of several acetone extracts of many plants have been reported. Of these several studies, Mishra et al. (2009), Nkomo et al. (2011) and Ghamba et al. (2012) shows that acetone extracts of many plants have demonstrated significant antimicrobial activity against many tested organisms. In this study, the acetone extract of the bark of Z. mucronata, also, demonstrated remarkable activity against all the test organisms. The active components of this plant, having a good antimicrobial potential, may be highly soluble in acetone. The degree of antimicrobial activity may be accounted for by the flavonoids synthesized by plants in response to microbial infection (Hernandez et al., 2000; Schinor et al., 2007) and the exclusive ability of acetone to extract most flavonoids and steroids more than other solvents (Eloff et al., 2008; Afolayan and Lewu, 2009).

Considering the pathogenic potential of the tested organisms and upshots in microbial resistance, globally, the exhibited degree of antimicrobial activity of this crude extract indicated a positive step towards curtailing organisms with pathogenic potentials in both immunocompetent and immunocompromised individuals if, further, prosecuted clinically and the most potent bioactive compounds isolated. Since lower MIC and MBC values indicate higher efficacy (Cowan, 1999) and phytochemicals are routinely classified as antimicrobials when susceptibility tests had MICs in the range of 100 to 1000 µg/ml (Simões et al., 2009), the MIC/MBC and MIC/MFC ratios showed that the extract is bactericidal and fungicidal at lower concentrations while at higher concentrations, significant bactericidal and fungicidal activities were exhibited against these potential pathogens. Where MIC equals MBC/MFC, the bactericidal and fungicidal potency with a broad spectrum and great therapeutic potential of the plant is indicated.

Also, the brine shrimps, being highly sensitive to a variety of chemical substances, are considered a useful tool for preliminary toxicity assessment of plant extracts (Solis et al., 1993). The brine shrimp lethality is an indicative of many pharmacological actions (Klare et al., 2003) while toxicity study is considered essential in the identification and isolation of new compounds from crude extracts (Sadidharan et al., 2008). The degree of toxicity exhibited by the acetone extract could be attributed to the tannins, phenolic contents, flavonoids and proanthocyanidins (Olajuyigbe and Afolayan, 2011) present in the stem bark. Tannins possess remarkable pharmacological activities including antibacterial (Jagetia and Baliga, 2002), and anti-infective (Baglin et al., 2003; Cichewicz and Kouzi, 2004) properties. The results could form a good basis for further phytochemical and pharmacological investigation.

In conclusion, though resistance is a global challenge and ethnotherapy or phytomedicine are often neglected because of civilization, western medicine, crude preparatory methods and lack of dosage regimen in traditional medicines, the observed antimicrobial and toxicity activities indicated that Z. mucronata could be a good source of essential and pharmacologically active compounds and justify the use of this plant in the treatment of microbial infections and its use in complementary and alternative medicines in South Africa.

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