In vitro antimicrobial activity of Harungana madagascariensis and Euphorbia prostrata extracts against some pathogenic Salmonella sp.

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ABSTRACT

Harungana madagascariensis Lam (Hypericaceae) and Euphorbia prostrata Ait (Euphorbiaceae) are commonly used in Cameroon as traditional medicines for the treatment of typhoid fevers. Aqueous extracts were prepared from the leaves of H. madagascariensis and the whole plant of E. prostrata and tested in vitro for antibacterial activity against Salmonella Typhi, Salmonella Paratyphi A, Salmonella Paratyphi B and Salmonella Typhimurium, using broth dilution technique. Phytochemical screening was performed using standard methods. Acute toxicity study of the various extracts was also done on mice. Results obtained showed that H. madagascariensis extract exhibited minimum inhibitory concentrations (MICs) ranging from 390.625 to 1562.5 µg/ml. The median lethal dose (LD\textsubscript{50}) of H. madagascariensis extract as shown by the acute toxicity studies were 11.6 g/kg and 13.2 g/kg body weight for female and male mice, respectively. The MIC values of E. prostrata extract varied from 1024 to 2048 µg/ml. The LD\textsubscript{50} values obtained for this extract were 23.2 g/kg and 26.4 g/kg body weight for female and male mice, respectively. Phytochemical analysis showed the presence of phenols, tannins, saponins, anthraquinones, anthocyanins, triterpenoids, flavonoids and alkaloids in both plant extracts. These data suggest that the aqueous extracts of H. madagascariensis and E. prostrata contain antibacterial principles which may be non toxic.

Keywords: Harungana madagascariensis, Euphorbia prostrata, antibacterial activity, Salmonella sp., phytochemical screening, acute toxicity.

INTRODUCTION

Typhoid fever continues to be a marked public health problem in developing countries in general and in sub-Saharan Africa in particular, where it is endemic (Gatsing et al., 2003). Typhoid fever is caused by Salmonella Typhi, whereas paratyphoid fevers are caused by S. Paratyphi A and S. Paratyphi B (Gatsing et al., 2006; Elgroud, 2009). Conventional antityphoid drugs are becoming more and more unavailable to the common man in Africa due to increased cost (Gatsing and Adoga, 2007). Moreover, the typhoid causative organism, Salmonella Typhi, has rapidly gained resistance to the previously efficacious drugs like ciprofloxacin (Madhulika et al., 2004). Hence, there is a need for new antityphoid agents.

Recently, there has been considerable interest in the use of plant materials as an alternative method of controlling pathogenic microorganisms (Aqil et al., 2005), and many...
compounds from plants have been shown to be effective against resistant pathogenic bacteria (Nostro et al., 2006). According to WHO (1996), medicinal plants are the best sources to obtain a variety of new herbal drugs. About 80% of individuals from developing countries use traditional medicine, which has substances derived from medicinal plants (WHO, 1996). Therefore, such plants should be investigated to better understand their properties, safety and efficacy (Nascimento et al., 2000).

*Harungana madagascariensis* (Hypericaceae) is a species of flowering plants widely spread in all inter-tropical Africa and Madagascar (Gbolade et al., 2009). The fruits are small, drupe-like, yellow or red at maturity. In the African traditional medicine, the leaves and stem bark are used as herbal medicines in treating anaemia, malaria, skin diseases (Gbolade et al., 2009). Other properties, including antiamoebic, antidiarrhoeal and spasmyloytic (Tona et al., 2000), antioxidant (Kouam et al., 2005), as well as anti-trichomonal (Iwalewa et al., 2008) activities have also been documented. In Cameroon, the leaves of *H. madagascariensis* are commonly used as traditional medicines for the treatment of typhoid fever.

*Euphorbia prostrata* is an annual herb, which belongs to family Euphobeaceae and is abundantly found in India and Africa. It is been traditionally used in several digestive system disorders (Alarcon-Aguilara et al., 1998; Gupta, 2011). In Burkina Faso, the leaves are used as a remedy against the bites of venomous insects (wasps, scorpions, etc.). In Togo, this plant is used to fight against infertility and menstrual pain (Schmelzer and Gurib-Fakim, 2008) and in the western rural parts of Cameroon, the whole plant of *Euphorbia prostrata* are very often used for the treatment of dysentery and typhoid whole fever.

In a continuation of our search for therapeutic agents from natural sources with potential for the treatment of typhoid and paratyphoid fevers (Aliyu et al., 2002; Gatsing et al., 2003; Teponno et al., 2006; Gatsing et al., 2007a; Gatsing et al., 2007b; Gatsing and Adoga, 2007; Djemgou et al., 2007; Gatsing et al., 2008; Gatsing et al., 2009), the antimicrobial activity of aqueous extracts of the leaves of *H. madagascariensis* and the whole plant of *E. prostrata* was investigated against *S. Typhi*, *S. Paratyphi A*, *S. Paratyphi B* and *S. Typhimurium*. Phytochemical screening and the acute toxicity study of the various extracts were also done.

**MATERIALS AND METHODS**

**Plant material**

The leaves of *Harungana madagascariensis* Lam were collected from trees growing in Dschang, West region of Cameroon, in January 2010. The plant was identified at the Cameroon National Herbarium (Yaoundé) where a voucher specimen was deposited (Ref. N° 3239). The whole plant of *Euphorbia prostrata* Ait was collected from Dschang in April 2010, and identified by the Cameroon National Herbarium (Yaoundé), where a voucher specimen was deposited (Ref. N° 33585/HNC).

**Test bacteria and culture media**

The test microorganisms, including *Salmonella Typhi*, *Salmonella Paratyphi A*, *Salmonella Paratyphi B* and *Salmonella Typhimurium*, were obtained from Pasteur Centre, Yaoundé, Cameroon. The culture media used, namely Mueller Hinton Agar (MHA), Salmonella-Shigella Agar (SSA) and Mueller Hinton Broth (MHB) were manufactured by AccumixTM (Belgique). SSA was used for the activation and isolation of the *Salmonella* species and for the screening of contaminants from the inoculum. Mueller Hinton Agar and Mueller Hinton Broth were used for antibacterial tests.

**Chemicals for antimicrobial assays**

Ciprofloxacin (Sigma-Aldrich, St Quentin Fallavier, France) and p-Iodonitrotetrazolium chloride (INT) were used...
as reference antibiotic and microbial growth indicator, respectively.

**Experimental animals**

In this study, 60 Swiss albino mice (30 males and 30 females, aged 10-12 weeks and weighing 20-30 g) were used. These animals were bred in the animal house of the Department of Biochemistry, University of Dschang, in the ambient environmental conditions (23 ± 2 °C).

**Preparation of plant extracts**

The leaves of *H. madagascariensis* were collected and air-dried at room temperature after which they were ground into powder. The extract (decoction) was prepared, according to traditional healer indications, by introducing 100 g of powder in one liter of distilled water and bringing the mixture to boil for 15 min. After cooling, the plant mixture was filtered using Whatman No. 1 filter paper. The filtrate (extract) was concentrated by allowing it to stand in an oven (Memmert) at 45 °C.

The whole plant of *E. prostrata* was collected and air-dried at room temperature and then ground into powder. The extraction (infusion) was done according to traditional healer indications. Thus 97.20 g of the powder were soaked for 15 min in 2 L of boiled distilled water. The preparation was filtered using Whatman No. 1 filter paper. The filtrate (extract) was concentrated by allowing it to stand in an oven (Memmert) at 45 °C.

**Antimicrobial assay**

The MIC values of the various extracts on the studied bacteria were determined using rapid INT colorimetric assay (Eloff, 1998; Mativandlela et al., 2006). Briefly, the test sample was dissolved in distilled water and the solution obtained was added to MHB, and two-fold serially diluted (in a 96-wells microplate). This was followed by the addition of 100 µl of inoculum (1.5 × 10⁶ CFU/ml) prepared in MHB. The plates were covered with a sterile plate sealer, agitated to mix the contents of the wells using a shaker and incubated at 37 °C for 18 h. Wells containing MHB and 100 µl of inoculum served as negative control. The total volume in each well was 200 µl. Ciprofloxacin was used as reference antibiotic. The MICs of samples were detected after 18 h incubation at 37 °C, following addition (40 µl) of 0.2 mg/ml INT and re-incubation at 37 °C for 30 minutes. Viable bacteria reduced the yellow dye to pink. MIC was defined as the lowest concentration of the sample that prevented this change and exhibited complete inhibition of microbial growth.

**Phytochemical screening**

The phytochemical screening was done using standard methods described by Harborne (1973), Odebiyi and Sofowora (1978). The plant sample was screened for the following classes of compounds: phenols, tannins, saponins, anthraquinones, anthocyanins, triterpenoids, flavonoids, alkaloids and steroids.

**Acute toxicity test**

For acute toxicity studies, 60 Swiss albino mice (30 males and 30 females) were used. Animal treatment was done according to the method previously used by Gatsing et al. (2005). The animals in all the groups were observed during the first 3 h after a single oral administration of the extract, for behavioural changes. The deaths were counted within the first 48 h and the lethal dose 50 (LD₅₀) was determined using the method of Behrens and Karber (1983); the surviving animals were further observed for two weeks, during which their weight, food and water consumption were recorded.

**Statistical analysis**

Data are presented as mean ± SEM (standard error of mean). Statistical analyses were performed with the aid of SPSS for Windows software program (release 12.0). Group comparisons were done using One-Way ANOVA and the Waller-Duncan test. A
P value < 0.05 was considered statistically significant.

Ethics
This work was carried out with respect for the welfare of animals, as recommended by WHO (1992).

RESULTS
Antimicrobial assay
The crude aqueous extracts obtained from the leaves of *H. madagascariensis* and the whole plant of *E. prostrata* were tested against four *Salmonella* species, namely *Salmonella Typhi*, *Salmonella Paratyphi A*, *Salmonella Paratyphi B* and *Salmonella Typhimurium*, and the results are presented in Table 1. The data obtained show that both plant extracts exhibited *in vitro* antibacterial activity against the various *Salmonella* species, with the MIC values ranging from 390.625 to 1562.5 µg/ml for *H. madagascariensis* extract and from 1024 to 2048 µg/ml for *E. prostrata* extract.

Phytochemical composition of the extracts
Phytochemical screening of *H. madagascariensis* and *E. prostrata* aqueous extracts revealed the presence of phenols, tannins, saponins, anthraquinones, anthocyanins, triterpenoids, flavonoids and alkaloids in both plants (Table 2). Sterols were absent in these extracts.

Acute toxicity

Behavioural observations
The mice were observed during the first 3 h after a single oral administration of the extract of each plant, for activity (locomotion), reaction to noise, reaction to pinch, state of excrement, and for mortality (within 48 h). For *H. madagascariensis* extract, a reduction in activity was observed in both male and female mice as from the dose 16 g/kg. The reactions to noise and to pinch were reduced in female mice as from the dose 20 g/kg. Male mice had soft excrement as from the dose 20 g/kg, whereas the excrement of female mice was soft as from 16 g/kg. For *E. prostrata* extract, locomotion, reaction to noise and reaction to pinch were reduced as from the dose 16 g/kg for both male and female mice.

The median lethal doses (LD₅₀) of *H. madagascariensis* extract as shown by the acute toxicity studies were 11.6 g/kg and 13.2 g/kg body weight for female and male mice, respectively. The LD₅₀ values obtained for *E. prostrata* extract were 23.2 g/kg and 26.4 g/kg body weight for female and male mice, respectively.

Food and water consumptions
Food and water consumption of mice during acute toxicity study with *H. madagascariensis* extract are shown in Table 3. Except for the dose of 8 g/kg in males, generally there was an increase in food consumption in all treated male and female animals, compared to the control group. This increase was significant (p < 0.05) in the males. In male mice, there was a decrease [significant (p < 0.05) at doses of 4 g/kg and 16 g/kg] of water consumption in all animals treated during the first week. However, in the females, there was an increase [significant (p < 0.05) at the dose of 8 g/kg] of this parameter during the same period. At the second week of treatment, there was a general increase in water consumption in all treated animals (except in male group receiving 16 g/kg and female group receiving 4 g/kg), compared to the control group.

Food and water consumption of mice receiving *E. prostrata* extract are presented in Table 4. During the first week, there was a significant decrease of food consumption in females at doses of 2, 16 and 24 g/kg and a significant (p < 0.05) increase at the dose of 8 g/kg, while in males, there was a significant (p < 0.05) increase at doses of 4, 8 and 16 g/kg and a significant decrease at doses of 2 and 24 g/kg. At the second week, in general, there was a significant (p < 0.05) decrease of food consumption in male and female mice. There was a significant (p < 0.05) decrease in water consumption during the first week in male
Body weight variation

The weight variations of the surviving female (A) and male (B) mice as affected by doses of *H. madagascariensis* extract during acute toxicity study are shown on Figure 1(A&B). Female mice generally showed increases in weight gain throughout the period of study, even though the values were significantly (p < 0.05) lower than those of the control group (Figure 1A). At doses ≥ 4 g/kg in male, there was a decrease in the percentage of weight gain in the first five days following the administration of the extract to animals. From the sixth day, there was an increase in the percentage of weight gain in these animals (Figure 1B). In general, the percentage of weight gain was significantly (p < 0.05) greater in the control than in the treated groups.

Weight variations of female (A) and male (B) mice as affected by doses of *E. prostrata* extract are shown on Figure 2 (A & B). In the female groups, the percentage of weight gain was significantly (p < 0.05) lower in the treated animals, compared to that in the control group throughout the period of study. In the male groups, except for the dose of 24 g/kg which showed a significant (p < 0.05) reduction in weight gain percentage during the first six days, in general, there was an increase in the percentage of weight gain during the fourteen days of acute toxicity study.

Table 1: MIC values of *Harungana madagascariensis* and *Euphorbia prostrata* extracts against *S. Typhi*, *S. Paratyphi A*, *S. Paratyphi B* and *Salmonella Typhimurium*.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Bacterial strains and CMI (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ST</td>
</tr>
<tr>
<td><em>H. madagascariensis</em></td>
<td>1562.5</td>
</tr>
<tr>
<td><em>E. prostrata</em></td>
<td>2048</td>
</tr>
<tr>
<td>Ciprofloxacin (standard)</td>
<td>0.625</td>
</tr>
</tbody>
</table>

ST: *Salmonella Typhi*; SPA: *Salmonella Paratyphi A*; SPB: *Salmonella Paratyphi B*; ST<sub>2</sub>: *Salmonella Typhimurium*.

Table 2: Phytochemical composition of *Harungana madagascariensis* and *Euphorbia prostrata* extracts.

<table>
<thead>
<tr>
<th>Phytochemical composition</th>
<th>EHM</th>
<th>EEP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anthocyanins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sterols</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

(+): Present; (−): Absent
EHM: Extract of *H. madagascariensis*;
EEP: Extract of *E. prostrata*
### Table 3: Food and water consumption of mice during acute toxicity study of *Harungana madagascariensis* extract.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Doses (g/kg)</th>
<th>Food consumption (g)</th>
<th>Water consumption (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1st week</td>
<td>2nd week</td>
</tr>
<tr>
<td>Female</td>
<td>0</td>
<td>6.10 ± 1.12a</td>
<td>5.97 ± 1.04a</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6.52 ± 1.59ab</td>
<td>6.61 ± 0.90ab</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>6.07 ± 1.56a</td>
<td>5.07 ± 0.97a</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>6.90 ± 1.09ab</td>
<td>5.95 ± 0.87ab</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>6.12 ± 2.79a</td>
<td>5.57 ± 1.13a</td>
</tr>
<tr>
<td>Male</td>
<td>0</td>
<td>5.49 ± 1.14a</td>
<td>5.64 ± 1.28a</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6.45 ± 1.66b</td>
<td>6.03 ± 0.90b</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>6.60 ± 1.15b</td>
<td>6.36 ± 0.61b</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>5.19 ± 1.35a</td>
<td>5.45 ± 0.92a</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>6.25 ± 2.76b</td>
<td>6.71 ± 0.75b</td>
</tr>
</tbody>
</table>

Tabulated values are Mean ± SEM of 5 determinations. Data in the same column in the same sex with different superscript letters are significantly different (p < 0.05).

### Tableau 4: Food and water consumption of mice during acute toxicity study of *E. prostrata* extract.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Doses (g/kg)</th>
<th>Food consumption (g)</th>
<th>Water consumption (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1st week</td>
<td>2nd week</td>
</tr>
<tr>
<td>Female</td>
<td>0</td>
<td>33.60 ± 5.63a</td>
<td>37.93 ± 3.14a</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>29.57 ± 3.47bc</td>
<td>32.14 ± 8.75b</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>32.57 ± 7.40b</td>
<td>24.57 ± 3.63d</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>36.21 ± 6.89a</td>
<td>30.79 ± 8.19b</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>26.07 ± 2.76d</td>
<td>27.39 ± 6.02c</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>13.86 ± 1.01e</td>
<td>11.57 ± 1.79e</td>
</tr>
<tr>
<td>Male</td>
<td>0</td>
<td>30.14 ± 4.74bc</td>
<td>34.50 ± 3.33c</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>26.85 ± 4.95b</td>
<td>25.29 ± 6.87d</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>36.07 ± 5.36a</td>
<td>32.57 ± 6.44ab</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>34.64 ± 5.21a</td>
<td>29.43 ± 6.96c</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>35.64 ± 5.16b</td>
<td>29.97 ± 8.09bc</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>20.00 ± 4.81d</td>
<td>20.86 ± 3.00c</td>
</tr>
</tbody>
</table>
Figure 1: Weight variations of female (A) and male (B) mice as affected by doses of *H. madagascariensis* extract during acute toxicity study. Data are expressed as mean ± S.E.M of 5 determinations.
Figure 2: Weight variations of female (A) and male (B) mice as affected by doses of *E. prostrata* extract during acute toxicity study. Data are expressed as mean ± S.E.M of 5 determinations.
DISCUSSION

Antibacterial activity

Both plant extracts showed in vitro antibacterial activity against the various Salmonella species used, with the MIC values ranging from 390.625 to 1562.5 µg/ml for *H. madagascariensis* extract and from 1024 to 2048 µg/ml for *E. prostrata* extract. In fact, according to criteria described by Fabry et al. (1998), extracts showing MIC values below 8000 µg/ml have noteworthy antimicrobial activity. Therefore, *H. madagascariensis* and *E. prostrata* aqueous extracts are active, and the antibacterial activities recorded with the two plant extracts can, in general, be considered as moderate. These results showed that *H. madagascariensis* and *E. prostrata* contain substances with antibacterial activities which may be used in the treatment of typhoid and paratyphoid fevers.

The phytochemical studies revealed the presence of secondary metabolite such as alkaloids, anthocyanins, anthraquinones, flavonoids, phenols, saponins, tannins and triterpenoids. Though the detection of such metabolites does not automatically predict the antimicrobial activity of a plant extract, it has clearly been demonstrated that several compounds belonging to the investigated classes of metabolites showed antibacterial activities (Bruneton, 1999; Gatsing et al., 2008; Ogutu et al., 2012). In fact, some flavonoids have shown several pharmacological activities including antibacterial and antifungal (Leung, 1980). Some triterpenes have presented a protective function by participating in the fight against microbial attacks and also acting as insectifuge (Harborne, 1973). Compounds belonging to the anthraquinone class such as norindone, dammacanthal and nordamnacanthal have been reported to exhibit high antimicrobial activities (Ali et al., 2000). Tannins prevent bacterial growth by precipitating their proteins (Fluck, 1976). Some saponins have demonstrated antifungal activities (Etsuji et al., 1984). The antibacterial activity of the two plant extracts used in this study may thus be due to the presence of flavonoids, triterpenoids, alkaloids, tannins or saponins.

Acute toxicity

A World Health Organization (WHO) survey indicated that about 70-80% of the world’s populations rely on non-conventional medicine, mainly of herbal sources, in their primary healthcare (Chan, 2003). Besides, there has been erroneous belief that these medicines are free from adverse effects (Ermst, 2005). Although medicinal plants may produce several biological activities in humans, generally, very little is known about their toxicity and the same applies for *H. madagascariensis* and *E. prostrata*. To determine the safety of drugs and plant products for human use, toxicological evaluation is carried out in various experimental animals to predict toxicity and to provide guidelines for selecting a ‘safe’ dose in humans (Okoli et al., 2002).

In general, acute toxicity study did not reveal any negative behavioural changes at low doses (≤ 4 g/kg) of extract as compared to the control in both sexes and for both plant extracts. However, reduced activity and reaction to noise were observed at doses ≥ 16 g/kg, as compared to the control group, suggesting that *H. madagascariensis* and *E. prostrata* extracts may have a depressant or sedative effect on the central nervous system at high doses. The extract may act as myorelaxant or tranquiliser on the nervous centres or on the motor fibres (Schmitt, 1973). Plants containing chemical constituents like coumarin, flavonoids, monoterpenes, proanthocyanidines and glycolipids, have been reported to possess central nervous system (CNS) depressant activity (Abid et al., 2006). Trofimiuk et al. (2005) reported that the sedative, muscle relaxant and anxiolytic effects of plant extracts could be due to the interaction of isoflavonoids with the GABA/benzodiazepine receptor complex in the brain. The depressant effect of *H. madagascariensis* and *E. prostrata* extracts may therefore be due to the presence of flavonoids in the extract.
The reduction of reaction to pinch was observed at doses ≥ 16 g/kg in both sexes and for both plant extracts. The effect of the extract on the perception of pain may be due to its action on the nociceptors or to the inhibition of the production of algogenic substances (e.g. prostaglandins, histamines), or to the inhibition of the painful message transmission, at the central level (Nguelefack et al., 2004). As mentioned above, preliminary phytochemical screening of *H. madagascariensis* and *E. prostrata* extracts revealed the presence of flavonoids. Flavonoids are known to target prostaglandins which are involved in the late phase of acute inflammation and pain perception (Chakraborty et al., 2004). Hence, the presence of flavonoids may potentiate the action of this extract on pain perception.

The method of Behrens and Karber (1983) was used for the calculation of the LD₅₀ which, generally, reflects the lethal nature of a substance (Bidie et al., 2010). The LD₅₀ values of *H. madagascariensis* extract were 11.6 g/kg and 13.2 g/kg for female and male mice respectively, whereas those of *E. prostrata* extract were 23.2 g/kg and 26.4 g/kg for female and male mice, respectively. Based on the Hodge and Steiner criteria (Delongeas et al., 1983), *H. madagascariensis* and *E. prostrata* extracts can be considered practically non-toxic, since the LD₅₀ values were greater than 5 g/kg. Moreover, the LD₅₀ values of *E. prostrata* extract are about 158 times the dose corresponding to the maximum MIC of the extract obtained in this study (i.e. 0.146 g/kg), whereas those of *H. madagascariensis* extract are about 103 times the dose corresponding to the maximum MIC of the extract (i.e. 0.112 g/kg).

Female mice treated with the various doses of *E. prostrata* extract (2, 4, 8, 16 and 24 g/kg) and male mice treated with the dose 24 g/kg showed significant reduction in body weight during this study, as compared to control. This reduction in weight may be due to less food and water intake, as observed during the study, which may be secondary to loss of appetite after administration of the extract (Joseph et al., 1989; Gatsing et al., 2005).

**Conclusion**

In the light of the foregoing, *H. madagascariensis* and *E. prostrata* contain antimicrobial substances which are active against *Salmonella Typhi*, *Salmonella Paratyphi A*, *Salmonella Paratyphi B* and *Salmonella Typhimurium*. Moreover, the aqueous extracts of these plants may be practically non-toxic and could be used in the treatment of typhoid and paratyphoid fevers. However, *in vivo* antimicrobial study and subchronic toxicity study should be done to further ascertain the antityphoid activity and determine the side effects of the extracts at the level of tissues and organs.

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