ANTIMICROBIAL ACTIVITY OF THE EXTRACT OF *Solenostemma Argel* (*Harjal*) PLANT

By

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**ABSTRACT**

The present study investigated the antimicrobial activity of *harjal* aqueous extracts against two fungi (*Aspergillus niger* and *Penicillium italicum*) and two Gram-negative bacteria (*E. coli* and *Salmonella typhi*). The *harjal* aqueous extracts were found to inhibit mycelial radial growth of both fungi. The effect was clear at the first days and at the last days of the experiment, the effect was insignificant. Mycelial fresh and dry weights of both fungi were also greatly reduced with the *harjal* extracts. The higher concentration gave the maximum effect which decreased with dilution. The effect on mycelial growth was more pronounced on *P. italicum* than on *A. niger*. The effect of *harjal* leaves extract on the two bacteria (*E. coli, S. typhi*) was evaluated by the inhibition zone and dilution methods. A clear zone of inhibition was shown by the extracts against both bacteria, although the effect was less against *E. coli*. The results of the dilution plate method, showed that the log number of colonies of both bacteria was highly decreased with *harjal* extracts, however, *S. typhi* was more susceptible and greatly affected.

**KEY WORDS:** Harjal (*Solenostemma argel*), antimicrobial activity, herbalism, radial growth.

**الملخص:**

أجرت هذه الدراسة لمعرفة خصائص المستخلص المائي للحرجل المضاد للميكروبات على إثنين من الفطريات (*A. niger* and *P. italicum*) وِ إثنين من البكتريا السالبة لصبغة غرام (*E. coli* و *S. typhi*).
INTRODUCTION
Herbal medicine sometimes referred to as herbalism or botanical medicine, is the use of herbs for their therapeutic or medicinal value. A herb is a plant or a plant part valued for its medicinal, aromatic or savory qualities. Herb plants produce and contain a variety of chemical substances that act upon the body (Shelef, 1983).

It has been estimated that approximately 80% of the world's inhabitants rely mainly on traditional medicines for their primary health care; where plant based systems still play a vital role in health care. In developed countries, plant drugs are also extremely important, currently at least 119 chemicals derived from plant species can be considered as important drugs in use (Mullholland, 2000). Spices and herbs have been used for thousands of centuries by many cultures to enhance the flavor and aroma of food. Early cultures also recognized the value of using spices and herbs in preserving food and for their medicinal value (Shelef, 1983). Scientific experiments since the late 19th century have documented the antimicrobial properties of some spices, herbs, and their components (Zakia, 1988).

Ten Sudanese plants were screened for their antibacterial activity, seven of them showed promising results (Mustafa et al., 1982). Crude extracts solution obtained from the plant Gordenia lutea, showed antibacterial activity against Bacillus subtilis, Staphylocous aureus, Escherichia coli and Pseudomonas typhi.
aeruginoza (Ahmed et al., 1984). Badreledin (2006) reported that ginger oil showed antimicrobial activity against *Staphylococcus aureus*, while, ELboshra (2005) reported that *Staphylococcus aureus* was sensitive to clove oil. The fenugreek oil was also found to inhibit *Salmonella typhimurium* (Sulieman, 2009). Despite of the growth of global market for herbal products, following complementary and/or alternative medicines, homeopathy, health foods and natural-pharmaceuticals therapy, yet the majority of these herbs were not assessed for quality, safety and efficacy.

The plant *harjal* (*Solenostemma argel*) is a member of the family Asclepiadaceae, that comprises numerous medicinal plants, like *Calotropis procera*, *Marsdenia obyssinicna* and *Huernia mecrocarpa*, known for their cardiac activity. *Harjal* grows naturally in the northern parts of the Sudan and extends from Berber to Abu-Hamad, especially the Rubatab area. It is also widely distributed throughout North Africa (Egypt, Libya and Algeria) and the Saudi Arabia (Ahmed, 2004). *Harjal* leaves are used in indigenous medicine for the treatment of some diseases such as the disease of liver and kidney. It is an effective remedy for bronchitis and is used to treat neuralgia. It is used as incense in the treatment of measles and sometimes crushed and used as remedy for healing wounds. The leaves are infused to treat gastro-intestinal cramps and stomach colic.

The present study was therefore, aimed to investigate the antimicrobial activities of the aqueous extracts of *harjal* against two fungi (*Aspergillus niger* and *Penicillum italicum*) and two bacteria (*E. coli* and *Salmonella typhi*).

**MATERIALS AND METHODS**

**Collection of samples**

Samples of *harjal* (*Solenostemma argel* L.) Leaves were obtained from Wad Medani local market during 2008. The samples were taken from retailer’s stores. The leaves were freed from foreign materials like stones, sand and dust, before kept in the lab., for further investigation. The leaves were then washed with water, dried, and milled using laboratory mill into fine powder. Aqueous extract of the powder (50g/500ml) was used to prepare different concentrations (0.00, 50 and 100% extract).
Source of cultures
The two bacterial isolates, *Escherichia coli* and *Salmonella typhi* and the two fungi, *Aspergillus niger* and *Penicillium italicum* were obtained from the Microbiological Laboratory of the Department of Food Science and Technology, Faculty of Engineering and Technology, University of Gezira, Wad Medani, Sudan.

Effects of harjal extracts on fungal growth
Effect on mycelial dry weight
The method used was as described by Abdel-Rahim *et al.* (2002). The Potato Dextrose Broth (PDB) medium was prepared and then dispensed in 100 ml in conical flasks volume (250ml).

The extracted solution was added to each flask to make serial dilutions of (0.0, 50 and 100%). The suspension in each flask was sterilized in an autoclave at 121°C (15-lb/ in²) for 15 minutes, and then allowed to cool at room temperature, before inoculation. Each flask was inoculated by three discs (5.0 mm diameter), taken from an edge of an actively growing culture of the fungus *Aspergillus niger* or *Penicillium italicum* grown on solidified PDA (medium). Inoculated flasks were incubated at room temperature (28-30°C) for 8 days. After incubation mycelia were collected by filtering the culture through a Whatman No.1 filter paper and fresh weight recorded and then dried at 80°C for 24 hours, before being weighed. All treatments were done in triplicates.

Effect on radial growth
The method used was as described by Abdel-Rahim *et al.* (1997) The Potato Dextrose Agar (PDA) medium was prepared and dispensed in 100 ml in conical flasks (250 ml), then the extract solutions were added to each flask to obtain several dilutions (0.0, 0.50 and 100%). The media were finally sterilized in an autoclave at 121°C (15 lb/In²) for 15 minutes.

After sterilization the solution (medium +extract) of each flask was poured in sterile Petri dishes and left to solidify at room temperature (28-30°C) for 24-40 hour. Each solidified medium was then inoculated centrally by a fungal growth disc cut by a sterile cork-borer (No.5) from an edge of an actively growing
culture of the fungus *A. niger* or the fungus *P. italicum* grown on PDA. The inoculated Petri dishes were then incubated at room temperature for 8 days and the radial growth was measured every two days. All treatments were done in triplicates.

**Calculation**

Every 48 hours, the diameter of growth was measured by taking the average of two crossed dimensions for each disc in the Petri dish. The radial growth was then calculated as a percentage from the diameter of the glass Petri dish.

**Effect of *harjal* extract on bacterial growth**

Two methods were used for this test:

**The Cup-Plate agar diffusion (Inhibition zone) method**

This method was used, using Nutrient Agar (NA). In this method 2ml of a standardized bacterial cell suspension \((10 \times 10^5)\) of *E. coli* or of *S. typhi* were thoroughly mixed with 200 ml of sterile molten nutrient agar, then the medium was distributed into sterile Petri-dishes and was left to solidity at room temperature for 24 hours. Sterile Whatman glass fiber discs (No. 5) were saturated with the extract of *harjal*, then allowed to dry and transferred centrally on the surface of the solidified medium in each plate. The plates were then incubated at room temperature for 72 hours and the inhibition zones were measured as described by Barry *et al.* (1970) and Cruickshank *et al.* (1975). Three replicates were made for each treatment.

**The Dilution Plate Method**

The effects of the aqueous extracts of *harjal* on bacterial growth, were made by the Dilution Plate Method. Nutrient broth medium batches containing different concentrations of the *harjal* extracts were distributed into McCartney bottles, each containing 9 ml. The medium was autoclaved and each bottle was inoculated by 1 ml of the bacterial suspension prepared as above. Inoculated bottles (three per treatment) were incubated at room temperature and the bacterial growth was measured by the dilution plate method, where 1 ml was
drawn from each bottle and placed in a tube cooling to 9 ml sterile distilled water and serially diluted.  
From each dilution 0.1 ml was removed by a sterile pipette and placed on the surface of an already solidified Nutrition Agar (NA) in Petri dishes and spreaded on that surface, using a sterile glass rod. Inoculated plates were kept at room temperature and the number of colonies was counted at different intervals of time. The number was calculated as a log number of colonies per 1 ml. The logs of the numbers were plotted against time.

**RESULTS**

The present study investigated the inhibitory effects of *harjal* extracts on both fungi (*Aspergillus niger* and *Penicillium italicum*) and two bacteria (*E. coli* and *Salmonella typhi*).  
For fungi, the aqueous extracts were tested against mycelial growth (mycelial radial growth and mycelial fresh and dry weights), using PDA and PDB media, respectively. As shown in Fig.1, extracts of *harjal* greatly affected mycelial radial growth of *A. niger*. However, this effect was pronounced at the first days but after further incubation the fungus was able to grow better at the lower concentrations and the effect was not significantly different from the control at the 8th day.  
The effect of *harjal* extract on the mycelial weight of the fungus *A. niger* followed the same pattern of the radial growth, with the higher concentration giving the maximum inhibition detected, and the effect was decreasing with dilution (Fig. 2).  
Figure (3) showed the effect the effect of *harjal* extract on radial growth of *P. italicum*. From the results, it could be seen that the radial growth was greatly reduced with the higher concentration of the *harjal* extracts. The effect on *P. italicum* was more pronounced than on *A. niger*. More than 50% reduction was recorded for the higher concentration. The result on the effect on the fresh and dry weight (Fig. 4) indicated that both weights were highly reduced with the aqueous extract of *harjal*.  

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Figure (1): Effect of different concentration of harjal extract on radial growth of *A. niger*.

Figure (2): The effect of different concentration of harjal extract on Mycelial fresh and dry weight of *A. niger*. 
Figure (3): Effect of different concentration of *harjal* extract on radial growth of *P. italicum*.

Figure (4): The effect of different concentration of *harjal* extract on mycelial fresh and dry weight of *P. italicum*. 
Table (1) compares the effect of harjal extracts on radial growth of both fungi (*A. niger* and *P. italicum*). It is clear that *A. niger* was less affected than *P. italicum*. Growth of the latter fungus was comparatively slower.

Comparison of the effect of the Harjal extracts on the fresh and dry weights of both fungi was shown in Table (2). Mycelial dry weight of both fungi was greatly reduced, especially with the higher concentration of the extract.

The present study also investigated the effect of harjal extract on growth of the two bacteria mentioned above using the Cup Plate (inhibition zone) method. The two bacteria included *E. coli* and *S. typhi*. From the results, it is clear that harjal extract showed a clear inhibition zone of both bacteria. Although the extracts inhibited both bacteria, they were less effective against *E. coli*, however, the zone of inhibition of this bacterium showed faint bacterial growth.

The dilution plate method was also used in this work to study the effects of the harjal extract on growth of the two bacteria (*E. coli* and *S. typhi*). It could be seen that the log number of the bacterium *E. coli* was greatly reduced compared to the control treatment (Fig. (5)). The log number was gradually decreasing with time. The higher concentration resulted in large reduction.

Figure (6) showed the effect of harjal extract in growth of the bacterium *S. typhi*. The log number of the bacterium was highly decreased with treatment. The log number was almost zero after 72 hours of incubation with the higher concentration. However comparing the effect of the extract on *E. coli* and *S. typhi*, it could be noticed that the later (*S. typhi*) was more susceptible and highly affected.

### Table (1): Comparison of the effects of Harjal extracts on the *A. niger* and *P. italicum* radial mycelial growth

<table>
<thead>
<tr>
<th>Incubation time (days)</th>
<th><em>A. niger</em></th>
<th></th>
<th><em>P. italicum</em></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.0</td>
<td>0.5</td>
<td>1.0</td>
<td>0.0</td>
</tr>
<tr>
<td>2</td>
<td>7.5</td>
<td>4.5</td>
<td>3.25</td>
<td>4.25</td>
</tr>
<tr>
<td>4</td>
<td>8.5</td>
<td>6</td>
<td>3.9</td>
<td>8.58</td>
</tr>
<tr>
<td>6</td>
<td>8.5</td>
<td>5.5</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>8</td>
<td>9</td>
<td>7.5</td>
<td>5</td>
<td>9</td>
</tr>
</tbody>
</table>
Table (2): Comparison of the effects of harjal extracts on mycelial fresh and dry weight of the *A. niger* and *P. italicum*

<table>
<thead>
<tr>
<th>Concentration (%)</th>
<th>Fresh weight</th>
<th>Dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>P</td>
</tr>
<tr>
<td>0.0</td>
<td>8.95</td>
<td>4.12</td>
</tr>
<tr>
<td>50</td>
<td>8.85</td>
<td>3.66</td>
</tr>
<tr>
<td>100</td>
<td>8.35</td>
<td>2.22</td>
</tr>
</tbody>
</table>

Figure (5): The effect of the harjal extracts on log number of the bacterium *E. coli*

Figure (6): The effect of harjal extract on the log number of the bacterium *S. typhi*
DISCUSSION

The present work was conducted in order to study the inhibitory effects of Harjal extracts on the growth of two fungi (A. niger and P. italicum) and two Gram-negative bacteria (E. coli and S. typhi). Mycelial radial growth and mycelial dry and fresh weights of both fungi were greatly reduced by harjal extracts. Plant extracts of many species of plants were reported to have antifungal activities (Bullerman, 1974, Abdel-Rahim et al., 1989 and Al-Jali et al., 1997). Extracts of many plants were found to have inhibitory effects on A. niger and other fungal species such as (A. flavus, A. parasiticus etc) (Alderman and Marth, 1976, Abdel-Rahim et al., 2002). According to Al-Jali et al. (1997) essential oils were more effective than plant extracts, in certain cases. On the other hand, Zainal et al. (1988) reported a pronounced effect of the leaf litter extracts of Mesquite (Prosopis juliflora) on A. niger and Candida albicans. Abdel-Rahim et al. (1989, 2002), and Al-Jali et al. (1997), showed that plant extracts of Cupress, Juniper and Rosemary were highly effective against A. flavus and A. parasiticus growth, while the extracts of Karkadi and Eucalyptus were non-effective against both fungi. In another study El-Shayeb and Mabrouk (1984) and Osman (1986) reported that the extracts of Sweet pepper and a Hibiscus sp. caused a complete inhibition of aflatoxin production by A. flavus, although both were non-effective on fungal growth. However, there were no reports about the direct effect of Harjal extracts on fungi and bacteria in the literature. Lai (2004) said that many herbs and spices included Harjal can yield medicinal compounds. Spices and herbs have been used for thousands of years by man to enhance the flavour and aroma of foods.

The present study also investigated the effect of harjal extracts on growth of two bacteria (E. coli and S. typhi). Two, methods; the dilution plate method and the Cup plate (inhibition zone) method were employed for that test. From the results it was found that Harjal extracts were highly effective against both bacteria, although the effect was more pronounced in the case of S. typhi. Antibacterial activity of spices and other plants are well documented (Alicia, 1981). Vlietinek et al. (1995) screened about 100 medicinal plants, used by traditional healers to treat infections in Rwanda, for their antibacterial, antifungal and antiviral properties. Their study showed that 45% were active
against bacteria; Staph aureus, 2% against E. coli, 16% against Pseudomonas aerogenosa, and 7% against fungus; Candida albicans. However, about 27% of the plant species tested exhibited antiviral properties. In Sumatra (Indonesia), 114 plant species extracts were assayed for their antibacterial activity (Ahmed, 2002). About 82% of the extracts were active against bacteria; Staph aureus, while 35% of them were active against E. coli and about 20% of the extracts inhibited growth of the tested fungi (Saccharomyces cerevisiae and Fusarium oxysporum).

In Sudan many studies were carried out for testing the antimicrobial activity of some medicinal plants. Ahmed (2004) tested the extracts of 10 Sudanese medicinal plants against gram-positive and gram-negative bacteria as well as on Candida albicans. He found a marked antibacterial effect against the gram-positive bacterium, Staph aureus, followed by the E. coli and Candida albicans.

The fenugreek oil was also found to inhibit Salmoenlla typhimurium, the inhibition zone diameters were 15mm when the oil concentrations were 100%. No reports in the literature were found regarding the effects of fenugreek oil against this bacterium, however, ginger oil and clove oil were reported to be effective against this bacterium (James et al., 1999; Elboshra, 2005 and Badreldin, 2006).

CONCLUSIONS
The present study indicated that aqueous harjal extracts have antimicrobial activity against the tested organisms. The study also revealed that the highest inhibitory effect of the harjal extract was found against P. italicum. The inhibitory effect against the tested organisms was more effective when using the concentrated extract.

From the present work it could be recommended that: harjal extracts can be used as antibacterial and antifungal agents. And it can be used in the food industry to flavour food and to compact contamination by micro-organisms.
REFERENCES


