ORIGINAL RESEARCH

Phytochemical composition of *Anastatica hierochuntica* L., can it fight the toxigenic bacterial agents responsible for food poisoning?

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**ABSTRACT**

The present work aims to study the biological activity of *Anastatica hierochuntica* L., against four bacterial strains considered as toxigenic responsible for food-borne infection. The plant was collected from Tindouf region (Far Southwest of Algeria). In this study we performed a phytochemical screening and evaluation of antibacterial activity of three macerates of two vegetative parts (seeds and stems) by two methods (disk and wells diffusion method). The yield of aqueous, methanolic and etheric macerates of the seeds and stems were (5.1; 3.8), (5; 1.4) and (2; 0.95)% respectively. Also, it appears that macerates obtained were rich in bioactive phyto-constituents particularly the seed of the plant. They showed the presence of ten large chemical groups. The antibiotic resistance profile of the bacterial strains tested showed an increased resistance to several families of antibiotics. The evaluation of the antibacterial activity of the extracts showed that methanolic and aqueous macerates of the seed were more active against Gram positive bacteria. The methanolic macerate of the stems was less active. However, other macerates were ineffective. The results obtained show that the plant has an average antibacterial activity and that depends on extract concentration used.

**Key Words:** *Anastatica hierochuntica*: phytochemical screening; antibacterial activity; foodborne infection; Tindouf (Algeria)

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**Introduction**

First ever estimates of the global burden of foodborne diseases caused by 31 agents (bacteria, virus, parasite, toxins and chemicals) states that each year as many as 600 million, or almost 1 in 10 people in the world, fall ill after consuming contaminated food and 420000 die as a result. Children under 5 years of age at particularity high risk, with 125000 children dying from foodborne diseases every year, where African and South-East Asian regions have the highest burden of this diseases (WHO, 2015).

Meanwhile, Plant biodiversity of the Sahara is characterized by the presence of medicinal plants having a great therapeutic potential against several diseases. For a long time, natural remedies, and especially medicinal plants were the principal recourse of medicine for our grandparents, despite the significant development of the pharmaceutical industry that allowed modern medicine to treat a large number of often fatal diseases (Bruneton, 1999).

The Brassicaceaes family, is among the ten families of the most economically important plants (Warwick, 1993). Indeed, many species are used as food plants, ornamentals, condiments, and others are considered as industrial sources of vegetable oils. According to Bellakhdir (Bellakhdir, 1997), Brassicaceaes are very used in traditional Moroccan medicine.

The species of *Anastatica hierochuntica* L., is known for its therapeutic properties as a hepato-protective plant, hypoglycemic and diuretic. It is used in traditional medicine for uterine bleeding and to facilitate the expulsion of dead fetus, to treat gastrointestinal disorders, depression, high blood pressure, indigestion, headache, fever, malaria, epilepsy, heart disease and infertility (Hegazy and Kabel, 2007).

It’s in this context that we were interested in phytochemical properties of the species *A. hierochuntica* L., and highlight the antibacterial activity of its some excerpts namely the methanolic, aqueous and etheric macerates against four bacterial strains responsible for poisoning and foodborne diseases.

**Materials and methods**

This study was conducted at the University of Bechar (Algeria), after preparing the plant as follows;

**Harvesting plants**

The plant studied was collected after being identified during the months of February-March 2015 in the far southwest of Algeria (Algerian-Moroccan border). The dried plant was put into clean bags.

**Qualitative phytochemical screening**

About tri-phytochemical study, three extractions were conducted according to the protocol developed by Emad (2014). The crude extracts were obtained by successive extractions with solvents of increasing polarity. In this order, petroleum ether, methanol and distilled water were used. Preparing extracts allowed performing a qualitative phytochemical screening of two vegetative parts (seeds and stems).

**Table 1** : Phytochemical screening of *A.hierochuntica* L.

<table>
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<tbody>
<tr>
<td>Seeds</td>
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<td>+</td>
<td>+</td>
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<tr>
<td>Stems</td>
<td>-</td>
<td>+</td>
<td>+</td>
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### Extraction processes

The aqueous, methanolic and etheric macerates of the plant studied were obtained by maceration with the method described below:

**Preparation of macerates**

A test sample 10 g of the dried plant was mixed with 100 ml of distilled water. The mixture is stirred for 24 hours. After filtration through a filter paper, the filtrate is evaporated (rota vapor), till it dried under reduced pressure at 100°C in order to obtain the aqueous macerates residue.

### Table 2: Values of inhibition zone diameters and inhibition percentages (1%) of antibiotics against the bacterial strains responsible for food-borne Infection.

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>E. coli</th>
<th>S. aureus</th>
<th>S. typhimurium</th>
<th>L. monocytogenes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATB</td>
<td>D (mm)</td>
<td>I (%)</td>
<td>P&lt;sub&gt;ATB&lt;/sub&gt;</td>
<td>D (mm)</td>
</tr>
<tr>
<td>Fosfomycin</td>
<td>9</td>
<td>10</td>
<td>R</td>
<td>28</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>22</td>
<td>24,44</td>
<td>S</td>
<td>-</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>6</td>
<td>6,66</td>
<td>R</td>
<td>-</td>
</tr>
<tr>
<td>Amox-CA</td>
<td>24</td>
<td>26,66</td>
<td>S</td>
<td>-</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>6</td>
<td>6,66</td>
<td>R</td>
<td>-</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>6</td>
<td>6,66</td>
<td>R</td>
<td>-</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>30</td>
<td>33,33</td>
<td>S</td>
<td>25</td>
</tr>
<tr>
<td>Tetracyclin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>Clindamycine</td>
<td>24</td>
<td>26,66</td>
<td>S</td>
<td>-</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>6</td>
<td>6,66</td>
<td>R</td>
<td>-</td>
</tr>
<tr>
<td>Olsitoxin</td>
<td>22</td>
<td>24,44</td>
<td>S</td>
<td>-</td>
</tr>
<tr>
<td>Imipenem</td>
<td>32</td>
<td>35,55</td>
<td>S</td>
<td>-</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Amikacin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>21</td>
</tr>
<tr>
<td>Fusidic acid</td>
<td>19</td>
<td>21,11</td>
<td>S</td>
<td>-</td>
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<tr>
<td>Ampicillin</td>
<td>-</td>
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</table>


Results and Discussion

**Qualitative phytoc hemical screening**

The phytochemical tests results of the plant exhausted by water, methanol and diethyl ether, are summarized in Table 1. The phytochemical screening performed showed the presence of ten major chemical groups in parts seeds and stems as following: tannins, coumarins, fatty acids, reducing compounds, steroids or triterpenes,sterol heterosides triterpenic, flavonoids, free quinones, terpenoids and aldehyds salts. These tests showed the presence of flavonoids and aldehyds salts but only in the seeds. As well as the absence of saponin, arachinacenosides, starch, emodols, anthocyaninoids and alkaloids bases in the two parts of the plant studied. These results were corroborated by the work of Dar (2012), which showed that all parts of the *A.hierochuntica* L., have been proved rich in total phenolic content. This representation of nearly all chemical families could justify the multiple use of A. hierochuntica. Also, these results were similar to those given by Bouhadjera et al., (2005) which has been shown the presence of almost all secondary metabolites of *Brassicaceae* family such as aldehyds salts, flavonoids, saponins, tannins, anthocyanins, sterols and steroids with little differences. This difference in composition was probably due to various conditions including the environment, genotype, geographical origin, harvest time, location, temperature and drying time (Svoboda and Hampson, 1999; Lis-Balchin and Hart, 1999).

**Antibiotic resistance profile of the bacterium used in this study:**

The antimicrobial resistance profile results of the bacterial strains against antibiotics was determined by the disk diffusion method and well diffusion method on agar medium (Sacchetti et al., 2005; Celiktas et al., 2007).
Antibacterial properties of the plant extracts; Agar diffusion method (Vincent Technique and wells method)

Faced to the problems of bacterial resistance to synthetic antibiotics, many researches have been conducted on the antimicrobial activity of natural products and plant extracts. The results of antibacterial test performed by the agar diffusion method, which are measured by measuring the inhibition zones around the discs, are shown in Table 3 and 4. The results showed that the methanolic macerate of the seeds presented an antibacterial effect only against the Gram-positive bacterial strains; *S. aureus* and *L. monocytogenes*, with no inhibition against *E. coli* and *S. typhimurium*. In addition, the aqueous macerate of the seeds presented an antibacterial effect on *S. aureus*. In parallel, no inhibition was observed against *E. coli*, *S. typhimurium* and *L. monocytogenes* for the aqeous and etheric macerates. For the stems macerates, the results show that the methanic macerate presented only an antibacterial effect on *L. monocytogenes* with inhibition zones 10, 8 and 7 mm corresponding to the concentrations 235; 117.5 and 56mg/ml for the methanolic macerate. Other macerates were revealed ineffective and have not shown any effect on the bacterial strains tested.

The results of the antibacterial test demonstrate low efficiency of the three macerates of *A. hierochuntica* L., against the bacterial strains tested. These results are consistent with the work of Al-Fatimia et al., (2007); Mohamed et al., (2010) which have not found any antibacterial activity against the same bacterial species we tested. The aqueous and methanolic macerates of the seeds has an average antibacterial activity against *S. aureus* and *L. monocytogenes*. While the methanolic macerate of the stems was only active on *L. monocytogenes*. According to the work of Rahmoun et al., (2014) who tested the antimicrobial activity of hydro-methanolic and chloroformic extracts of *A. hierochuntica*, gave a mediocre activity against *Achenobacter baumanni*, *Citrobacter freundii*, *Enterobacter cloacae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *S. typhimurium*. This activity is attributed to phenolic compounds which have a large spectrum of activity against Gram negative bacteria. However, they did not show any interesting activity against Gram positive bacteria we tested on. The Sitosterol and the Sigmaticaster, are the phytosterols the most encountered in higher plants, they represent a wide spectrum of activity against the microorganism and the ecosystem in which it is located; the phytochemicals grouped into several classes; phenols, phenolic acids, flavonoids, anthocyanins, tannins, coumarins and quinones (Arimboor et al., 2008), which are exploited in phytotherapy, having a vasculo-protectrices, antispasmodic and antioxidants properties (Shon et al., 2004; Machex et al., 2005), but also to the alkaloids by their different physiological effects, which these compounds are mainly represented by tropanic, imidazole and indolic in the Brassicaceae family (Berghious et al., 2009). These are substances that possess various activities: anticancer (Charpentier et al., 2008), local anesthetic and antimicrobial activity (Waller and Novack, 1978).

The flavonoids have a very large and diverse antibacterial activity. Indeed, they attack a lot of bacteria with different intensity depending on the microorganism and the ecosystem in which it is located; the flavonoids are able to inhibit the growth of various types of Gram positive and Gram negative bacteria namely *S. aureus*, *E. coli*, *P. mirabilis* and *Enterococcus faecalis*. Also, terpenoids have strong activity against *S. aureus*, and also low activity against Gram negative bacteria (Bouhadjera et al., 2005). It should also be noted that methanol can extract bioactive compounds; anthocyanins, terpenoids, saponins, tannins, xanthohylin, flavones, polyphenols) more than other solvents used (Cowan, 1999). It argues that the inhibitory effect of methanolic macerate observed as well as in the work of Mohamed et al., (2010) on the same species.

Conclusion

The bioactive phytochemicals detected and the antibacterial activities of *A.hierochuntica* L., against toxigenic bacterial strains responsible for food born infection support their medicinal properties which are used in traditional medicine. The modest results of the antibacterial activity of methanolic and aqueous macerates of the seeds reflects the need for further investigation regarding fractionation and purification of bioactive compounds, as well as the mode of action in microbial cells.

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Conflict of interest

The authors have declared no conflict of interest

References


