Alternative Treatment of Infection by Compounds Isolated from *Globularia Eriocephala* Leaves

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ABSTRACT

**Objective:** The prevalence of antimicrobial resistance among clinical pathogens is reviewed and its clinical impact on management is increased. Continued surveillance of resistance rates among clinical pathogens is needed to ensure that appropriate recommendations can be made for treatment of infected patients. Since, vancomycin-resistant enterococci (VRE) and methicillin-resistant *Staphylococcus aureus* (MRSA) are the most important bacteria isolated in this case. Further studies addressing the clinical and bacteriological outcomes of patients infected with a resistant pathogen are needed. The leaves of *Globularia eoriopehala* Pomel (family Globulariaceae) named “Tasselgha” are widely used in North region of Algeria as a folk medicine. Chloroform, ethyl acetate and hydromethanol extracts of *Globularia alypum* and essential oil were tested against some clinical pathogens bacteria. **Methods used:** Antibacterial activities were tested against bacteria isolated from surgery in peroperative period using disc-diffusion method and MIC was determined. The compound of essential oils was confirmed using chromatography techniques. **Results:** The hydromethanolic extract of *Globularia eriocephola* leaves demonstrated antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and inactivity against *Proteus vulgaris* and *Escherichia coli*. Antibacterial activities were observed in ethyl acetate and hydromethanolic extracts of *Globularia eriocephala* leaves. The MIC values of the compound against *Staphylococcus aureus* (75.0mg/ml), *Pseudomonas aeruginosa* (63.5mg/ml) and *Klebsiella pneumonia* (62.0 mg/ml). The combination of Chloroform extract and essential oils exerted wielded a synergistic effect for the inhibition against the growth of the Gram-negative and Gram-positive bacteria when the MICs were applied. **Conclusion:** Ethyl acetate extract showed promising antibacterial activity against bacterial responsible of nosocomial infection, and the essential oil compounds showed at all a moderate activity against these bacteria. These results suggested *Globularia eriocephala* could be a potential source of antibacterial agents. Further investigations are in progress to determine the active constituent(s) for their application in medical research.

**Key words:** Antimicrobial, Medicinal plants, *Globularia eriocephala*, Polyphenols, Algeria.

Introduction

The plants of *Globularia eriocephala* Pomel (family Globulariaceae) named in Arabic “Tasselgha” are traditionally used in north Africa especially in Tiaret region of Algeria as folk medicine in the treatment of many illnesses. The leaves and stems of *Globularia eriocephala* are used as decoction in the treatment of hypoglycemia, rheumatic and infectious diseases, its leaves are reported to be used in the treatment of diabetes, in renal and cardiovascular diseases [1,2,3]. Little is now about this specie

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Globularia eriocephala but others species were studied; for example, Çalis et al. [4,5] have isolated the Iridoid glycosides from Globularia trichosantha and G. davisiana, Sugar esters from G. orientalis [6]. Elbetieha et al. [7] showed the phytotoxic potentials of G. arabisca and G. alypum and Es-Safi et al. [8], Saglam et al., [9], Djeridane et al., [10] have determined the antioxidant constituents and the α-tocopherol of G. alypum. In our research group, many compounds with significant pharmacological effects have been isolated from this plant. The aim of this study was to evaluate the antibacterial effect of plant extracts isolated from Globularia eriocephala leaves against Clinical Pathogens Bacteria.

Materials and Methods

Plant material

Fresh Globularia eriocephala was collected during the florescence phase in 2008 from the region of Tiaret (Algeria). Taxonomic identification was performed by Pr. K. Benabdeli (Ecobiological Laboratory, S.N.V, Faculty, Sidi Belabes-Algeria).

Isolation of the Essential Oil (EO):

Oil extracts were obtained from 100 g of fresh leaves after submition for 3 h, to hydrodistillation water-distillation using a Clevenger-type apparatus. The obtained essential oil (EO) was filtered and stored at 4°C until antibacterial tested against pathogens.

Preparation of plant extracts:

Fresh aerial parts (leaves) were washed and air-dried in shade at room temperature. They were then mechanically powdered and sieved. The powdered plant material (50 g) was extracted, for 3 h under occasional shaking with 250 ml of chloroform and The obtained crude preparation was centrifuged at 5000 x g for 30 min. After filtration, the supernatant filtrates were concentrated under reduced pressure at 45°C and the crude extracts were filtered over Whatman no 1 filter paper. The combined extracts were reduced and pooled, the w/w yield in terms of dry starting material was 1.43%. The obtained residue was dried and re-extracted with Ethyl acetate and filtered for a yield of 2.82%. The final residue was dried and re-extracted with methanol-water (1:1, v/v) for 24 h at room temperature in darkness, filtered and concentrated for yielding 19.36%. The extracts were sterilized by membrane filtration using 0.45 μm pore size filters, and were kept at 4°C for further investigation. All the extractions were repeated three times.

2.4. Microbial strains:

Five clinical microbial strains tested were isolated from surgery patients in perioperative period in Mascara Hospital. Four Gram negative-bacteria, Pseudomonas aeruginosa, Klebsiella pneumonia, Proteus vulgaris, Escherichia coli; one Gram-positive bacteria, Staphylococcus aureus. All microorganisms were identified by recommandation of bergy’s manual Methods by culture in specific media followed by Gram coloration and biochemical test using automate microbiological system identification (API system). Inoculum for the assays were prepared by diluting scraped cell mass in 0.85% NaCl solution, adjusted to McFarland scale 0.5 and confirmed by spectrophotometric reading at 580 nm. Cell suspensions were finally diluted to 10⁶ CFU/ml. The microbial isolates were maintained on agar slant at 4 °C in the Laboratory LRSBG (Faculty of S.N.V, University of Mascara) where the antimicrobials tests were performed.

2.4.1. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bacteriocidal Concentration (MBC) by microdilution:

MIC test were carried out according to Ellof [11] using Muller Hilton Broth on a tissue culture test plate (96-well). Briefly, 100 ml of each concentration from the substance (compounds or essential oils) was added in the first well, and serial dilutions were performed (1/2) and at the last 100 ml of cell suspension was added to all wells. Hydro Methanol extract was dissolved in DMSO (2%), it used as negative control. The plates were agitated and incubated at 37°C for 48 h.

The lowest concentration showing no culture was considered as the MIC and its express as (μg/ml, or ml/ml). For The MBC determination, 100 ml of liquid from each well that showed no change culture was plated on GNA and incubated at 37°C for 24 h. The lowest concentration that yielded no growth after this sub-culturing was taken as the MBC.

2.4.1. Sensitivity test: Agar Disc Diffusion Assay:

Antibacterial activity was carried out using a disc-diffusion method. Petri plates were prepared with 20 ml of sterile Mueller Hinton Agar (MHA) (Sigma, France) inoculate by suspension of cell (1ml) adjusted by McFarland 0.5 method (10⁶ CFU /ml). The test cultures were swabbed on the top of the solidified media and allowed to dry for 10min. The tests were conducted at different concentrations of the plant extract in Sterile filter paper discs (6 mm). The loaded discs were placed on the surface of the medium and left for 30 min at room temperature for compound diffusion. The inhibition zones produced
by the plant extract were compared with the inhibition zones produced by commercial standard antibiotics: gentamycin and vancomycin (50mg/disc). DMSO were used as the negative control. The zone of inhibition (mm) was measured from the boundary of the disc after cultivation at 37°C for 48 h.

2.5. Statistical analysis:

Results were expressed as the mean±SD of five replicates. Data were analyzed using one-way analysis of variance (ANOVA), P values <0.05 were considered significant.

Statistical analysis:

Results and Discussion

The results of the experiments assessing the bacteriostatic effects of essential oil and solvent extracts compounds of the plants under study on Gram-negative and Gram-positive bacteria *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Proteus vulgaris*, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus aureus* are presented in Table 1. Effectively The ethyl acetate extract from *Globularia eriocephala* leaves was the best compounds demonstrated antibacterial activity against all clinical pathogens bacteria with lower concentrations, the MIC were including 55 and 75 mg/ml. Hydromethanolic extract showed activities against only *Klebsiella pneumonia*, *Pseudomonas aeroginosa* and *Staphylococcus aureus*, with concentration between 62 and 75 mg/ml respectively. Ethanolic extracts of the same species have been mentioned in the littérature for their antibacterial activity on *S. aureus*, *P. aeruginosa* and *E. coli* [12]. The essential oil compounds showed at all a moderate activity (150-500mg/ml) against these bacteria. The most binteresting result from this study was the combination of Chloroform extract and essential oil wich exerted yielded a synergistic effect for the inhibition against the growth of the Gram-negative and Gram-positive bacteria especially, *S. aureus* (67mg/ml), *P. aeruginosa* (74,2mg/ml) and *K. Pneumonae* (83,6mg/ml) (Table 1). Also, the bacteriocidal activity (MBC) its extent varied, and depending on the extract compounds as shown in Table 1. The literature indicates that the antibacterial activity is due to different chemical agents in the extract, including essential oils, flavonoids and other phenolic compounds or free hydroxyl groups [13].

As follows from the Table 2, ethyl acetate extracts tested by means of agar disc diffusion assay showed activities against all pathogens bacteria with an inhibition zone more than 20mm (Fig. 1). An inhibition zone of 10 or greater was considered as good antimicrobial activity [14]. The hydromethanolic extracts showed an activity more important with an inhibition zone from 25 to 30 mm, against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* respectively (Photo 1). Thus, no growth of *E.coli* and *Proteus vulgaris* tested was observed in the presence of this compound (Table 2). Clinical pathogens strains were less susceptible to Essentials oil with an inhibition zone from 10 to 16 mm (Fig. 1). The chloroform extracts provided a moderate zone of inhibition from 8 to 10 mm diameter on *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* (Table 2). The solvent used as control DMSO, exerted no effect against the bacteria. Thus, both extracts plants by solvents and essential oils exhibit pronounced bacteriostatic effects against Gram-positive and Gram-negative pathogens bacteria. Solvent extracts and essential oils differed qualitatively in the relative content of polar compounds. Ethyl acetate extracts contained compounds with greater polarity than that of their counterparts present in others solvents or aqueous extracts. This may be the likely explanation for significant differences in the bacteriostatic activity between different compounds extracts of *Globularia eriocephala* leaves

The mechanism of bactericidal activity of plant extracts has not been fully understood; it is believed that the active compounds of G. eriocephala leaves exert direct effects against Clinical pathogens Bacteria. Generally, the mechanism is probably due to their ability to complex with extracellular and soluble proteins and then to complex with bacterial cell walls [15]. The availability of efflux pumps inhibitors [17]. Inhibition and interferences with some virulence factors Enzymes, coagulase, Dnase, lipase, thermonuclease [16]. Inhibition of conjugative R___ plasmid transfer in enteric bacteria [17,18]

Conclusion:

The plant extracts from *Globularia eriocephala* leaves may be a promising alternative treatment of localized infections even with severe hospital-acquired strains (Nosocomial Infection). Further investigations are in progress to determine the active constituent(s) for their application in medical research. It would be advantageous to standardize methods of extraction, activity-guided fractionation, and in vitro testing so the search could be more systematic and reproducible, and the interpretation of results would be facilitated. In vitro activity should be to determine their efficacy, stability, and bioavailability and their effects on beneficial normal microbiota.
Table 1: MIC and MBC Results (mg/ml) from *G. eriocephala* extract leaves against different clinical pathogens bacteria

<table>
<thead>
<tr>
<th>Sample</th>
<th>Staphylococcus aureus</th>
<th>Pseudomonas aeruginosa</th>
<th>Klebsiella pneumoniae</th>
<th>E. coli</th>
<th>Proteus vulgaris</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC</td>
<td>MBC</td>
<td>MIC</td>
<td>MBC</td>
<td>MIC</td>
</tr>
<tr>
<td>Hydromethanolic</td>
<td>75</td>
<td>150</td>
<td>63.5</td>
<td>127</td>
<td>62</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>73</td>
<td>146</td>
<td>60</td>
<td>120</td>
<td>55.5</td>
</tr>
<tr>
<td>Chloroform</td>
<td>200</td>
<td>400</td>
<td>250</td>
<td>500</td>
<td>200</td>
</tr>
<tr>
<td>Essential oil</td>
<td>150</td>
<td>300</td>
<td>150</td>
<td>300</td>
<td>200</td>
</tr>
<tr>
<td>Chloroform + Essential oils</td>
<td>67</td>
<td>134</td>
<td>74.2</td>
<td>148.4</td>
<td>83.6</td>
</tr>
<tr>
<td>DMSO –water (1:1)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tbody>
</table>

Table 2: Growth Inhibition zones of clinical pathogens bacteria (mm)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Staphylococcus aureus</th>
<th>Pseudomonas aeruginosa</th>
<th>Klebsiella pneumoniae</th>
<th>E. coli</th>
<th>Proteus vulgaris</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC</td>
<td>MBC</td>
<td>MIC</td>
<td>MBC</td>
<td>MIC</td>
</tr>
<tr>
<td>Hydromethanolic</td>
<td>25 ± 0.2</td>
<td>25±0.36</td>
<td>30±0.20</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>30 ±0.41</td>
<td>30 ±0.21</td>
<td>35 ±0.25</td>
<td>25 ±0.6</td>
<td>30 ±0.15</td>
</tr>
<tr>
<td>Chloroform</td>
<td>08 ±0.02</td>
<td>10 ±0.21</td>
<td>08 ±0.21</td>
<td>10 ±0.60</td>
<td>10 ±0.15</td>
</tr>
<tr>
<td>Essential oils</td>
<td>16 ±0.25</td>
<td>14 ±0.77</td>
<td>12 ±0.22</td>
<td>10 ±0.60</td>
<td>10 ±0.15</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>21 ±0.20</td>
<td>25 ±0.21</td>
<td>20 ±0.21</td>
<td>15 ±0.20</td>
<td>25 ±0.2</td>
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<tr>
<td>Vencemycin</td>
<td>22 ±0.09</td>
<td>18 ±0.60</td>
<td>22 ±0.15</td>
<td>20 ±0.22</td>
<td>15 ±0.6</td>
</tr>
<tr>
<td>DMSO –water (1:1)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</table>

References


