Phytochemical Screening and Antibacterial Activity of Menthe Piperita, Artemisa Compestris L and Pelargonium Adoraitissimum Leaves Extracts from North Libya

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Abstract: Phytochemical screening of the menthe piperita, artemisa compestris L and pelargonium adoraitissimum leaf extracts from north Libya revealed the presence of some bioactive components. Various chemical tests showed that the bioactive compounds are found in methanolic extracts of these plants such as flavonoid, alkaloids, resins, terpineol, volatile oils and tannin groups. Saponin is present in Pelargonium adoraitissimum while it is absent in Menthe piperit and Artemisa compestris. The effects of methanolic extracts on some pathogenic: two strains of gram negative bacteria [Escherichia coli and Klebsiella pneumonia] and two strains of gram positive bacteria [Staphylococcus aureus and streptococcus spp.] indicated that the menthe piperita has greater inhibition zone against two tested Gram negative bacteria and strains of gram positive bacteria.

Keywords: Phytochemical Screening, Antibacterial Activity, Medicinal Plants

1. Introduction

Most plants are important as a "food source". For example, proteins and fats have high nutritional value [1] because they contain energy sources. Of carbohydrates they also have medical and therapeutic benefits as they are used in treating many diseases[2, 3], including asthma, worms, and also in urinary tract infections, Bronchial infections, aldecy cough, and skin allergies [4, 5]. There are many medicinal plants such as Lawsonia inermis, Cuminum cyminum, Coriandrum, Zingiber officinale, Castor oil, and Black Cumin. These plants contain chemical compounds which have a clear biological effect against various bacteria and fungi [3].

The menthe piperita L. is one of the most important medicinal and aromatic plants. It has many immense therapeutic uses and has been used in medicine since thousands of years. For example, the babylonians used it in treating indigestion and the Chinese used it treating colic whereas the Romans used it in treating detoxification and poisoning [6, 7]. It is used as a disinfectant and in mouth washes, gums and toothpastes. As it relaxes the muscles, it may also help in relieving painful cramps, in treating colds and flu [8]. Some experiments have shown that menthe has the ability to treat asthma, inflammation of the sinuses and to reduce abdominal pain, and colic [9].

Artemisia campestris L. is an Asteraceae species and a perennial herb, commonly known as a field worm wood. It is widespread in Asia, North America, and Europe and predominates the arid regions of North Africa. Artemisia campestris has many medicinal actions, including: antivenom [10, 11], anthelmintic [12], antimicrobial, antifungal, anticancer [13], antidiabetic [14, 15, 16], antihypertensive. It has been utilized in treating many conditions, including: digestive, respiratory, cutaneous and genital diseases [17-22]. Some scientific studies on Artemisia campestris L showed the presence of constituents which belong mainly to the groups of essential oils, fatty acids flavonoids, coumarins, isocoumarins and phenolic acids.

The Pelargonium adoraitissimum belongs to Geraniaceae
family and represents important sources of food, medicines and cosmetics and of distilled volatile oils [23-27]. These genera have been found to possess significant pharmacological and biological activities, including antioxidant, anti-neuroinflammatory, antitumor, anticancer, antimicrobial and antifungal activity [23, 24, 27-30]. It is used in folk medicine in Libya and in the world. as a food and tea, additive drinks and in relieving some gastrointestinal, topical, dental and cardiovascular disorders [24, 25, 27, 31]. The phytochemical profile of Pelargonium has shown abundance of essential oils, phenolics and flavonoid [24, 27-29, 32-35]. The interest in secreted materials of menthe piperita, Artemisa compestris L and Pelargonium adoraitissimum for pharmaceutical products and spices led the researchers to study them. This study therefore aims to carry out a preliminary screening for the important phytochemical bioactive compounds and to investigate in vitro the antibacterial activity of methanolic leave extracts.

2. Materials and Methods

2.1. Collection of Plant Materials

Fresh leaves of menthe piperita, artemisa compestris L and pelargonium adoraitissimum were collected locally from Zawia, Libya and were identified by the Department of Botany, University of Zawia. The menthe piperita, artemisa compestris L and pelargonium adoraitissimum leaves were exposed to shed drying and grind by an electric grinder to turn it into a fine powder. Then, the powder was saved in a sterilized and tightly packed glass container which was stored in a cool place not exposed to light until use.

2.2. Preparation of Extracts

5 grams of each selected dried plant powder were weighed and added to a 50 ml of methanol in a conical flask of a 200 ml capacity. The flask was covered and left aside for 24 hours. The plant mixture was mixed by a magnetic mixer and was left to be filtered. Then, the plant extract was kept in the refrigerator until use.

2.3. Preliminary Phytochemical Screening

Screening of the above three selected medicinal plants for various phytochemical constituents were carried out through using standard methods [36-38]. Qualitative phytochemical screening of plant extracts were carried out using the following methods to test only for the presence of secondary metabolites.

Test for Tannins: 5 ml of distilled water was added to 1 ml of the extract in a test tube. 1 ml of 0.02 M Ferric chloride containing 0.1N hydrochloric acid was also added. A blue-black coloration was observed.

Test for Saponins: 5 ml of distilled water was added to crude extract. The mixture was shaken in vigorously with some drops of olive oil in a test tube. Stable foam was observed which was considered as an indication of the presence of saponins.

Test for Flavonoids: Crude extract was added to 5 ml of diluted ammonia solution and concentrated H2SO4. Yellow coloration which disappeared on standing indicates the presence of flavonoids.

Test for Terpenoids (Salkowski test): 2 ml of chloroform and 3 ml of concentrated H2SO4 was added to 5 ml of extract to form a layer of reddish brown colouration.

Test for resins: The plant extract was boiled and added to distilled water in case turbidity in solution was visible; it is considered an indication of the existence of resins.

Test for volatile oils: During the vaporization of plant extract by a vaporizer, the emergence of an oily layer that was separated from the alcohol layer directly after several minutes of the vaporization process represents an indication of volatile oils content.

Test for Alkaloids: Curde extract was dissolved with 2ml of 1% HCl and heated gently. Mayers reagents were added to the mixture. Turbidity of the resulting precipitate was taken as a confirmation for the presence of alkaloids.

2.4. Antibacterial Assay

The antibacterial activity of menthe piperita, artemisa compestris L and pelargonium adoraitissimum leaves methanolic extract was evaluated using the agar diffusion technique [39]. The tested organisms were Gram-positive bacteria [Staphylococcus aureus and Streptococcus spp] and Gram-negative bacteria [Escherichia coli and Klebsiella pneumonia]. The bacteria were maintained in nutrient agar media, respectively. After 24 h incubation at 37 °C, the diameter (mm) of the inhibition zones was measured.

3. Result & Discussion

3.1. Phytochemical Screening

The extract of the leaves of the tested plants (menthe piperita, artemisa compestris L and pelargonium adoraitissimum) showed a positive result for the presence of medicinally active constituents. The Preliminary phytochemical screening of the tested plants is summarized in Table 1. In the methanolic extract; tannins, resins, flavonoids, alkaloids, terpenoids, alkaloids and volatile oils were most commonly found in the tested plants. Saponin is present in pelargonium adoraitissimum while it is absent in menthe piperit and artemisa compestris. These finding correlated well with several earlier publications [40]. Plants were rich in a wide variety of secondary metabolites such as terpenoids, alkaloids, tannins, flavonoids appears biological and pharmacological activities. These may have potential to be used as chemotherapeutic agents or serve as starting material in the developing of new antibiotics [41].
important role in our health such as flavonoids, essential oils, polyphenol and Terpen 
[43]. Widely of pathogens and resistant's bacteria were urgent needed of new antimicrobial agents, natural products which have inhibited of many organism causing harm disease of human [44].

Our results are in agreement with the results of Modupe [45], which found that, the antibacterial activity is attributed to high concentration of carvon; which is essential oil. the lipophilic nature of essential oils enables the oil to cross the cell membrane and reach the cytoplasm to play an active biological role [46].

4. Conclusion

Phytochemical analysis and an antimicrobial study of medicinal plants is a very significant way to establish that the selected plant species may be used as potent drugs. The high cost and side effects of synthesized drugs are forcing the scientists to research alternative sources for the treatment of diseases. Saponins, terpenes, glycosides, Essential oils and polyphenols are a very promising drug discovery.

In this study, it is found that the presence of tanins, resins, flavonoids alkaloids, terpenoids, alkaloids and volatile oils in the tested plants can be used as an important source of phytochemical and antimicrobial activity. On the basis of this antibacterial study, it is clear that the selected plant shows a significant antibacterial activity against selected bacteria strains. Therefore, this study indicates that these plants may be used as potent antibacterial drugs of natural origin. A further work may emphasize the isolation and characterization of effective compounds.

Table 1. Preliminary phytochemical screening of menthe piperita, artemisa compestris L and pelargonium adoraitissimum leaves extract.

<table>
<thead>
<tr>
<th>Plant extracts</th>
<th>phytochemical compounds</th>
<th>Tannins</th>
<th>Flavonoids</th>
<th>Saponins</th>
<th>Alkaloids</th>
<th>Resins</th>
<th>Terpenoids</th>
<th>Volatile oils</th>
</tr>
</thead>
<tbody>
<tr>
<td>Menthe piperita</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Artemisa compestris</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Pelargonium adoraitissimum</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

(+++) high (+++) medium (+) poor (-) no found

3.2. Antibacterial Activity

Methanolic extracts of tested plants show a varying degree for antibacterial activity (Table 2). The leaf menthe extract, showed a maximum zone of inhibition (35mm) against Staphylococcus aureus which is a gram-positive bacteria. This kind of bacteria can cause various skin and soft tissue infections [42] particularly when skin or mucosal barriers have been breached. On the other hand, it shows a moderate activity against Escherichia coli by menthe extract. The methanolic extracts of artemisia campesints and pelargonium adoraitissimum showed an inhibition zone from 10-30mm against organism tested. The bioactive compounds plays an

Table 2. Inhibition zone (mean diameter of inhibition in mm), of menthe piperita, artemisa compestris L and pelargonium adoraitissimum leaves extract.

<table>
<thead>
<tr>
<th>Plant extracts</th>
<th>Gram-positive bacteria</th>
<th>Streptococcus spp</th>
<th>Staphylococcus aureus</th>
<th>Escherichia coli</th>
<th>Klebsiella pneumonia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inhibition zone (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pelargonium adoraitissimum</td>
<td>10</td>
<td>14</td>
<td>12</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Menthe piperita</td>
<td>25</td>
<td>35</td>
<td>14</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Artemisa compestris</td>
<td>20</td>
<td>30</td>
<td>20</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

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