Investigation of chemical components and antibacterial activity of yarrow (Achillea wilhelmsii) extract

Sara Naebi 1, Masoomeh Shirzad 2,*

1Department of Microbiology, Ahar Branch, Islamic Azad University, Ahar, Iran
2Drug Design and Bioinformatics Unit, Medical Biotechnology Department, Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran

*Corresponding author: Masoomeh Shirzad, Drug Design and Bioinformatics Unit, Medical Biotechnology Department, Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran. Email: masishirzad@hotmail.com

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ABSTRACT

Herbal medicine is one of the oldest human approaches to treating diseases and yarrow (Achillea wilhelmsii) one of the most famous medicinal plants. Yarrow essential oil has anti-inflammatory, anti-spasm, anti-scalp, hair growth stimulation and topical skin healing due to chamazulene, cineole and borneol. Staphylococcus aureus (S.aureus) and Escherichia coli (E.coli) are the most important bacteria causing nosocomial infections with multiple antibiotic resistances. Also, S.aureus strain is more sensitive than E.coli to extract of yarrow. Alpha-pinene, phenolic compounds and flavonoids are major constituents of yarrow. This review shows how methanol extract of yarrow prevented the growth of Gram-positive bacteria such as S.aureus and E.coli. So these extracts can be substituted for chemical drugs.

Keywords: Antimicrobial effects; extract; Staphylococcus aureus; Escherichia coli

INTRODUCTION

The use of herbs for the prevention and treatment of diseases has long attracted the attention of medical professionals [1-2]. As such, plants can be considered as a source of potentially useful chemicals that are only partially exploited. Nowadays, compounds identified in plants are used as new drugs could be used as a key for identifying low-
cost therapies and with fewer side effects in the treatment of many diseases [3-4]. The overuse of synthetic antimicrobial drugs has led to increase drug resistance against different antibiotics in most bacteria [5-6]. This has been one of the reasons for growing use of herbs as low-risk, affordable, and inexpensive natural ingredients in the treatment of bacterial infections compared to synthetic antibiotics. Also, these herbal remedies are more widely used in people [7-12]. Among the many varieties used in traditional medicine, yarrow (Achillea wilhelmsii) is the most prominent because of its variety in use and dates back to 3000 BC. So far, 85 species of this plant have been identified, 7 species of which are found exclusively in Iran. Indigenous species of yarrow plants grow in lowland, alongside roads and mountainous areas [13,14]. In a 2014 study, Sales found that ethanolic extract of yarrow had the greatest effect on S. aurous [15]. In a 2003 study by Sokmen et al. The antimicrobial properties of essential oils and aqueous and methanol extracts of yarrow on 12 bacterial and two yeast strains were compared [16]. This review was performed to evaluate the effect of methanol extract of yarrow on S.aureus and E.coli.

**Prepare yarrow extract**

The aerial parts were collected from natural areas. The specimens were cleaned and collected after being transported and dried in a large and suitable environment in sunny conditions. After the specimens were completely dried and the shoots were removed from the roots, they were prepared for grinding. After grinding, the essential oils and extracts were prepared using clevenger and soxhlet extractor apparatus. The essential oil sample was injected into the gas chromatography–mass (GC/MS) after obtaining a thermal planning system. Then identification of compounds using the device database and comparing their mass spectrometry was carried out. Concentrated extracts of 5 % dimethyl sulfoxide with 20, 30, 50 and 400 mg/ml were prepared for use in MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) assay. The microorganisms studied in this study were: S.aureus ATCC 25923 and E.coli ATCC 25922, which were cultured separately on Muller Hinton agar medium to allow for the detection of colonies. Solution was prepared using 0.5 McFarland turbidity (1.5*10^6 cfu/ml). Then, pits of diameter 6 mm and 2.5 cm apart were created at the agar surface. Subsequently, 100 μl of concentrations of 20, 30, 50 and 400 mg/ml were injected from methanolic extract into each well. Negative control was obtained by using a solution of extracts (5 % DMSO). Chloramphenicol was used as a positive
control. Then the plates were incubated at 37 °C for 24 h. Microbial cultures were measured in millimeters for the presence or absence of growth zone. To determine the MIC and MBC of methanol extract was performed by dilution method with dilutions of 6.25, 12.5, 25, 50, 100 and 200 mg/ml.

Yarrow (Achillea wilhelmsii) essential oil and extract was extracted by clevenger and soxhlet extractor distillation and different concentrations of the extract were prepared. Then MIC and MBC extracts of S.aureus and E.coli isolated from patients were determined using the broth dilution and agar dilution methods. Then, MIC and MBC of extracts on S.aureus and E.coli were determined using agar well diffusion and dilution test. The results revealed that, the methanolic extract of yarrow inhibits the growth of S.aureus and E.coli. Alpha-pinene is a major constituent of yarrow. Due to the remarkable antibacterial effect of methanol extract of yarrow on pathogenic bacteria involved in developing various infectious and nosocomial infections, these extracts could be substituted for chemical drugs.

The effective constituents of yarrow were identified and their percentages in essential oils shown in Tables 1. About 23 Compounds had been identified in the essential oils of yarrow, which make up 94 % of all essential oils. The major constituents of the essential oils were α-pinene (17.66 %), piperitenone (11.84 %), caryophyllene oxide (9.47 %) and β-pinenetrans (8.62 %). Comparison between concentrations of 20, 30, 50 and 400 mg/ml of methanolic extract of yarrow by well diffusion method against E. coli and S. aureus showed that the extract of yarrow had antibacterial activity against the studied bacteria; S. aureus was the most sensitive to methanol extract of yarrow. However, E. coli was less sensitive to the extract of mothers. Also, with increasing the extract concentration, diameter of bacterial growth zone was significantly increased (P <0.005), indicating that the antibacterial effect of the extracts was dependent on concentration (Table 2). Results of MIC and MBC experiments such as well diffusion method, yarrow extract on S.aureus showed higher bactericidal activity than E.coli (Table 3).
Table 1. Chemical compounds identified from yarrow essential oil by GC/MS

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Iranian yarrow, Ahar (%)</th>
<th>India [50]</th>
<th>Brazil [46]</th>
<th>Iranian yarrow, Tabriz [47]</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Pinene</td>
<td>17.66</td>
<td>0.8–11.7 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caryophyllene oxide</td>
<td>9.47</td>
<td></td>
<td>10.27 %</td>
<td></td>
</tr>
<tr>
<td>Camphene</td>
<td>3.22</td>
<td>0.2–5.7 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Terpineol</td>
<td>4.21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Pinene</td>
<td>8.62</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sabinene</td>
<td>2.89</td>
<td>8.77 %</td>
<td>8.2 %</td>
<td></td>
</tr>
<tr>
<td>Valencene</td>
<td>6.81</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-Cymene</td>
<td>4.41</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Borneol</td>
<td>5.08</td>
<td>4.7–24.9 %</td>
<td>7.5 %</td>
<td></td>
</tr>
<tr>
<td>1,8-Cineole</td>
<td>1.34</td>
<td></td>
<td>16.8 %</td>
<td></td>
</tr>
<tr>
<td>Camphor</td>
<td>4.29</td>
<td>0.6–17.6 %</td>
<td>5.8 %</td>
<td></td>
</tr>
<tr>
<td>Carveol&lt;trans-&gt;</td>
<td>0.17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carveol&lt;cis-&gt;</td>
<td>0.19</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Piperitenone</td>
<td>11.84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>trans-Piperitol</td>
<td>5.54</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Eudesmol</td>
<td>1.41</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isopiperitenone</td>
<td>1.87</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spathulenol</td>
<td>0.47</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n-alkane</td>
<td>1.21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thymol</td>
<td>1.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Germaacrene D</td>
<td>0.26</td>
<td>1.1–46.6 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mintfuranone</td>
<td>1.97</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Cadinol</td>
<td>0.52</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Farnesene</td>
<td></td>
<td></td>
<td>31.66 %</td>
<td></td>
</tr>
<tr>
<td>Chamazulene</td>
<td>0.8–9.6 %</td>
<td></td>
<td>17.17 %</td>
<td></td>
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</tbody>
</table>
In recent years there has been a great deal of research into the antimicrobial effects of various herbs, which have shown that some herbs have similar or far greater effects than chemical drugs [17]. In general, plant products cause cytoplasmic granulation, cytoplasmic membrane disruption, inactivation or inhibition of intracellular and extracellular enzymes, and cell wall disintegration [18-20]. The yarrow was reported to be rich in flavonoid and sesquiterpene lactones [21]. It is also the best solvent for the extraction of methanolic flavonoids [22-23]. On the other hand, methanol extract in addition to flavonoids, alkaloids, saponins, tannins, anthraquinones and terpenes [24-25]. It seems that the antimicrobial properties of methanolic extracts could be generally attributed primarily to flavonoids, secondarily to terpenes, and to thirdly saponins [26]. Yaghoubi and his colleagues in 2007 also confirmed the antibacterial effects of phenolic compounds and flavonoids [27]. Stojanovic and his colleagues reported the antimicrobial effect of methanol extract of yarrow due to the compounds of sesquiterpene and flavonoids [25].

The major chemical components identified in this study were alpha α-pinene (17.66 %), piperitenone (11.84 %), caryophyllene oxide (9.47 %) and β-pinenetrans (8.62 %). Qaderi et al. showed that 19 compounds isolated from yarrow essential oil, alpha-pinene accounted for 19.76 % of all compounds [28]. Lori-Gooini et al., reported the major constituents of yarrow essential oil, respectively, chrysanthene (14.31 %), camphor (13.95 %), 1-8 cineole (10.01 %), and trans-pinocarveol (9.50 %) [29].

In this review, it was found that methanolic extract of this plant at concentrations of about 30 mg/ml prevented the growth of Gram-positive bacteria tested. However, higher concentrations are needed for the effect on Gram-negative bacteria. Among the medicinal plants of yarrow, due to its wide range of therapeutic properties, it was considered. It had been used for thousands of years in the treatment of various diseases, especially infectious diseases [30]. In 2003, chalabian et al. determined that the essential oil of yarrow had an antibacterial effect on S.aureus [31]. Mohammadi-Sichani et al. in 2011 showed that methanol extract of yarrow flowers inhibited the growth of S.aureus,
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*B. cereus* and *E. coli*. The growth inhibitory concentration of these extracts varied from 6.25 to 25 mg/ml [32]. In a 2012 study, Ghaderi and *et al.* found that yarrow had inhibitory effects on *E. coli, B. cereus, B. licheniformis*, and *P. aeruginosa* [33]. In the Aljancic *et al.* studied that yarrow in vitro inhibited the growth of *B. subtilis, C. albicans* and *A. niger*. They identified the flavonoids isolated from the extract as antimicrobial effects [34]. Kermanshah *et al.* investigated the antibacterial effect of hydroalcoholic extract of *Salvia officinalis* and yarrow on microorganisms causing tooth decay, and found that both extracts inhibit the growth of *S. mutans, L. rhamnosus* and *A. viscosus* [35]. Ahmadi and his colleagues in 2011 showed that camphor was the most important composition of yarrow essential oil. Also, *S. aureus* strain was more sensitive to *E. coli* than extract of yarrow [36]. Amjad and his colleagues showed that methanol extracts of yarrow flowers and leaves had the most effects on *B. cereus* and *S. aureus*, which increased their antibacterial activity as well as their effects on *E. coli* [37]. The different effect of the yarrow extract on the growth of gram-positive and gram-negative bacteria may be due to the structural differences between the walls of these two groups of bacteria [38].

Antibacterial effects of yarrow extract

The content of flavonoids, camphor, comarins, proazulene, tannins and polyphenol are important in extract quality [41,44,47,50]. Yarrow in the markets have more content of fat and saturated fatty acids, proteins, ash, energy value, sugars and flavonoids, while the real sample contained more levels of carbohydrates, organic acids, unsaturated fatty acids, tocopherols and phenolic acids [43,53]. Chamazulene (15.84%) was the main chemical component found in the yarrow essential oil which has inhibitory effect against *Staphylococcus* spp [46,47]. The minimum inhibitory concentration (MIC) of ethanolic extract was in ranges 6.5 and 13 mg/ml [39,49]. The extracts were examined for antibacterial effects. Growth of the highly resistant bacterium was inhibited by mullein extract and red clover extract [40]. The phenolic content and antibacterial activity of ethanolic extract of yarrow was evaluated and in yarrow *m*-hydroxybenzoic acid was the most abundant (12.1 mg/L), while from the group of cinnamic acids, the main compound was *o*-coumaric acid [42]. The complexity of the important compounds of yarrow species explains their activity. Based on the researches, the pharmacological effects are related to the essential oil, proazulenes, dicafeoylquinic acids and flavonoids [45].
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Yarrow essential oil prevented the initial cell binding of the *Listeria* cells on polystyrene, stainless steel and polyethylene surfaces until 6 h preformed biofilms formed on polystyrene surface. Metabolic activity of the biofilms reduced mainly after incubation with the oil. Yarrow essential oil, or its constituents, may be useful additives for the development of new disinfectant and sanitizer formulations for application in the food industry [51].

Antibacterial effect of yarrow essential oil was improved by four to eight times over the free yarrow oil after encapsulation of yarrow essential oil in hydroxypropyl-ß-cyclodextrin (HPβCD) through freezedrying technique [48].

Endophytic bacteria were isolated from yarrow and tragacanth. Their antifungal and antibacterial activities against *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATTC 35218), These isolates showed inhibitory effects against the tested fungi [52].

Table 2. Antibacterial effect of *Achillea wilhelmsii* on *S.aureus* and *E.coli*(Mean ± SD)

<table>
<thead>
<tr>
<th>Concentration of methanol extract (mg/ml)</th>
<th>20</th>
<th>30</th>
<th>50</th>
<th>400</th>
<th>Negative control</th>
<th>Positive control</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>0.71 ±13.26</td>
<td>0.94 ±18.33</td>
<td>0.81 ±24</td>
<td>0.74 ±24</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>0</td>
<td>0</td>
<td>0.83 ±13.8</td>
<td>1.09 ±17.8</td>
<td>0</td>
<td>27</td>
</tr>
</tbody>
</table>

Table 3. Determination of MIC of *Achillea wilhelmsii* extract on *S.aureus* and *E.coli*

<table>
<thead>
<tr>
<th>Concentrations of methanol extract mg/ml</th>
<th>Ahar</th>
<th>Tabriz [39]</th>
<th>Brazil [46]</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>6.25</td>
<td>6.5</td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>12.5</td>
<td></td>
<td>150</td>
</tr>
</tbody>
</table>

*HBB. 4(3): 6-18*
CONCLUSION

*S. aureus* is more sensitive to the plant than other Gram-positive bacteria is not clear. On the other hand, the subspecies of yarrow are different due to the differences in their active constituents in terms of growth inhibition on bacteria. In addition to the intrinsic properties of the biological activity of the compounds, the methods of extraction and preparation, the geographical area and the type of climate can also have different results. Due to the remarkable antibacterial effect of methanol extract of yarrow on pathogenic bacteria that are involved in the development of various infectious and nosocomial infections, they can be considered as a natural herbal supplement.

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