Antibacterial Activity of Extracts of Ajuga Iva, and Teucrium Polium

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**ABSTRACT**

Antibiotics provide the main basis for the therapy of microbial (bacterial and fungal) infections. Since the discovery of these antibiotics and their use as chemotherapeutic agents there was a belief in the medical fraternity that this would lead to the eventual eradication of infectious diseases. However, overuse of antibiotics has become the major factor in the emergence and dissemination of multi-drug resistant strains of several groups of microorganisms. Many plants which are used in traditional medicine contain antimicrobial compounds. In this study, the antibacterial activity of methanolic extracts of *Teucrium polium* and *Ajuga iva* were tested against five bacteria, *E. coli*, *Bacillus subtilis*, *Pseudomonas diminutus*, *Paracoccus paratrophus* and *Micrococcus luteus*. *T. polium* and *A. iva* plants were collected and allowed to dry in the dark at room temperature. Dried plant material (100g) was added to 1 L of methanol and incubated at room temperature for three days. The crude solution was filtered through muslin cloth, and the filtrate evaporated to dryness. The dried material was dissolved in 2 ml of methanol. Bacterial suspensions (100 µl) were spread on tryptone soya agar (TSA) medium. Plant extracts (10 µl) were applied to discs of filter paper and placed on agar plates containing the microorganisms. The plates were incubated at 37°C for 48h. After incubation the zones of inhibition around the discs was measured. Extracts of *T. polium* gave zones of inhibition against *Bacillus subtilis*, *Pseudomonas diminutus*, *Paracoccus paratrophus* and *Micrococcus luteus* of 3.7, 2.0 and 2.0 mm, respectively. *A. iva* extract only inhibited the growth of *Paracoccus paratrophus*, giving a zone of inhibition of 3.0 mm. The present results showed that extracts of *T. polium* inhibited the growth of three bacterial species. Extracts of *A. iva*, on the other hand, inhibited only one bacterium.

**Key word:** Ajuga iva, antibacterial activity, plant extracts, Teucrium polium.

**Introduction**

Antibiotics provide the main basis for the therapy of microbial (bacterial and fungal) infections. Since the discovery of these antibiotics and their use as chemotherapeutic agents there was a belief in the medical fraternity that this would lead to the eventual eradication of infectious diseases. However, overuse of antibiotics has become the major factor in the emergence and dissemination of multi-drug resistant strains of several groups of microorganisms [10,13]. Numerous studies have shown that aromatic and medicinal plants are sources of diverse nutrient

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and non nutrient molecules, many of which display antioxidant and antimicrobial properties which can protect the human body against both cellular oxidation reactions and pathogens. Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids and flavonoids, which have been found in vitro to have antimicrobial properties [2,26,21]. Compounds that inhibit pathogens but have little toxicity to host cells are candidates for developing new antimicrobial drugs [21]. There are several reports on the antimicrobial activity of different herbal extracts [2]. A number of phytotherapy manuals have mentioned various medicinal plants for treating infectious diseases owing to their availability, few side effects and low toxicity [15].

Thus, it is important to characterize different types of medicinal plants for their antioxidant and antimicrobial potential [2,25,21]. In the light of evidence for the rapid global spread of isolates of pathogenic microorganisms with antibiotic resistance, the need to find new antimicrobial agents is of paramount importance. However, the past record of rapid, widespread emergence of resistance to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectancy [6]. For this reason, researchers are increasingly turning their attention to herbal products, looking for new leads to develop better drugs against strains of microorganisms with multi drug resistance [4]. According to the world health organization (WHO), medicinal plants would be the best source for obtaining a variety of drugs [19]. Therefore, in this report information on the antimicrobial activity of methanolic extracts of two plants is given.

Materials and methods

Plant Material:

_Teucrium polium_ and _Ajuga iva_ plants were collected and allowed to dry in the dark at room temperature. Dried plant material (100g) was added to 1 litre of methanol and incubated at room temperature for three days. The crude solution was filtered through muslin cloth, and the filtrate evaporated to dryness.

Bacteria Strains:

Five bacterial strains were used in this study, _E. coli_ MC 4100, _Bacillus subtilis_, _Pseudomonas diminutus_, _Paracoccus paratrophus_ and _Micrococcus luteus_ obtained from the laboratory of Dr. Jane Nicklin, School of Biological and Chemical Sciences, Birkbeck College University of London, UK

Antibacterial Assay:

The dried material was dissolved in 2 ml of methanol and was tested against the five mentioned bacteria. Bacterial suspensions (100 µl) were spread on Tryptone Soya Agar (TSA) medium. Plant extracts (10 µl) were applied to discs of filter paper (Whatmann AA, 6 mm diam.) and placed on agar plates containing the microorganisms. The plates were incubated at 37°C for 48h.

Results and discussion

After incubation the zones of inhibition around the discs was measured. Extracts of _T. polium_ gave zones of inhibition against _Bacillus subtilis_, _Micrococcus luteus_ and _Paracoccus paratrophus_ of 3.7, 2.0 and 2.0 mm, respectively. _A. iva_ only inhibited the growth of _Paracoccus paratrophus_, giving a zone of inhibition of 3.0 mm (Figure 1, Table 1).

The antimicrobial compounds from plants may inhibit bacterial growth by different mechanisms than those presently used. Antimicrobials therefore, may have a significant clinical value in treatment of resistant microbial strains. In particular, the antimicrobial activity of plant oils and extracts have formed the basis of many applications including raw and processed food preservation, pharmaceuticals, alternative medicine, and natural therapies [20].

_T. polium_ L. (Lamiaceae) has numerous pharmacological properties. These include calcium antagonistic, anorexic, intestinal motility and hypertension, anti-ulcer, anti-inflammatory, antipyretic and antibacterial actions, glycemic and hypolipidemic effects; treat liver disease, and diabetes. The extract is also used as an antiemic, an antisapmodic, an anti-inflammatory, an antipyretic, an analgesic, and an anti-carminative [17].

The antibacterial activity of _T. polium_ extracts can be attributed to its contents in flavonoids. In general the methanol extraction of _T. polium_ plant material yielded to flavonoids [22]. The obtained results were similar to the results in other studies, the methanolic extract of _T. polium_ inhibited the growth of several bacteria with different minimal inhibitory concentration (MIC) This extract inhibited the growth of _Staphylococcus aureus_, _Salmonella typhi_ with a MIC of 40 mg/mL, this concentration was 10 mg/mL _Bordetella bronchiseptica_ and _Bacillus anthracis_. This concentration of 10 mg/mL
represents also the minimal bactericidal concentration (MBC) against *Bacillus anthracis*. The plant extract was also active against *Bordetella bronchiseptica*. The hydroalcoholic extract of *T. polium* had a relatively satisfactory effect on *Salmonella typhi*. All flavonoids isolated from leaves of *Psidium guajava* inhibited with bacteriostatic mode of action all of the fish pathogens used in study, *Aeromonas hydrophila; Aeromonas salmonicida* subsp. *salmonicida* ATCC 14174; *Flavobacterium columnare* ATCC 23463; *Lactococcus garvieae* ATCC 49156; *Streptococcus agalactiae; Vibrio salmonicida* ATCC 43839 [18].

Maximum activity of flavonoid fraction of callus tissue from *Gossypium* species was observed of several tested bacteria, *Bacillus cereus* (NCIM 2156), *Staphylococcus aureus* (NCIM 2654) *Staphylococcus epidermidis* (NCIM 2493), *Mycobacterium smegmatis* (NCIM 5138), *Pseudomonas aeruginosa* (NCIM 5032), *Proteus vulgaris* (NCIM 2027), *Salmonella typhimurium* (NCIM 2501), *Escherichia coli* (NCIM 2027) [5]. In the same way the propolis products which contained flavonoids, expressed bactericidal activity against *B. subtilis* and *S. aureus* with a zone of inhibition of 19 mm and 17 mm respectively [14]. In other study six flavonoids (7'-dimethoxyflavone-4'-O-_-D-glucopyranoside, 5,7- dimethoxyflavanone-4'-O-[2''-O-(5'''-O-trans-cinnamoyl)-_-D-apiofuranosyl]-_-D-glucopyranoside, 5,7,3'-trihydroxyflavanone-4'-O-_-D-glucopyranoside, naringenin-7-O-_-D-glucopyranoside and nicotiflorin) was tested against several strains of *K. pneumoniae*, all these flavonoids, showed *in vitro* antimicrobial activity similar to that produced by the control antibacterial (ofloxacin) at the concentration of 32-64 µg/ml [16]. Similarly, the aqueous extract of *T. polium* inhibited the growth of *Saccharomyces cerevisiae* (Ki = 29 µg/l) and *Yarrowia lipolytica* (Ki = 61 µg/l) [10], in contrast the Ethanolic extract of *T. polium* had no effect on *Micrococcus luteus* NRRL B- 4375 [1].

Ethnopharmacological surveys have revealed that some 20 species of *Ajuga* plants are used in traditional medicine mostly in Africa, Asia and China). In North Africa, *Ajuga* plants are used to treat diabetes and hypertension [9,24]. Other reported activities of *Ajuga* plants include antibacterial, antifungal, anti-inflammatory, antimalarial/antiplasmodial, antimycobacterial, antioxidant, antipyretic, larvae and insect antifeedant and insect growth inhibitor activity [11]. This activities are related to there contents in active compounds, they contains a wide range of compound such as ajugapyrin A, bracteonin A and lupulin C and irodoids which had a wide range of biological and pharmacological activity. The plant contains also, a class of secondary metabolites which are produced by plants primarily as a defence against herbivores or against infection by microorganisms [23,11].

Fig. 1: Antibacterial effect of *Teucrium polium* (8) and *Ajuga iva* (8) against *Bacillus subtilis* (2), *Micrococcus luteus* (5) and *Paracoccus paratrophus* (6).
Table 1: Antibacterial effect of *T. polium* and *A. iva* extracts

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th><em>Teucrium polium</em> extracts</th>
<th><em>Ajuga iva</em> extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus subtilis</td>
<td>3.7</td>
<td>0.0</td>
</tr>
<tr>
<td>E. coli MC 4100</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Micrococcus luteus</td>
<td>2.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Paracoccus paratrophus</td>
<td>2.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Pseudomonas diminutus</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

While the *A. iva* plants contain all these active compounds, in our study the extracts of *A. iva*, on the other hand, inhibited only one bacterium. This result can be explained that the methanolic extraction do not allow the extraction of more antibacterial active compound such as Iridoids, for which the most efficient isolation techniques are, Pressurized hot water extraction and hot water extraction which were the [23]. In contrast other workers have demonstrated that aqueous extracts of *A. iva* reduced the formation of calcium oxalate crystals in artificial urine, suggesting that preparations from this plant might be helpful in decreasing the incidence of kidney stones [3] and caused mortality of larvae and adults of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) [12].

References


