

Research Letter

Antimicrobial activity of phenolic extract from *Teucrium polium geyrii* (Lamiaceae) plant

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ABSTRACT: This work presents a contribution to the detection of biological activity of some secondary metabolites of *Teucrium polium geyrii*, (Lamiaceae) from Tamanrasset (southern Algeria). The plant was subjected to two types of phenolic compounds extraction; maceration and Soxhlet apparatus. The concentration of total phenols and flavonoids, was determined by colorimetry. This showed that the ethyl acetate extract obtained by maceration contains the largest amount of total phenols, 89.05 ± 0.50 mg/g equivalent of gallic acid, as well as flavonoids with 0.45 ± 0.001 mg/g equivalent of vitexin. The antibacterial activity was measured on bacterial strains (pathogenic and alterations) *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus cremoris*, *Clostridium perfringens*, *Klebsilla pneumoniae*, *Escherichia coli* and *Proteus mirabilis*, by the Aromatogramme method. These activity determinations showed that the extracts tested are endowed with interesting antibacterial activities, with inhibition zones reaching as much as 3.4 ± 0.3 cm in diameter on *Proteus mirabilis*.

KEYWORDS: *Teucrium polium geyrii*, Lamiaceae, medicinal plant, polyphenols, flavonoids, antibacterial activity

INTRODUCTION

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects.^[1,2,3] Thus, study of plant species that traditionally have been used as pain killers may be seen as a logical research strategy, in search for new drugs with therapeutic effects.^[3] *Teucrium polium* is a wild-growing flowering plant belonging to the family Lamiaceae and it is found abundantly in south western Asia, Europe and North Africa. The sub species *Teucrium polium geyrii*, Takmazzut appointed by the Touareg, is common in the Saharan Atlas. It grows mainly in rocky beds at altitude between 1200 and 2600 m.^[4–7] *Teucrium polium* is a medicinal plant used in folk medicine for various purposes including anti-inflammatory, anti-nociceptive, anti-bacterial, anti-hypertensive and anti-hyperlipedemic. In African traditional medicine, this species is used in times of stress. It also has a beneficial effect on digestion. Its anti-stress

activity and anti-oxidants help to fight against aging of the skin.^[8] It is used to flavor tea. This plant has also been described as the aspirin of the Touareg. In traditional medicine, it has an important place because of its therapeutic indications.^[4] However, it seems to have been insufficiently studied chemically, unlike other sub species of the genus. This work presents a contribution to the detection of biological activity of some secondary metabolites of *Teucrium polium geyrii*, (Lamiaceae) from Algeria.

MATERIALS AND METHODS

Plant material

The plant material consists of the aerial part of the plant *Teucrium polium geyrii*, harvested in Tamanrasset (south Algeria) in November 2007. The samples were dried protected from light and moisture, at room temperature and stored carefully in a dry place for their analysis. The plant was identified by Benhouhou S., botanist at the National Agronomic Institute in El-Harrach, (Algeria).

Extraction of phenolic compounds

Extraction by maceration

A sample of 5 g of plant powder was subjected to extraction by soaking in a water-alcohol mixture (methanol/water 80/20: v/v) for 36 h at room temperature in

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darkness, with renewal of the solvent at 24 h. The hydro-alcoholic extracts are evaporated under vacuum at a temperature of 40 °C. After treatment with petroleum ether to remove pigments,^[9] the aqueous phase was extracted with ethyl acetate. The organic phase obtained was subsequently dried over anhydrous sodium sulfate (Na₂SO₄), followed by evaporation under vacuum at 40 °C.^[10,11]

Soxhlet extraction

A 50 g sample of the aerial part of *Teucrium polium geyrii* was subjected to extraction with methanol in a Soxhlet apparatus for 7 h. After complete evaporation of the solvent, 100 ml of hot water are added to the dry residue and stored overnight. A liquid-liquid extraction step was then carried out with a solvent system of increasing polarity: petroleum ether, hexane, chloroform, ethyl acetate and finally n-butanol.^[12]

Quantification of phenolic compounds

The determination of total phenols was carried out by a method adapted from Singleton *et al.*, using Folin-Ciocalteu reagent.^[13] Flavonoids were quantified by direct determination by aluminum trichloride (AlCl₃).^[13,14]

Antibacterial activity of extracts

The aromatochromogrammes method was used to search antibacterial activity in the bacteriology laboratory of the Hospital BOUDIAF and laboratory quality control of Ouargla (Algeria). The culture medium used was Mueller-Hinton agar. The bacterial strains chosen were: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus cremoris*, *Clostridium perfringens*, *Klebsilla pneumoniae*, *Escherichia coli* and *Proteus mirabilis*. The agar plates were inoculated with of 1 ml of inoculums on the surface. Disks for aromatochromogrammes (0.5 cm diameter) were deposited onto the surface of the plate. Each

disc is impregnated with varying amounts (between 1 and 10 µl) of the extract dissolved in DMSO. The plates were placed in an oven at 37 °C for 12 to 18 hours. Following incubation, the absence of microbial growth resulting in a halo around the disk was defined as antibacterial activity. To quantify the antibacterial activity, the diameter of inhibition was measured and expressed in centimeters. The results were compared with antibiotic standards. Each tests was repeated three times.

RESULTS AND DISCUSSION

Extraction of phenolic compounds

Table 1 shows the appearance, color and performance of each phenolic extract obtained by the two methods outlined in materials and methods section.

The results show higher returns in the case of extraction conducted by Soxhlet hexane (7.50% of mass of original material extracted). The lowest extraction yield was seen for chloroform (2.66% of mass of original material extracted). This difference may be attributed to the presence of lipophilic compounds (fatty acids, carotenoids, chlorophylls) of high molecular weight which are more soluble in hexane. However, these extractions can be considered complementary to the extent that natural products have quite different polarities.

Quantitative analysis of polyphenols

The phenolic content of each plant extract is expressed in milligrams equivalents gallic acid per gram of dry plant material. The quantification of flavonoids is expressed in milligrams equivalents Vitexin per gram of dry material plant. The results are summarized in Table 2.

Table 1: Characteristics of phenolic extracts obtained by maceration and Soxhlet extraction from *Teucrium polium geyrii* plant

Extracts	Color	Aspects	Yield (%)
Hexane (Soxhlet).	Dark green	Viscous	7.50
Chloroform (Soxhlet)	Green yellow	Viscous	2.66
Ethyl acetate (Soxhlet)	Yellow	Powder	5.31
n-butanol (Soxhlet)	Yellow brown	Powder	5.58
Ethyl acetate (maceration)	Yellow green	Powder	3.70

Table 2: Total phenols and flavonoids quantifications from *Teucrium polium geyrii* plant

Extracts	Total Phenols extract (mg/g) equivalent Gallic acid	Flavonoids (mg/g) equivalent Vitexin
Ethyl acetate (maceration)	89.05 ± 0.50	0.45 ± 0.001
Ethyl acetate (Soxhlet)	1.81 ± 0.05	0.06 ± 0.097
n-butanol (Soxhlet)	49.87 ± 0.004	0.16 ± 0.001
Chloroform (Soxhlet)	8.48 ± 0.07	0.16 ± 0.035
Hexane (Soxhlet)	7.52 ± 0.02	0.12 ± 0.008

The polyphenol content in the plant *Teucrium polium geyrii* studied varies between extracts. The ethyl acetate extract obtained by maceration contains the largest amount of total phenols (89.05 ± 0.50 mg/g) as well as the highest level of flavonoids (0.45 ± 0.001 mg/g). The lowest content of total phenols with (1.81 ± 0.05 mg/g) was observed for the ethyl acetate Soxhlet extract. A comparison between the results of the present study with the results of previous studies^[11,15] shows that *Teucrium polium geyrii* plant from south Algeria is rich in phenolic compounds.

Antibacterial activity of extracts

Figure 1 shows the results of antimicrobial activity of extracts from *Teucrium polium geyrii* plant against the following bacterial strains: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus cremoris*, *Clostridium perfringens*, *Klebsilla pneumoniae*, *Escherichia coli* and *Proteus mirabilis*.

Figure 1 illustrates an antibacterial effect of *Teucrium polium geyrii* plant against different bacterial strains (both Gram-positive and Gram-negative). This efficiency may be due to the presence of flavonoids, which are secondary metabolites known for their antibacterial effects.^[16,17] Also, the significant effect of the n-butanol extract of *Teucrium polium* against all the strains studied is noteworthy. The effect of this extract is notable compared to other extracts tested, with maximum inhibition of (3.4 ± 0.3) cm in diameter on *Proteus mirabilis* (Figure 2). As *Proteus mirabilis* has been linked with inflammatory conditions such as gout, it would therefore also be of interest to examine the potential anti-inflammatory effect of this extract. The extract with ethyl acetate has a large

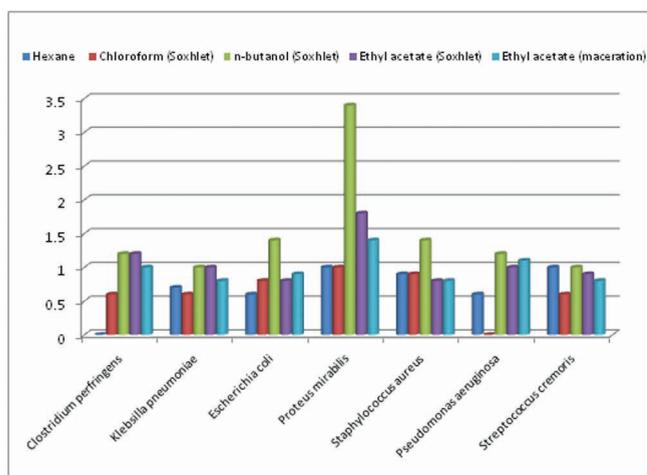


Figure 1. The Effect of extracts of *Teucrium polium geyrii* on different bacterial strains. All results represent the mean of triplicate determinations.



Figure 2. Antibacterial effect of *Teucrium polium geyrii* extracts on *Proteus mirabilis*.



Figure 3. Antibacterial effect of *T. polium* extracts on *Klebsilla pneumoniae*.

spectrum of actions covering Gram-positive and Gram-negative, in the present study. The chloroform and hexane extracts have similar effects, but with different zones of inhibition (Figure 2 and Figure 3).

A study of Oganesyanyan *et al.* (1992)^[18] noted the antimicrobial effect of the methanol extract of *Teucrium polium* on *Klebsilla pneumoniae* and *Escherichia coli*.

CONCLUSIONS

This work represents a contribution to the study of biological activities of some secondary metabolites of *Teucrium polium geyrii* plant from the region of Tamanrasset (Algeria). The plant is subjected to two types of extraction,

maceration and Soxhlet. Quantitative analysis by spectrophotometry revealed considerable levels of polyphenols with appreciable amounts of flavonoids. The antibacterial activity tests of different extracts showed that all microbial strains tested are inhibited by the extracts. The n-butanol extract is the most important effect of all extracts tested, with maximum inhibition zone of 3.4 ± 0.3 cm in diameter against *Proteus mirabilis*.

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