Preliminarly Study of Composition and Antimicrobial Activity of Essential Oil of *Glechoma sardoa* Bég.

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Abstract

***Glechoma sardoa* Bég. (Labiatae), is an endemic plant growing wild only in Sardinia and Corsica: it is a perennial, green-grey, hairy herb, with a prostrate, creeping stem, radicating at the base, 1-3 dm long. The essential oil of *G. sardoa* shows an interesting and particular chemical composition and microbiological tests show that the essential oil has a fair activity against Gram + bacteria as *Staphylococcus aureus* (MIC 0.5 mg/mL) and *Staphylococcus epidermidis* (MIC 1mg/mL) while no activity has been observed (MIC 2mg/mL) against *Candida albicans* and the other Gram – bacteria (*Escherichia coli* and *Pseudomonas aeruginosus*) tested.**

INTRODUCTION

Glechoma sardoa Bég. (Moris, 1859; Pignatti, 1982), Labiatae family, is an endemic plant growing wild in Sardinia and Corsica**:** it is perennial, green-grey, hairy herb, with a prostrate, creeping stem, radicating at the base, 1-3 dm long. The leaves are petiolate, opposite, roundish, cordate-reniform, crenate, hairy, and glaucous on both sides, though often purplish beneath. The floral leaves are of the same form. The flowers are bluish-purple, about 3 together in axillary whorls. The corolla is about 3 times as long as the calyx, with a variegated throat. The calyx is long, curved, villous, the limb oblique, the teeth triangulate and with the base large (1.8-2 mm.) Morphologically it is very similar to the well known *G. hederacea*, but for this species no study has been performed on the chemical composition and the activity of the essential oil and very few on the botanical characteristics. The current research presents a preliminary study about the essential oil composition and antimicrobial activity of *Glechoma sardoa* Bég. extracts.

MATERIAL AND METHODS

Plant Material

Plants of *Glechoma sardoa* Berg. were collected during their flowering period (July) in a station of North Sardinia (Badde Salighes) and were identified by Prof. A. Atzei. Voucher specimens have been deposited at the Herbarium S.A.SS.A of the Dipartimento di Scienze del Farmaco, Università di Sassari.

Oil Distillation and Yield and Aqueous Extract

The plant material was submitted for 4 hours to hydro distillation using a Clevenger-type apparatus (F.U.I., 1991); the yield obtained was 0.05% (w/w). The oil was dried over anhydrous sodium sulphate and stored at –20°C until it was analysed.

After hydro distillation of the essential oil, the aqueous residue was taken to dryness and stored at –20°C until it was used for microbiological assays.

Oil Analyses

1. GC- Four replicates of each sample were analysed using a Hewlett-Packard Model 5890A GC equipped with a flame ionisation detector and fitted with a 60 m x 0.25 mm,

thickness 0.25 μm AT-5 fused silica capillary column (Alltech). Injection port and detector temperature were 280°C. The column temperature was programmed from 50°C to 135°C at 5°C/min (1 min), 5°C/min up 225°C (5 min), 5°C/min up 260°C and held for 10 min. The samples (0.2 μL each), generally analysed without dilution (using 2,6-dimethylphenol as internal standard) were injected using a split/splitless automatic injector HP 7673 and using helium as the carrier gas. Measurements of peak areas were performed with a HP workstation; the threshold was set at 0, peak width at 0.02. The data reported in Table I are the average of four GC injections. The quantitation of each compound was expressed as an absolute weight percentage using internal standard and response factors. The detector response factors (RFs) were determined for key components relative to 2,6-dimethylphenol and assigned to other components on the basis of functional group and/or structural similarity, since oxygenated compounds have a lower detectability by F.I.D. than hydrocarbons (Dugo G. et al., 1983). The standards were > 95% pure, and actual purity was checked by GC. Several response factor solutions were prepared that consisted of only four or five components, plus 2,6-dimethylphenol, to prevent interference from trace impurities.

2. GC/ms- GC/ms analyses were carried out with a Hewlett Packard G1800B-GCD System using the same conditions and column described above. The column was connected with the ion source of the mass spectrometer. Mass units were monitored from 10 to 450 at 70 eV. The identification of compounds was based on comparison of their retention times with those of authentic samples and/or by comparison of their mass spectra with those of published data and HP Libraries **(**Guenter, 1952; NIST98; Adams, 1995) or on the interpretation of the EI-fragmentation of the molecules.

Antibacterial Assays

The oil of *G. sardoa* was dissolved at 10% w/vol in PEG 200 (Sigma). Preliminary tests with PEG 200 were performed to assure that no microorganism inhibition occurred at the concentrations used

Aqueous extract was dissolved at 10% w/vol in sterile distilled water.

The antibacterial activity of the oil was evaluated as the minimum inhibitory concentration (M.I.C.), in comparison with chlorhexidine gluconate, by using an agar dilution technique (Barry, 1986). Microorganisms included both Gram + (*Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* of clinical isolation) and Gram – strains (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853).

Two-fold serial dilutions of the solutions (2 mg/mL to 0.0039 mg/mL) were prepared in triplicate in molten (45°C) Mueller Hinton Agar (MHA; Oxoid) and poured into 50 mm Petri plates. Each plate was inoculated with about $1x10^4$ bacteria, applied as a spot of about 5 mm in diameter. M.I.C.s were recorded after 18-24 hours of incubation at 35°C as the lowest concentration which completely inhibits bacterial growth.

RESULTS

In this preliminary study we report for the first time the chemical composition of the oils obtained from *Glechoma sardoa* Berg. collected in a station in North Sardinia and its antibacterial activity, as well as antibacterial activity of the aqueous extract.

A chromatographic profile of the essential oil is reported in Figure 1.

GC and GC/ms analyses of the oil led to the identification of the main components with their percentages. The constituents identified and quantified from the oil of *G. sardoa* represent 89.7 % of the total oil. In Table 1 are reported the major chemical groups and their percentages.

The oil composition was characterised by a high elemene content: β-elemene (25.36%), δ-elemene and γ-elemene (5.14% and 4.03% respectively) and isogermafurene reached 7.69%. The essential oil of *G. sardoa* is characterised by the presence, in high content, of germacrene D (9.55%) and β-elemol (3.29%) that are not common essential oil constituents.

The antibacterial activity of the oil was evaluated as the minimum inhibitory

concentration (M.I.C.), in comparison with that of chlorhexedine gluconate, by using an agar dilution technique.

Microbiological tests using essential oil and aqueous extract showed a fair activity (Table 2) against Gram + bacteria such as *Staphylococcus aureus* (MIC 0.5 mg/mL) and *Staphylococcus epidermidis*, (MIC 1 mg/mL) while no activity has been observed (MIC> 2mg/mL) against Gram – bacteria (*Escherichia coli* and *Pseudomonas aeruginosus*) tested.

DISCUSSION

As we have stated previously, this is a preliminarily work on an endemic plant growing wild in Sardinia (Italy). There has not been previous chemical work on this plant and little on *G. hederacea*, which is not indigenous to Sardinia.

From the first analyses of the essential oil it was possible to see that the main constituents were δ, γ, and in particularly β-elemene (25.3%); this constituent is not usually among the major constituents of an essential oil except in only a few plants, such as in *Protium heptaphyllum* (Aubl.) March. (Bursaceae) (Senatore, 2000), where it reachs a high concentration, with a percentage very close to that found in *Glechoma sardoa*.

Also germacrene D (9.55%) is not usually found in essential oils, especially when the GC separation is made with an apolar column because it cyclizes to γ-elemene. This compound is considered a precursor of sesquiterpene hydrocarbons.

These compounds form the main chemical group in this essential oil, but it also contains 24.5% of alcohols. There are very few aldehydes, that are normally flavour compounds, and esters reached only 1.08% of the total. This work will be continued with a more accurate separation of single constituents and definitive assignments will be made using NMR techniques.

The antimicrobial analyses show an interesting selective action of this essential oil in particularly against *Staphylococcus aureus* (MIC 0.5 mg/mL). Moreover, the aqueous extract obtained from the water used for essential oil hydrodistillation also showed this activity, and for this reason, future work will try to identify the constituents that give this activity. The investigation will also be extended to the chemical constituents of the whole plant.

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Tables

Table 1. Main chemical groups (%) identified in *Glechoma sardoa* Berg. essential oil

Chemical groups	**%**
Aldehydes	0.07
Ketones	0.57
Esters	1.08
Alcohols	24.50
Monoterpene hydrocarbons	1.98
Sesquiterpene hydrocarbons	60.27
Acids	1.18

Table 2. M.I.C. (mg/mL) of essential oil of *Glechoma sardoa* Berg. (**Untested)

PI	***Glechoma sardoa*** **essential oil (2000)**	***Glechoma sardoa*** **essential oil (2001)**	***Glechoma sardoa*** **aqueous extract (2001)**	**Chlorhexidine gluconate**
St. aureus ATCC 25923	0.5	1.5	4	0.0016
St. epidermidis (clin. isol. cute)	1	1	1	0.001
St. epidermidis (clin. isol. urine)	1	1	1	0.001
E. coli ATCC 25922	>2	>2	>2	0.001
Ps. aeruginosa ATCC 27853	>2	>2	**	0.016

Figures

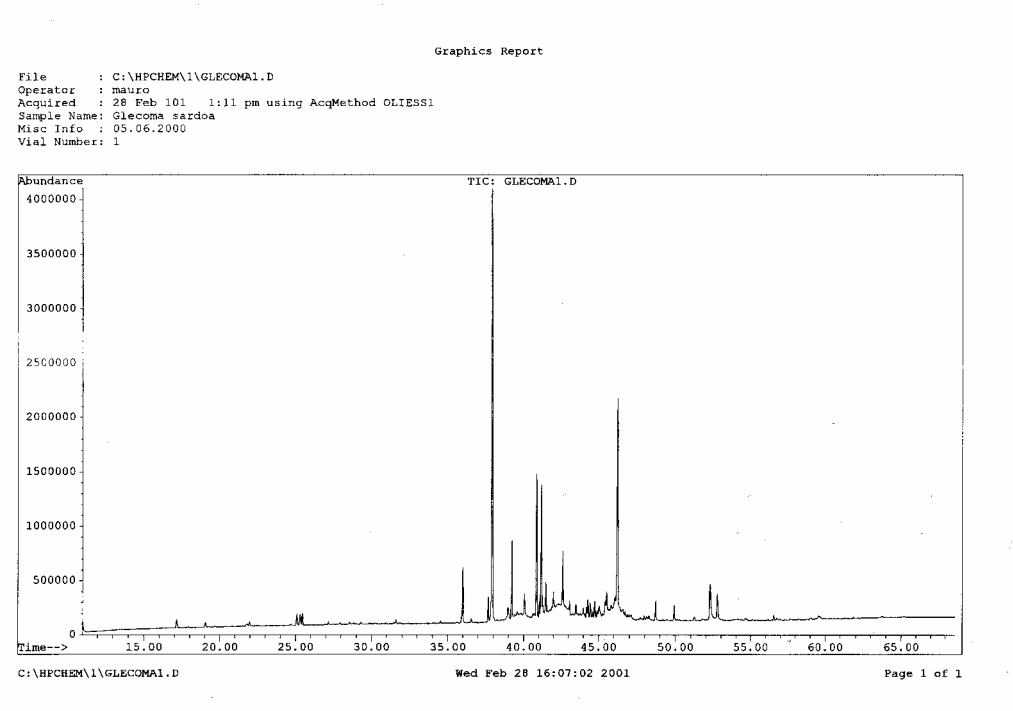

Fig.1. *Glechoma sardoa* Berg. essential oil chromatogram