ORIGINAL ARTICLE

ANTIBACTERIAL AND CYTOTOXIC ACTIVITY OF THE ACETONE EXTRACT OF THE FLOWERS OF *SALVIA SCLAREA* AND SOME NATURAL PRODUCTS

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ABSTRACT

The aim of this study was to investigate the antibacterial and the cytotoxic activity of the acetone extract of the flowers of *Salvia sclarea* and of some natural products (sclareol, sclareolide and ambrox). The antibacterial and the cytotoxic activity were determined by the dilution method. Sclareolide, ambrox and sclareol demonstrated a good antibacterial activity against *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27950, *Escherichia coli ATCC* 25922 and *Enterococcus faecalis* ATCC 29212. The acetonic extract of the flowers of *Salvia sclarea* has a significant cytotoxic activity against Hep-2 cells.

Keywords: *Salvia sclarea*, flowers, antibacterial activity, cytotoxic activity, natural products, hep-2 cells

INTRODUCTION

Salvia sclarea L. is a biennial or perennial medicinal and aromatic plant belonging to the Lamiaceae family. This shrub, 60 to 100 cm high, is known by the common name '*Oculus christis*' (Moretti *et al*., 1997). It is a plant native to the southern Europe and one of the most important aromatic plants cultivated worldwide as a source of the essential oils and many other compounds derived from different parts of the plant. The flowers have a very strong aromatic scent and essential oil characterized by a fresh floral and herbaceous odour also has an economic value for flavour and fragrance industries (Carrube *et al*., 2002). *Salvia sclarea* has shown diverse biological activities. It has been reported for its anti-inflammatory (Moretti *et al*., 1997), antitumoral (Ulubelen *et al*., 1992; Topcu *et al*., 1997), antituberculosis (Ulubelen *et al*., 1997)**,** genotoxic (Zani *et al*., 2000). Various diterpenoides have been isolated from *Salvia scalrea* (Ulubelen, 1994). Our aim is to investigate the antibacterial activity of the acetonic extract of the flowers of *Salvia sclarea* and of some natural products; sclareolide, sclareol and ambrox.

MATERIALS AND METHODS

Plant material was collected in 2002 from Monastir (Tunisia), the plant was identified by Pr. Mohamed Chaieb botanist in the University of Science (Sfax,Tunisia)

Extract preparation

Dried flowers of the plant (10 g) were macerated at 80°C with a mixture of (acetone/water) for 5 hours, and then the extract was filtered using Whatmann filter paper no.1 after cooling. The acetonic extract was evaporated to dryness under reduced pressure and then assayed for their antibacterial and cytotoxic activities immediately or after storage in the deep freezer at -20°C. Sclareolide, ambrox and sclareol were natural products isolated from a previous work (Fekih, 2001) in the Laboratory of Organic Chemistry, University of Pharmacy, Monastir (Tunisia) and were used for the biological assay.

Microorganisms

The strains of microorganisms employed were *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27950, *Enterococcus faecalis* ATCC 29212 and *Klebsiella pneumoniae* (clinical isolate).

Antibacterial activity assays

Minimal inhibitory concentration (MIC) for extracts against bacterial strains was determined based on a micro-well dilution method (Swanson *et al*., 1992), with two fold serial dilutions of plant extracts from 10- 0.01mg/ml. MIC values were taken as the lowest concentration of the extract that completely inhibited bacterial growth after 24 hours of incubation at 37°C.

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Cytotoxic activity

Cytotoxic or antitumoral activity was tested against Hep-2. This cancer cell was cultured in Dulbecco's Modified Egale's Medium supplemented with 10% foetal cell serum, 2 % L-glutamine and 5 mg/ml each of gentamycin, penicillin and streptomycin. The culture was maintained in a 5% CO_2 incubation at 37°C. It was grown to confluence for 2-3 days and adjusted to 10^5 cells/ml with fresh medium. Initial 10 ml culture was prepared in 25 cm^2 flasks before application of treatments (Davis JM, 1994).

The extract and the compounds were solubilised in DMSO and applied to the cell culture at final concentrations of 20, 40, 60, 80 and 100µg/ml. DMSO final concentration was adjusted to 0.2% in all treatment including the control. A normal control substituting DMSO with an equivalent volume of culture medium was also included. The culture was maintained in a 5% CO_2 incubation at 37°C for different periods. After incubations, the hep 2 cells viability was estimated by the measure of the optical density (OD) at 620 nm using methylene blue. The percentage of viability of hep-2 cells is calculated as (A-B) / A) x100. Where A and B are the OD_{620} of untreated and treated cells, respectively. The 50% inhibitory concentration for hep2-cells (IC50) was determined graphically.

RESULTS AND DISCUSSION

The results of the antibacterial activity are shown in table 2 the chemical composition of the acetonic extract of *S. sclarea* flowers are summarized in table 1, which shows that the acetonic extract containe diterpenoids (chromatogram 1) and the main constituent of this extract is sclareol; an alcohol diterpene. The results of the cytotoxic activity are shown in table 3. DMSO (0.2%) did not seem to have any noticeable effect on the cellular growth as compared to the normal control. *E. coli* and *S. aureus* which causes frequent urinary tract infection are the common organisms used by others studies for bacteriostatic activity (Roia and Smith, 1977) but *K. pneumoniae* was used as test organism because of its high resistance to antibacterial agent. In the present study, the acetonic extract of the flowers of *Salvia sclarea* inhibited the growth of *E.coli*, *P.aeurogenosa* and *S. aureus* with MIC of 630 to 2500 µg/ml while *K. pneumoniae* and *E. faecalis* showed resistance to the acetonic extract. Sclareolide is a natural product synthesised from the sclareol (Fekih, 1996), which inhibit the growth of *E. coli*, *S. aureus, E. faecalis* and *P. aeurogenosa* with a MIC of 20 µg/ml. Ambrox is a natural product with Ambra odorant used in perfumery (Ohloff, 1982) inhibited the growth of *E. coli,, E. faecalis* and *S. aureus* with the same MIC of sclareolide but it was inactive against *K. pneumoniae*. The acetonic extract of the flowers of *Salvia sclarea* showed the greatest cytotoxic or antitumoral activity against Hep-2 cells, with IC50 of 4 µg/ml. Considering their high cytotoxicity and moderate antimicrobial activity, it couldn't be applied to treat microbial infections. In contrast sclareol, sclareolide and ambrox which showed no cytotoxicity on Hep-2 cells line but good antibacterial activity may be applied to treat microbial infections. In a previous study (Alessandra *et*

Table 1: Chemical composition of the acetone extract of the flowers of *Salvia sclarea* by GC/MS

Compounds	Retention time (min)	Area (%)
Epi-oxide of manoyle	20,52	2.14
manoyle oxide	20,78	1.33
Manool	21,31	1.05
α-gurjunène	21,70	1,45
Aromadendrène	21,94	1,69
Caryophillène oxide	22,71	0,59
Sclareol	23,28	87,79
Farnesol	28,60	0,1
Carotene	28,87	0,17
Tétracosane	29,42	0,29

Table 2*:* Antibacterial activity of the acetone extract of the flowers of *Salvia sclarea* and of natural products

Strains	MIC (µg/ml)			
	Acetonic extract[a]	Sclareol	Sclareolide	Ambrox
Escherichia coli ATCC 25922	630	10	20	20
Enterococcus faecalis ATCC 29212	-	10	20	20
Pseudomonas aeruginosa ATCC 27950	1250	10	20	630
Staphylococcus aureus ATCC 25923	2500	10	20	20
Klebsiella pneumoniae (clinical isolate)	-	-	-	-

- : inactive; a) IC50: is the concentration of the 50% cytotoxic effect.

al., 1999) *S. sclarea* oil showed significant bacteriostatic action against *S. aureus*, *E. coli*, *S. epidermidis* and *C. albicans*.

Table 3: Cytotoxic activity of the extract and the compounds against Hep 2 cells

Compounds	IC50 (µg/:ml)a
Acetonic extract of the flowers	4
Ambrox	>100
Sclareolide	>100
Sclareol	53

ACKNOWLEDGEMENT

We are grateful to Pr BABA Hamouda and Khedher Mohamed for their help. We also thank Dr. Hammami Mohamed and Mr. Chrif Imed for the mass spectra.

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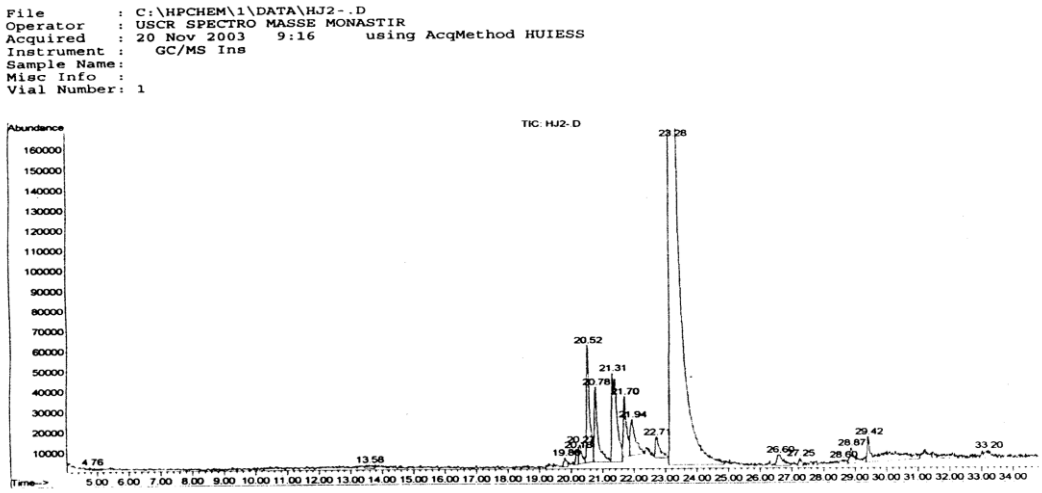
Received: 28-10-2006 – Accepted: 24-03-2006



Chromatogram 1 of the acetone extract of the flowers of *Salvia sclarea* by GC/MS.