INTERNATİONAL JOURNAL OF AGRİCULTURE & BİOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
10–360/ZIP/2011/13–1–134–136
http://www.fspublishers.org

Short Communication

Antimicrobial Activity of *Salvia trichoclada* in Southern Turkey

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ABSTRACT

Antimicrobial activity of chloroform and ethanol extract of *Salvia trichoclada* L. was investigated by disk diffusion method. Active components extracted showed an average antimicrobial activity against microorganisms mainly bacteria. The results showed that the gram negative bacteria were more sensitive than gram positive bacteria. Ethanol extract was more effective than the chloroform extract. Soxhlete extraction is more suitable than soaking for higher inhibition on test microorganisms. *S. trichoclada* contains antibacterial components against various microorganism, which could be important in various pharmaceutical preparations. © 2011 Friends Science Publishers

Key Words: *Salvia trichoclada*; Antimicrobial; Ethanolic extract

INTRODUCTION

Salvia trichoclada is a member of family Lamiaceae, which includes so many species used as herbs, spices, folk medicines and a source of fragrance (Vural & Adıgüzel, 1996). In folk medicine, *Salvia* species are used due to their antibacterial, antioxidant, antidiabetic and antitumor properties (Ulubelen, 2003). Therefore, many researchers have focused on biological properties of *Salvia* species and their components (Murakami *et al*., 1990; Tada *et al*., 1994; Sivropoulou *et al*., 1997; Velickovic *et al*., 2002; Gulcin *et al*., 2004; Dulger & Hacioglu, 2008).

This paper describes antimicrobial activity of *S. trichoclada* to rationalize its use in folk medicine for bacterial diseases.

MATERIALS AND METHODS

Plant samples: *S. trichoclada* was collected from Kahramanmaraş, Turkey in May 2009. The specimens were identified using Flora of Turkey (Davis, 1982) at University of Sutcu Imam.

Preparation of extracts: Aerial parts of the dried *Salvia* samples (40 g for each solvent) were ground in an omnimixer and extracted for 24 h in a Soxhlet extractor with 200 mL of chloroform and ethanol (Dulger *et al*., 1997). Another set of plant sample was also prepared for soaking (Senhaji *et al.*, 2005) at room temperature for two days to determine molecular aberration of active materials to be extracted. Finally, the samples were concentrated in a vacuumed rotary evaporator at 50°C after filtering with Whatman paper no. 1. After 3 mL of chloroform or ethanol addition, the extract was stored at +4°C until preparation of the discs (Tanis *et al.*, 2009). The preparation of the discs were accomplished by loading 25 and 50 µL of the sample to the sterile discs (whatman no 1; 6 mm in diameter). Chloroform and ethanol loaded discs were also used as control.

Microorganisms: *Enterococcus faecalis* ATCC 29212, *Micrococcus luteus* NRLL B-4375, *M. luteus* ATCC 9341, *Enterobacter aerogenes* ATCC 13048, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 39628, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 6897, *Listeria monocytogenes* ATCC 7644, *Pseudomonas* sp. (Clinic isolate), *Enterobacter cloacae* ATCC 13047D, *S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228, *Salmonella typhimurium* CCM 5445, *K. pneumonia* (clinical isolate), *Candida albicans* (clinic isolate), *S. cerevisia* and *Aspergillus flavus* were obtained from Celal Bayar University, Gazi University Biology departments and Sutcuimam University Medical Faculty Microbiology laboratory.

Antimicrobial assays: Antimicrobial activities of the *S. trichoclada* extracts were performed by using disc diffusion method. Mueller Hinton and Sabouraud dextrose Agar cultures of test microorganism were prepared with a standardized inoculum giving 1 x 10^8 bacteria and 1 x 10^6 yeast per mL (Collins *et al*., 1989).

To cite this paper: Karcıoglu, L., H. Tanıs, N. Comlekcıoglu, E. Dıraz, E. Kırecci and A. Aygan, 2011. Antimicrobial activity of *Salvia trichoclada* in southern Turkey. *Int. J. Agric. Biol*., 13: 134–136

Table I: Antimicrobial activity of ethanol and chloroform extract of Southern Turkey *Salvia trichoclada*

Microorganisms	Extraction with Soxhlete						Extraction with Soaking				Control		
	Ethanol		Chloroform		Chloroform(a)		Ethanol		Chloroform				
	25µL	50µL	25µL	50µL	25µL	50µL	25µL	50µL	25µL	50µL	A/S	Cep	Nys
Enterococcus faecalis ATCC 29212	-	-	-	-	-	-	-	-	-	-	-	-	NT
Micrococcus luteus NRLL B-4375	-	-	-	-	-	-	-	-	-	-	30	28	NT
Micrococcus luteus ATCC 9341	2	2	-	-	-	-	2	2	-	-	16	40	NT
Enterobacter aerogenes ATCC 13048	22	27	-	-	-	-	12	15	-	-	-	-	NT
Bacillus subtilis ATCC 6633	10	16	-	-	-	-	8	14	-	-	18	20	NT
Escherichia coli ATCC 39628	8	8	8	8	-	-	8	8	-	8	18	27	NT
Pseudomonas aeruginosa ATCC 27853	10	12	-	8	-	-	8	10	-	4	-	9	NT
Proteus vulgaris ATCC 6897	8	8	8	8	-	-	8	10	8	8	14	12	NT
Listeria monocytogenes ATCC 7644	10	12	8	8	-	-	8	10	8	8	6	10	NT
P. aeruginosa (Clinic isolate)	8	14	8	10	8	8	-	-	14	20	12	16	NT
Enterobacter cloacae ATCC 13047D	-	-	-	-	-	-	8	8	-	-	-	20	NT
S.aureus ATCC 25923	-	8	-	-	-	-	-	8	-	-	18	20	NT
S. epidermidis ATCC 12228	8	8	-	-	-	-	8	8	-	-	20	20	NT
K. pneumonia (Clinic isolate)	8	10	-	-	-	-	-	8	-	-	-	19	NT
Salmonella typhimurium CCM 5445	10	10	-	-	-	-	-	-	-	-	-	21	NT
Candida albicans (clinic isolate)	-	-	-	-	-	-	-	-	-	-	NT	NT	18
S.cerevisia	-	-	-	-	-	-	-	-	-	-	NT	NT	24
Aspergillus flavus	-	-	-	-	-	-	-	-	-	-	NT	NT	14

A/S: Ampicillin/sulbactam (20 µg); Cep: Cephazolin (30 µg); Nys: Nystatine (100U)
Control (Ethanol, Chloroform): No Inhibition Zone, NT: Not Tested
(a): The plant samples were subjected to chloroform extraction after ethanol extraction

The inoculated plates with bacterial strains were incubated overnight at 36°C and the fungal plates incubated for two days at 30°C. After incubation period, the diameters of the inhibition zones were measured and evaluated.

RESULTS AND DISCUSSION

The antimicrobial activity of ethanol and chloroform extracts of *S. trichoclada* were examined against 15 bacterial and 3 fungal strains. *S. trichoclada* had no effect on fungal strains and two of the bacterial strains as reported earlier by Gulcin *et al*. (2004) result for *S. sclarea.* However, Dulger and Hacioglu (2008) reported a strong antifungal effect of extract from *S. tigrina.* The tested ethanolic extracts showed relatively an average level of antimicrobial activity against the tested microorganisms. Among the bacteria tested, *E. aerogenes* (ATCC 13048) was the most sensitive to ethanolic extracts. *L. monocytogenes* ATCC 7644 and *P. aeruginosa* showed an average sensitivity. The results showed that the gram negative bacteria were more sensitive than gram positive bacteria. Ethanol extract was more effective than the chloroform extract. If the extraction process type considered, soxhlete extraction was more suitable than soaking for higher inhibition. However, soaking in chloroform at room temperature produced higher inhibition on *P. aeruginosa* (clinic isolate), which also developed larger inhibition than the reference antibiotic. Our results also showed that the active components were completely extracted with ethanol, therefore there was no remaining antimicrobic components to be extracted with chloroform (Table I). According to Sivropoulou *et al.* (1997) reports antibacterial activity of the component from *S. fructicosa* was mainly due to α- and *β*-thujone and 1,8-cineole. Another major component, camphor, had no antibacterial effect. Mayekiso *et al*. (2008) has also identified the main components of *S. repens* such as camphor *para*–cymene, sabinene, *1*– *β* –pinene , myrcene, terpinene, *trans*- *β* – Ocimene, terpinene-4-ol, nopol, α-terpinolene, *β*-caryophllene with some inhibitory effect on some gram positive and negative bacteria. Some of these constituents varying in quantity and composition were also reported from *Salvia* species by other researchers (Croteau *et al*., 1981; Velickovic *et al*., 2002; Lima *et al*., 2004). The differences in composition is probably due to climate, soil composition, altitude and age as well as species (Bakkali *et al*., 2008).

CONCLUSION

Results of the study support existence of the antimicrobial components and traditional use of *S. trichoclada* in diseases caused by the susceptible microorganisms.

Acknowledgement: The authors thank to Mr. Orhan Sari for some technical support and Dr. Ahmet ILCIM for collection and scientific identification of plant samples.

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(Received 08 July 2010; Accepted 13 September 2010)