African Journal of Biotechnology Vol. 5 (19), pp. 1795-1798, 2 October, 2006
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2005 Academic Journals

Full Lenght Research Paper

Antibacterial activities of *Allium vineale*, *Chaerophyllum macropodum* and *Prangos ferulacea*

Hisamettin Durmaz[1]*, Emrullah Sagun[2], Zekai Tarakci[2] and Fevzi Ozgokce[3]

[1]Harran University, Veterinary Faculty, Food Hygiene and Technology Department, Sanliurfa, Turkiye.
[2]Yuzuncu Yil University, Veterinary Faculty, Food Hygiene and Technology Department, Van, Turkiye.
[3]Yuzuncu Yil University, Science and Arts Faculty, Biology Department, Van, Turkiye.

Accepted 15 September, 2006

***Allium vineale L.*, *Chaerophyllum macropodum Boiss.* and *Prangos ferulacea (L.) Lindl.* have been used for cheese production in Turkiye for many centuries. In addition, it is traditionally believed by local people that these plants have antibacterial activity. The purpose of this study was to evaluate the antibacterial activity of these plants. Four solvent extracts (in methanol, ethanol, *n*-hexane and water) of the plants were investigated against *Bacillus cereus*, *Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus aureus, Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Salmonella enteritidis* and *Salmonella typhimurium* by using disc diffusion method. The methanol, ethanol and *n*-hexane extracts of all the plants showed antibacterial activity against *B. cereus*, *B. subtilis*, *M. luteus* and *S. aureus*, while the methanol extract of *Allium vineale* was also active against *P. mirabilis*. However, the water extracts of these plants had no antibacterial activity against any of the bacteria tested. The methanol extracts had the higher activity followed by the extracts of ethanol and *n*-hexane. *A. vineale* showed the higher antibacterial activity as compared with *C. macropodum* and *P. ferulacea*. As a result, organic solvent extracts (especially methanol and ethanol extracts) of these plants can be used as natural antibacterial additives for incorporation in cheese and various food products.**

Key words: *Allium vineale, Chaerophyllum macropodum, Prangos ferulacea,* antibacterial activity.

INTRODUCTION

It has been well known since ancient times that plants and spices have antimicrobial activity (Ayres et al., 1980; Zaika, 1988). There has been a considerable interest to use plants and spices for the elimination of microorganisms because of increasing antibiotic resistance of microorganisms (Kunin, 1993; Finch, 1998; Smid and Gorris, 1999). In Turkey, *Allium vineale* L. (*Liliaceae*), *Chaerophyllum macropodum* Boiss. (*Apiaceae*) and *Prangos ferulacea* (L.) Lindl. (*Apiaceae*) are traditionally added to herby cheese for their aroma and flavor. Recently, we demonstrated that methanol, ethanol and *n*-hexane extracts of these plants used in cheese have inhibitor effect on *Listeria monocytogenes* (Sagun et al., 2006). In this study, we investigated the antibacterial activity of methanol, ethanol, *n*-hexane and water extracts of *A. vineal*e, *C. macropodum* and *P. ferulace*a against a panel of Gram positive and Gram negative bacteria. To our knowledge, antibacterial activities of these plants against a wide range of other microorganisms have not been studied in the literature.

*Corresponding Authors E-mail:hisamettindurmaz@yahoo.com. Tel.: +90 414 312 8456. Fax: +90 414 314 4158.

MATERIAL AND METHODS

Plant material

Aerial parts of *A. vineale*, *C. macropodum* and *P. ferulacea* were collected from Alacabuk (Pelli) mountain (Bitlis, Turkiye), with assistance of knowledgeable villagers. The plants were identified in the Department of Biology, Faculty of Science and Arts, Yuzuncu Yil University (Van, Turkiye), where the voucher specimens have been deposited (F 11 178, F 11 176, and F 11 175, respectively, VANF). The plants were separately dried in shade, pulverized by a mechanical grinder and stored in airtight glass containers in dark until extraction.

Table 1. The yields of plants extracted in different solvents.

Plant species	Extraction yields (%)			
	Methanol	Ethanol	n-hexane	Water
Allium vineale	13.6	7.25	2.30	1.47
Chaerophyllum macropodum	10.55	4.70	0.51	1.92
Prangos ferulacea	9.05	5.33	0.40	1.01

Preparation of extracts

For extraction, methanol, ethanol, *n*-hexane and water were used as solvents. Thirty grams of the dried and powdered plant materials were extracted with 300 mL of solvents by using Soxhlet apparatus for 10 h at a temperature not exceeding the boiling point of the solvents (Lin et al., 1999). The obtained extracts were filtered by using Whatman No. 1 filter paper and then concentrated under vacuum at 40 °C by using a rotary evaporator. The residual extracts were stored in a desiccator until further use.

Bacterial cultures

Four Gram positive bacteria including *Bacillus cereus* (ATCC 11778), *Bacillus subtilis* (ATCC 6633), *Micrococcus luteus* (clinical isolate) and *Staphylococcus aureus* Cowan I (NTCC 838) and five Gram negative bacteria including *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (clinical isolate), *Proteus mirabilis* (ATCC 7002), *Salmonella enteritidis* (clinical isolate) and *Salmonella typhimurium* (clinical isolate) were used in the study. The bacterial cultures were maintained at 4°C on tryptone soya agar (Oxoid) slants through bimonthly transfers.

Antibacterial assay

Antibacterial activities of the extracts were carried out by disc diffusion method (Lennette et al., 1985). The residual extracts were dissolved in their extracting solvents to yield the final concentration of 50 mg/mL and sterilized by filtration (filter pore size 0.47 µm). Sterile filter paper discs (Whatman No. 3, diameter 6 mm) were impregnated with the prepared extracts. For the preparation of the inoculation, the tested bacteria were cultured in tryptone soya broth (Oxoid) at 37°C for 24 h and standardized for the same absorbency, number 0.5 of the McFarland Nephelometer, which corresponds to the order of 10^8 CFU/mL (Barry and Thornsberry, 1985). One hundred microliters of prepared culture were spread on the surface of Mueller-Hinton agar (Oxoid). Previously prepared extract impregnated discs (Whatman No. 3, diameter 6 mm) placed on the culture medium. The plates were kept at ambient temperature for 30 min to enable diffusion of extracts and then incubated at 37°C for 24 h. Discs impregnated with only solvents were used as negative controls and antibiotic discs of streptomycin (10 µg) (Oxoid) were used as positive controls. The antibacterial activity was evaluated by measuring the diameter of inhibition zone. Each experiment was repeated at three times and mean of the diameter of inhibition zones was calculated.

RESULTS

In the study, among the extractions assayed, the methanol extracts of *A. vineal*e, *C. macropodum* and *P. ferulace*a contained the higher soluble solids than those of ethanol, *n*-hexane and water (Table 1). Solvents (negative control) used for extraction showed no activity against any bacteria tested (Table 2).

Methanol, ethanol and *n*-hexane extracts of the three species produced inhibition zones against Gram positive bacteria *B. cereus*, *B. subtilis*, *M. luteus* and *S. aureus*. In contrast, no inhibition zone was seen against Gram negative bacteria *E. coli*, *K. pneumoniae*, *S. enteritidis* and *S. typhimurium*. The methanol extract of *A. vineale* was the only one that produced inhibition zone Gram negative bacterium, *P. mirabilis*.

The inhibition zones varied depending on type of extract, plant species and bacterial species. In general, methanol extracts of the three species were found to be more effective than other extracts. The largest diameter of inhibition zone was observed from methanol extracts of *A. vineale* against *M. luteus* and *S. aureus*, which showed inhibition zones close to that of streptomycin. However, the water extracts prepared from the three plant species did not exhibit any activity against the bacteria tested. Among the plant species tested, *A. vineale* exhibited higher antibacterial activity compared with *Chaerophyllum macropodum and Prangos ferulacea* (Table 2).

DISCUSSION

The present study was designed to obtain preliminary information on the antibacterial activity of three plants used for cheese production in Turkey. The disc diffusion method was preferred to be used in this study. Our study showed a remarkable antibacterial activity of the methanol, ethanol and n-hexane extracts of *A. vineal*e, *C. macropodum* and *P. ferulace*a.

Most of the identified components with antimicrobial activity extracted from plants are aromatic or saturated organic compounds and they are more soluble in methanol and ethanol (Cowan, 1999). Several workers (Martin, 1995; Paz et al., 1995; Vlientinck et al., 1995) have generally reported that water extracts of plants do not have much activity against bacteria. Eloff (1998) reported that methanol was the most effective solvent for plant extraction than ethanol, *n*-hexane and water. Similarly, in our study the methanol extracts exhibited higher activity followed by the ethanol and *n*-hexane extracts. The water

Table 2. Antibacterial activity of the various extracts from plants added Herby cheese[a].

Plant species	Extracts	Inhibition zone diameters (mm)[b]								
		Bc	Bs	Ml	Sa	Ec	Kp	Pm	Se	St
Allium vineale	Methanol	15	17	21	19	-	-	11	-	-
	Ethanol	10	13	18	17	-	-	-	-	-
	n-hexane	10	11	18	14	-	-	-	-	-
	Water	-	-	-	-	-	-	-	-	-
Chaerophyllum macropodum	Methanol	12	14	18	18	-	-	-	-	-
	Ethanol	8	13	17	17	-	-	-	-	-
	n-hexane	9	11	15	13	-	-	-	-	-
	Water	-	-	-	-	-	-	-	-	-
Prangos ferulacea	Methanol	11	15	12	16	-	-	-	-	-
	Ethanol	8	11	16	16	-	-	-	-	-
	n-hexane	10	9	11	11	-	-	-	-	-
	Water	-	-	-	-	-	-	-	-	-
Negative control	Methanol	-	-	-	-	-	-	-	-	-
	Ethanol	-	-	-	-	-	-	-	-	-
	n-hexane	-	-	-	-	-	-	-	-	-
	Water	-	-	-	-	-	-	-	-	-
Positive control[c]		27	22	21	21	23	-	24	23	22

[a]Bacteria: Bc, *Bacillus cereus*; Bs, *Bacillus subtilis*; Ml, *Micrococcus luteus*; Sa, *Staphylococcus aureus*; Ec, *Escherichia coli*; Kp, *Klebsiella pneumoniae*; Pm, *Proteus mirabilis*; Se, *Salmonella enteritidis*; St, *Salmonella typhimurium*.
[b]No inhibition zone.
[c]Streptomycin (10 µg/disc).

extracts did not show any antibacterial activity. Our results indicated that methanol and ethanol extraction is a good method to extract antibacterial compounds found in these species.

Previous studies showed the high antibacterial activity of sulfur and other numerous phenolic compounds found in *Allium* plants (Rivlin, 2001; Griffiths et al., 2002). The higher antibacterial activity of methanol extract from *Allium vineale* may therefore be due to the higher phenolic content of this plant. Extracts from the genus *Prangos* were found to be rich in coumarin and terpenoids (Shikishima et al., 2001; Tada et al., 2002). The coumarin derivatives have been reported to have a slight antibacterial activity against *Escherichia coli* and *Candida albicans* (Ulubelen et al., 1995; Pedro et al., 1999). Terpenes have also been found in *Chaerophyllum* plants and they have been shown to be useful in the control of *L. monocytogenes* (Pedro et al., 1999; Aureli et al., 1992). The components with antibacterial activity in the extracts were not determined in our study. However, the compounds mentioned above may partially be responsible for antibacterial activity of the extracts investigated in our study.

The higher resistance of Gram-negative bacteria to plant extracts has previously been documented and related to thick murein layer in their outer membrane, which prevents the entry of inhibitor substances into the cell (Martin, 1995; Brantner et al., 1996; Palombo and Semple, 2001; Tortora et al., 2001; Matu and van Staden, 2003). Similarly, our results indicated that the antibacterial activities of the extracts were more pronounced on Gram positive than on Gram-negative bacteria. Only the methanol extract from *Allium vineale* displayed antibacterial activity against the Gram-negative bacterium *P. mirabilis*.

In conclusion, methanol and ethanol extracts of studied plants showed antibacterial activity. Among the plants tested, *Allium vineale* showed higher antibacterial activity against tested bacteria. This gives an indication of the presence of promising antibacterial compounds. Lastly, we think that further phytochemical studies are needed to elucidate the components responsible for antibacterial activity of these extracts against bacteria.

REFERENCES

Aureli P, Costantini A, Zolea S (1992). Antimicrobial activity of some plant essential oils against *Listeria monocytogenes*. J. Food Prot. 55: 344-348.

Ayres JC, Mundt JO, Sandine, WE (1980). Microbiology of Foods. San Francisco: WH Freeman and Company.

Barry AL, Thornsberry C (1985). Susceptibility Tests: Diffusion Test Procedures. In: Lennette EH, Balows A, Hausler WJ, Shadomy HJ (eds) Manual of Clinical Microbiology. Washington, DC: Am Soc. for Microbiol. pp 978-987.

Brantner A, Males Z, Pepeljnjak S, Antolic A (1996). Antimicrobial activity of *Paliurus spina-christi* Mill (Christ's thorn). J. Ethnopharmacol. 52: 119-122.
Cowan MM (1999). Plant products as antimicrobial agents. Clin. Microbiol. Rev. 12: 564-582.
Eloff JN (1998). Which extractant should be used for the screening and isolation of antimicrobial components from plants? J. Ethnopharmacol. 60:1-8
Finch RG (1998). Antibiotic resistance. J. Antimicrob. Chemother. 42: 125-128.
Griffiths G, Trueman L, Crowther T, Thomas B, Smith B (2002). Onions-a global benefits to health. Phytother. Res. 16: 603-615.
Kunin CM (1993). Resistance to antimicrobial drugs-a world-wide calamity. Ann. Intern. Med. 118: 557-561.
Lennette EH, Balows A, Hausler WJ, Shadomy HJ (1985). Manual of Clinical Microbiology (4th ed), Washington, DC: Am Soc. for Microbiol. pp 978-987.
Lin J, Opoku AR, Geheeb-Keller M, Hutchings AD, Terblanche SE, Jäger AK, van Staden J (1999). Preliminary screening of some traditional zulu medicinal plants for anti-inflammatory and anti-microbial activities. J. Ethnopharmacol. 68: 267-274.
Martin GJ (1995). Ethnobotany: A Methods Manual. London: Chapman and Hall.
Matu EN, van Staden J (2003). Antibacterial and anti-inflammatory activities of some plants used for medicinal purposes in Kenya. J. Ethnopharmacol. 87: 35-41.
Palombo EA, Semple SJ (2001). Antibacterial activity of traditional Australian medicinal plants. J. Ethnopharmacol. 77: 151-157.
Paz EA, Cerdeiras MP, Fernandez J, Ferreira F, Moyna P, Soubes M, Vázquez A, Vero S, Zunino L (1995). Screening of Uruguayan medicinal plants for antimicrobial activity. J. Ethnopharmacol. 45: 67-70.
Pedro LG, Silva JAD, Barroso JG, Figueiredo AC, Deans SG, Looman A, Scheffer JJC (1999). Composition of the essential oil of *Chaerophyllum azoricum* Trel., an endemic species of the Azores archipelago. Flavor Fragrance J. 14: 287-289.
Rivlin RS (2001). Historical perspective on the use of garlic. J. Nutr. 131: 951S-954S.
Sagun E, Durmaz H, Tarakci Z, Sagdic O (2006). Antibacterial activities of the extracts of some herbs used in Turkish Herby cheese against *Listeria monocytogenes* serovars. Int. J. Food Prop. 9: 255-260.
Shikishima Y, Takaishi Y, Honda G, Ito M, Takeda Y, Kodzhimatov OK, Ashurmetov O (2001). Terpenoids and ã-pyrone derivatives from *Prangos tschimganica*. Phytochemistry 57: 135-141.
Smid EJ, Gorris LGM (1999). Natural antimicrobials for food preservation. In: Shafiurr Rahman M (eds) Handbook of Food Preservation. New York: Marcel Dekker, Inc., pp 285-308.
Tada Y, Shikishima Y, Takaishi Y, Shibata H, Higutia T, Honda G, Ito M, Takeda Y, Kodzhimatov OK, Ashurmetov O, Ohmoto Y (2002). Coumarins and ã-pyrone derivatives from *Prangos pabularia*: antibacterial activity and inhibition of cytokine release. Phytochemistry 59: 649-654.
Tortora GJ, Funke BR, Case CL (2001). Microbiology: An Introduction. San Francisco: Benjamin Cummings.
Ulubelen A, Topcu G, Tan N, Olcal S, Johansson C, Ucer M, Birman H, Tamer S (1995). Biological activities of a Turkish medicinal plant, *Prangos platychlaena*. J. Ethnopharmacol. 45: 193-197.
Vlietinck AJ, van Hoof L, Totté J, Lasure A, Vanden Berghe D, Rwangabo PC, Mvukiyumwami J (1995). Screening of hundred Rwandese medicinal plants for antimicrobial and antiviral properties. J. Ethnopharmacol. 46:31-47.
Zaika LL (1988). Spices and herbs: their antimicrobial activity and its determination. J. Food Safety 9:97-118.