Asian Journal of Chemistry Vol. 19, No. 7 (2007), 5533-5536

Antimicrobial Activity of Black Mustard Seed (*Brassica nigra* L.)

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This study was made to determine the antimicrobial activity of mustard (*Brassica nigra* L.). For this purpose extract of mustard which was prepared in diethyl ether tested on test strains of *P. aeruginosa* ATCC 25853, *E. coli* ATCC 11239, *K. pneumoniae* FML 5, *M. luteus* ATCC 9345, *E. faecalis* ATCC 29212, *B. megaterium* DSM 32 and *S. aureus* ATCC 25923 with disk diffusion method as *in vitro*. The present investigation shows the different levels of inhibitory activity to test strains while the mustard has inhibitory activity on the all test strains.

Key Words: Mustard, Antimicrobial Activity.

INTRODUCTION

Many of the plants used today were known to the people of ancient cultures throughout the world and they were valued their preservative and medicinal powers. Scientific experiments on the antimicrobial properties of plants and their components have been documented in the late 19th century[1].

The success story of chemotherapy lies in the continuous search for new drugs to counter the challenge posed by resistant strains of microorganisms. The investigation of certain indigenous plants for their antimicrobial properties may yield useful results. A large number of plants indeed were used to combat different diseases and known to possess antimicrobial activity[2].

Spices and herbs are used in foods primarily because they impart desirable flavours and aromas, but they may fulfill more than one function in foods to which they are added. Thus, cloves, mustard, garlic, onion, oregano and others, in addition to imparting flavour, exhibit antimicrobial activity and may help preserve the food[3].

Annual herb up to 1.5 m or more, usually branched from about the middle. Stem covered below with stiff bristly hairs, glabrous in region

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of inflorescence. Basal leaves petiolate, lyrate-pinnatisect with a large terminal lobe, hispid on both surfaces. Upper leaves oblong-linear, entire, glabrous. Petals c. 8 × 3-4 mm, bright yellow. Siliquae 10-17 × 1.5-2 mm including a very narrow beak 1.5-3 mm. Seeds not mucilaginous[4]. The mustard seed contain fixed oil, (20-30 %), laxative (30 %) and glycozide[5].

The aim of this study was to compare the inhibitory effect of mustard extract on the growth of gram positive and gram negative bacteria.

EXPERIMENTAL

The mustard seeds were purchased. All samples collected were dried in shade and then powdered. *Pseudomonas aeruginosa* ATCC 25853, *Escherichia coli* ATCC 11239, *Klebsiella pneumoniae* FML 5, *Micrococcus luteus* ATCC 9345, *Enterococcus faecalis* ATCC 29212, *Bacillus megaterium* DSM 32 and *Staphylococcus aureus* ATCC 25923 strains were obtained from culture collection of Microbiology Laboratory, Medical Faculty, Yüzüncü Yil University.

Preparation of model extracts: The method suggested by Monsefi *et al.*[6] was applied to prepare model extracts. The mustard seed powder were extracted with diethil ether for 3 d and subsequently, the mixture was filtered and concentrated under reduced pressure (by a evaporator) at 50ºC. The extract was preserved in a refrigerator (4 ºC) until the end of the analysis.

Test of antimicrobial activity: In the present study, disc difusion technique as described by Hanafy and Hatem[7] was applied. The diethyl-ether extracts of mustard seed, were transferred into sterile bottles containing filter paper (Whatmann No:1; 6 mm diameter) Bottles were then placed in a water bath (50°C) with occasional shaking to allow an even distribution of the extract between discs until complete evaporation of ether had been achieved. To activate bacterial culture, Trypticase Soy Broth (TSB, DIFCO 0369-01-4) were used while Mueller-Hinton Agar (MHA,OXOID CM337) was used in antibacterial activity. Having taken from stock culture, the strains were diluted with TSB and incubated at 35 ± 0.1ºC for 24 h. At the end of incubation, bacterial suspension, *ca.* contain 10^8-10^9 cfu/mL levels of organisms, mixed with a steril loop and then, 0.1 mL were inoculated on MHA petri plates. The inoculum was spreaded on surface of plates completely and the inoculated petri plates were dried in room temperature. Then, paper discs penetrated of plant extract were placed on petri plates inoculated previously and were incubated at 35 ± 0.1°C for 48 h. Diameter of inhibition zone around discs were measured and expressed as mm. Antibacterial activity studies were carried out duplicate for each test strains and average measurement were calculated.

RESULTS AND DISCUSSION

The antimicrobial activity of mustard seeds were shown at Table-1. At the end of the antimicrobial activity tests it is determined that mustard seeds has inhibitory activity all test strain.

In this study 30 µg/disk concentration of extract of mustard seeds which was prepared in diethyl ether tested on 7 bacteria strains (*P. aeruginosa* ATCC 25853, *E. coli* ATCC 11239, *K. pneumoniae* FML 5, *M. luteus* ATCC 9345, *E. faecalis* ATCC 29212, *B. megaterium* DSM 32 and *S. aureus* ATCC 25923) with disk diffusion method as *in vitro*.

It is determined that 30 µg/disk mustard has inhibitory activity on all tested strains (Table-1). Inhibition zone diameters were determined as 10 mm for *P. aeruginosa*, 10 mm for *E. coli*, 11 mm for *K. pneumoniae*, 15 mm for *E. faecalis*, 19 mm for *M. luteus*, 15 mm for *S. aureus* and 8 mm for *B. megaterium*. It is observed that mustard effects on *M. luteus* one of the test strains was maximum.

TABLE-1
THE RESULTS OF ANTIBACTERIAL ACTIVITY OF MUSTARD SEED

Bacterium strains	Inhibition zone (mm) (30 µg/disk)
P. aeruginosa ATCC 25853	10
E. coli ATCC 11239	10
K. pneumoniae FML 5	11
E. faecalis ATCC 29212	15
M. luteus ATCC 9345	19
S. aureus ATCC 25923	15
B. megaterium DSM 32	8

Interest to medicinal plants and active substance which are originating from medicinal herbs and studies about this subject are increased in recent years. Some kinds of these herbs are used as pigment and drug in pharmacology and some kind of these herbs are used to give aroma to food in food industry[5].

Some spices (mustard, pepper and coriander) stimulate the growth of some lactic acid bacteria used as starter cultures[8-10]. In addition to this studies have been reported on antimicrobial effects of different herbs and their extracts used as spices or aromatic herbs including garlic, rosemary, onion, nutmeg, curry, mustard, black pepper, cinnamoni, thyme, oregano sage, Jamaican pepper, aniseed, dill, basal, paprika, cassia, turmeric, cardamom, cayenne pepper, clover, chives, coriander, ginger, savory and marjoram[2,9,12-22]. The difference between inhibition zone diameters showed that mustard seed has effects on bacteria strains at different level. This finding ensure a parallelism with the finding that is sensitivity of microor-

ganisms to chemotherapeutics shows differences according to type of strains which was stated by Çetin and Gürler[23]. Essential oils of coriander, mustard and pepper have been described as efficient on pathogens by other authors[8,15,24] however present extracts had different effect against the tested bacteria. Tegos *et al.*[25] in their study on herb antimicrobials stated that, *S. aureus* and *B. megaterium* were the most sensitive microorganism to antimicrobial substances. When Table-1 is examined; it is observed that mustard has one of the strains that greatest effect on *S. aureus*. This finding is in agreement with the findings of Tegos *et al.*[25].

As a result it is determined that mustard, which was collected from different parts of Turkey, has antimicrobial activity on some gram positive and gram negative microorganisms, which were important for food poison.

REFERENCES

1. L. Zaika, *J. Food Safety*, **9**, 97 (1975).
2. D.S. Arora and J. Kaur, *Int. J. Antimicrob. Agents*, **12**, 257 (1999).
3. S.M.N. Abbas and A.K. Halkman, *Int. J. Food Microbiol.*, **97**, 63 (2004).
4. P.H. Davis, Flora of Turkey and the East Aegean Island, Edinburg at the University Press, Vol. 8, p. 635 (1982).
5. T. Baytop, Türkiye'de Bitkiler ile Tedavi. I. Ü. Eczacilik Fak. I. Ü. Yayinlari. No:3255-Eczacilik Fak. No: 40, Istanbul (1984).
6. M. Monsefi, M. Ghasemi and A. Bahaoddini, *Daru*, **14**, 131 (2006).
7. M.S. Hanafy and M.E. Hatem, *J. Ethanopharmacol.*, **34**, 275 (1991).
8. C.N. Huntanen, *J. Food Prot.*, **43**, 195 (1980).
9. L.L. Zaika and J.C. Kissenger, *J. Food Sci.*, **46**, 1205 (1981).
10. I.F. Nes and R. Skjelkvale, *J. Food Sci*., **47**, 1618 (1982).
11. S.E. Aktug and M. Karapinar, *Int. J. Food. Microbiol.*, **3**, 349 (1986).
12. T. El-Khateib, H.A. El-Rahman, *J. Food. Prot.*, **50**, 310 (1987).
13. S.G. Deans and K.P. Svodoba, *J. Hort. Sci.*, **64**, 205 (1989).
14. R.S. Farag, Z.Y. Daw, F.M. Hewedy and G.S.A. El-Baroty, *J. Food Prot.*, **52**, 665 (1989).
15. P. Aureli, A. Constantini and S. Zolea, *J. Food Prot.*, **55**, 344 (1992).
16. M. Karapinar, *Int. J. Food. Microbiol.*, **10**, 193 (1990).
17. W.T.E. Ting and K.E. Deibel, *J. Food. Safety*, **12**, 129 (1992).
18. Y.A. Hefnawy, S.I. Mousta and E.H. Marth, *J. Food Prot.*, **56**, 876 (1993).
19. V.A. Pandit and L.A. Shelef, *Food Microbiol.*, **11**, 57 (1994).
20. D.L. Holt and N.G. Almonte, *J. Food Prot.*, **58**, 322 (1995).
21. A. Sivropoulou, E. Papanikolaou, C. Nikolaou, C. Kokkini, T. Lanaras and M. Arsenakis, *J. Agric. Food Chem.*, **44**, 1202 (1996).
22. Y.Y. Hao, R.E. Brackett and M.P. Doyle, *J. Food Prot.*, **61**, 307 (1998).
23. T.E. Çetin and N. Gürler, Bakterilerin Antibiyotiklere Duyarlilik Deneylerinin Yapilmasi, Kükem Dergisi, 12, 2 (1989).
24. M. Kivanç and A. Akgül, *Flavour Fragr. J.*, **3**, 95 (1988).
25. G. Tegos, F.R. Stermitz, O. Lomovskaya and K. Lewis, *Antimicrob. Agents Chemo.*, **46**, 3133 (2002).

(*Received*: 22 November 2006; *Accepted*: 19 June 2007) AJC-5735