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Full Length Research Paper

Effect of distillation and solvent extraction methods on oil extracts of Australian and Chinese garlic

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In this study the effect of distillation and solvent extraction methods were studied on many factors; oil percentage, physical and chemical properties, and percentage of decay of both Australian (white and red) and Chinese garlic. On the other hand, the antimicrobial effect of Australian and Chinese garlic plants was determined at various concentrations against *Staphylococcus aureus* ATCC®25923, *Pseudomonas aeruginosa* ATCC®9027, *Escherichia coli* ATCC®25922 and *Bacillus subtilis* ATCC®6633. Furthermore, the antimicrobial effect of Australian and Chinese garlic plants was compared with that of the antibiotics at definite minimum inhibitory concentrations. The highest percentage of extracted oil was obtained from the white Australian garlic followed by the red one. The solvent extraction method showed higher efficiency in oil extraction than the distillation method. Moreover, the white Australian garlic was found to have the best physical and chemical properties followed by the red one. It is worth mentioning that, the Chinese garlic has higher resistance to decay more than the red Australian garlic. Therefore, it is recommended to use the white Australian garlic due to its high oil content which has potential antibacterial activity and has the best physical and chemical properties, but it should not be stored to prevent loss on decay.

Key words: Antimicrobial activity, distillation, oil extraction, synergistic effect.

INTRODUCTION

Garlic was considered as one of the most important economic plants due to its efficient antimicrobial activity and contains powerful sulfur and other numerous phenolic compounds which have great interest (Rivlin, 2001; Griffiths et al., 2002). Moreover, FDA and WHO reported that the garlic plant had been recently extensively introduced in the treatment of many microbial infectious diseases as potential bactericidal (Gram-negative and Gram-positive) and fungicidal agent. Furthermore, it was considered a nutritive plant because it has sufficient content from essential mineral elements such as sulfur, which may be required as a cofactor for various enzymes which play important roles in the metabolism (Whitemore and Naidu, 2000). Moreover, allicin is a notable flavonoid in garlic forming with crushing (Ross et al. 2000). Han et al. (1995) reported that one mg of garlic allicin (allyl 2-propene thiosulfinate) has been equated to fifteen IU of penicillin. Garlic allicin exerted antibacterial activity against *Salmonella typhimurium*, primarily by interfering with RNA synthesis

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(Feldberg et al. 1988). Garlic also has been reported to produce various beneficial effects, including anti stress protection, growth promotion, appetite stimulation, immune stimulation and antimicrobial properties in fin fish and shrimp larviculture (Vaseeharan et al., 2011). Guo et al. (2012) investigated the *in vitro* antibacterial activity of garlic against *S. iniae* and the effect of garlic supplemented diets on growth and disease. Ruiz et al. (2010) evaluated in vitro the effects of two of these garlic derived compounds (PTS and PTS-O) on predominant faecal microbial populations of swine, and to determine the concentrations active against some of the most relevant populations of swine intestinal microbiota. Additionally, activity against *Escherichia coli* and *Salmonella typhimurium*, two common pathogens of pigs, was also tested. Karuppiah and Rajaram (2012) evaluated the antibacterial properties of *Allium sativum* (garlic) cloves and *Zingiber officinale* (ginger) rhizomes against multi drug resistant clinical pathogens causing nosocomial infection. Resistance in organge-spotted grouper challenged with *S. iniae*. Many investigations have also demonstrated an inhibitory effect of aqueous extracts on numerous bacterial and fungal species (Sivam et al., 1997; Hsieh et al., 2001; Ward et al., 2002). Garlic was used in China since many years ago as an efficient antibacterial agent against *Helicobacter pylori*. This pathogen led to an epidemic event where 241 Chinese adults were highly incident with gastric lesions, gastric dysplasia and gastric cancer (You et al., 2004). Koch and Lawson (1996) cited in their comprehensive book on garlic, at least 15 statistical studies and review articles that correlate garlic consumption to low cancer rates in Europe, Egypt, India, China, and other third world countries, which are favorable to garlic. Garlic also contains some sulphur-containing compounds such as alliin, ajoene, diallylsulphide, dithin, S- allylcysteine, enzymes and other non sulphur-containing compounds including vitamin B, proteins, minerals, saponins and flavonoids (Johnson et al. 2008). The root bulb of garlic has been used traditionally for thousands of years to treat many diseases because it has high concentrations of sulfur containing compounds (Tattelman, 2005). The elucidation of the chemical structures of these compounds has led to the synthesis and production of more potent and safer drugs (Bhattacharjee et al., 2005). The aim of this work is studying the antimicrobial activity and phytochemical properties of Australian and Chinese garlic plants such as oil content, physochemical properties, percentage of decay on storage.

MATERIALS AND METHODS

Extraction of crude oil from garlic using steam distillation method

Garlic pills were collected from the market. Two hundreds grams of garlic cloves were grounded in a mixer and placed in the distillation flask, 400 ml of distilled water were added and distillation was carried out for 4 h. The distillate was collected and dried over anhydrous sodium sulphate then filtered and kept in dark brown glass containers in the refrigerator at 2 to 5°C (Benkeblia, 2004).

Extraction of garlic oil using organic solvent method

The organic solvent was first distilled; garlic pills were then grounded into coarse particles using the mixer and soaked for 24 h in organic solvent (ethanol) in reflux condenser apparatus. The extract was dried over anhydrous sodium sulfate, and then filtered through Bunchner funnel using filter paper No. 52. The solvent was evaporated at 60°C (Bektas et al., 2005).

Determination of specific gravity of oil

The specific gravity of the oil was determined according to the USP 2006 using the pyknometer.

Determination of refraction index of the oil

The refraction index of the oil was determined according to USP 2006 using the refractometer digital ABBE.

Determination of Acid and Peroxide Values

The acid value of oil was determined according to USP 2006. The peroxide value of oil was determined according to the EP 2005.

Microorganisms

The bacterial cultures used in the present study are *Pseudomonas aeruginosa* ATCC®9027, *Bacillus subtilis* ATCC®6633, *Staphylococcus aureus* ATCC®25923 and *Escherichia coli* ATCC®25922.

Preparation of inoculums

A loopful of inoculum was taken from pure culture of the respective bacteria grown on slants and inoculated into 10 ml of nutrient broth. The broth suspension was then incubated at 37°C. The growth so obtained was inoculum for the sensitivity test.

Sensitivity of bacteria to garlic extract

The antibacterial activity of various extracts was tested by agar diffusion method. The plates containing Mueller Hinton agar were spread with 0.2 ml of bacterial inoculum (about 10^5 – 10^6 CFU/ml). Wells (8 mm diameter) were cut from agar plates using sterilized stainless steel borer and were filled with 0.1 ml of garlic extract. The plates were incubated at 37°C. The diameter of any resultant zone of inhibition was measured. Each combination of microorganism and antibiotic was repeated three times. Microorganism showing clear zone was considered to be inhibited.

Comparative sensitivity of bacteria to garlic extract and antibiotic

Comparative activity of different antibiotics and garlic extract on bacteria was assayed by agar diffusion method. The Muller Hinton

Table 1. Effect of extraction methods on garlic oil percentages and physicochemical properties.

Extraction method	Distillation			Solvent ethanol		
Garlic	Australian		Chinese	Australian		Chinese
	White	Red		White	Red	
Oil %	0.058	0.046	0.044	6.730	5.971	5.658
Specific gravity	1.0588	1.0547	1.0549	1.0545	1.0555	1.0528
Refractive index	1.4658	1.4669	1.4650	1.4712	1.4710	1.4705
Acid value	0.00	0.00	0.00	0.50	0.52	0.48
Peroxide value	0.08	0.05	0.05	0.30	0.33	0.41

agar plates were inoculated with 0.2 ml of bacterial standard commercial antibiotic discs (OXOID). Inoculated plates with antibiotic discs were incubated for 24 h and the diameter of any resultant zone of inhibition was measured. Each combination of microorganism and antibiotic was repeated three times. Interpretation of resistance was based on the National Committee for Clinical Laboratory Standards (1999) criteria. The antibiotics discs used were penicillin pen, 10 µg; amikacin AK, 30 µg; tetracycline TE, 30 µg; amoxicillin clavulanate AC, 30 µg; ciprocin CIP, 30 µg; erythrocin E, 10 µg; chloramphenicol C, 30 µg; piperacillin/tazobactam TZP, 110 µg.

RESULTS AND DISCUSSION

Garlic oil percentage

Data recorded in Table 1 showed that the percentage of oil differed according to the type of garlic; Australian (white and red), and the Chinese garlic. The oil percentage differed also according to the extraction method used (distillation and solvent extraction method), and it was obtained by the solvent extraction method in all garlic types, whereas the distillation method yielded the lowest oil percentage for all garlic types. It was concluded that the white Australian garlic yielded the highest oil percentage by either solvent extraction method (6.73%) or by distillation method (0.058%), whereas the Chinese garlic yielded the lowest oil percentage either by using the distillation method (0.044%) or by solvent extraction method (5.658%).

Physical properties

Specific gravity

The specific gravity of oil showed by determined especially during the transport or the storage of those oils in order to design the specific pipes and tanks. The specific gravity values of garlic oil extracted from different types of garlic by steam distillation and organic solvent as shown in Table 1. The highest specific gravity was 1.0588 for the white Australian garlic oil extracted by using the distillation method whereas the Chinese garlic oil extracted by solvent extraction method gave the lowest value of the specific gravity (1.0528).

Refractive index

Refractive index is an important physical parameter used for the identification of oils, fats and liquid wax, as it can be used to estimate the degree of their purity of oil. Results in Table 1 showed the effect of extracting method on the refractive index of garlic oil. It was found that the refractive index values differed according to the extraction method of garlic oil. The values were higher in case of using the solvent extraction method than those obtained by the distillation method. The highest value was (1.4712) for the Australian white garlic oil extracted by organic solvent, whereas the lowest refractive index was 1.4650 for Chinese garlic oil extracted by steam distillation.

Chemical properties

Acid value

Acid value is a parameter for the content of free fatty acids in oil and is used to detect the hydrolysis of fatty acids under the influence of lipase enzyme. Table 1 showed the effect of oil extracting method on the acid value of garlic oil. It was found that garlic oil extracted by distillation value has no acid value whereas the oil extracted by solvent method gave only low acid values it ranged from 0.48 to 0.52 for oil extracted from Chinese garlic and Australian red garlic respectively.

Peroxide value

Peroxide value is the quantity of active oxygen absorbed by one kilogram of oil; peroxide value of garlic was slightly affected by the extraction methods. Data in Table 1 showed that peroxide values ranged from 0.3 for oil extracted from Australian white garlic by ethanol to 0.41 for oil extracted by ethanol from Chinese garlic; but when

Table 2. Effect of storage on the decay percentage of garlic cloves.

Days	Australian garlic		Chinese garlic
	White	Red	
60	5.31	1.10	0.51
120	9.82	3.56	1.07
180	10.77	4.05	2.10
240	11.34	5.50	3.72

using the distillation method for extraction, the peroxide value was 0.08 for the oil extracted from white Australian garlic and 0.05 for the oil extracted from red Australian garlic, respectively.

Percentage of garlic decay

It was found that the Chinese garlic has a higher ability to resist decay which affects the cloves on storage followed by the red Australian garlic which was found to be more resistant than the white Australian garlic. The results in Table 2 showed that the percentage of decay after 60 days was 0.51% for the Chinese garlic, whereas it was 1.1% for the red Australian garlic, then the white Australian garlic in which the percentage of decay was 5.31%. There was also a relation between the percentage of decay and the duration of storage, the percentage of decay increased after 240 days of storage to be 3.72% for the Chinese garlic, 5.5% for the red Australian garlic and 11.34% for the white Australian garlic.

Sensitivity of bacteria to different concentrations of garlic extracts

The result of our present study shows that the antimicrobial activity of both of garlic (Chinese and Australian) extracts exhibited different inhibition levels against four medically important pathogens; *P. aeruginosa* ATCC®9027, *B. subtilis* ATCC®6633, *S. aureus* ATCC®25923 and *E. coli* ATCC®25922, as shown in Table 3. In dose response study, the inhibition zone increased with increasing concentration of extracts. Low concentration (50 – 100 ml/L) inhibited weakly the growth of bacteria and this result agreed with previous study (Benkeblia, 2004). At high concentrations (200, 300, and 500 ml/L) of garlic extract exhibited significant inhibition activity against bacteria and the inhibition of Chinese extract was more than Australian extract.

The result of our present study shows that, the inhibition of Chinese extract of garlic was more than those of Australian garlic extracts. Comparatively, *S. aureus* ATCC®25923 was less sensitive to the inhibitory activity of garlic extracts than *E. coli* ATCC®25922 which were more inhibited at same concentrations of Chinese extracts as shown in Table 4. *E. coli* ATCC®25922 > *B. subtilis* ATCC®6633 > *P. aeruginosa* ATCC®9027 > *S. aureus* ATCC®25923. Kyung et al. (2002) reported that, allicin of garlic extract showed strong antibacterial activity against *S. aureus* ATCC®25923 at 150 ml/L concentration. The antibacterial activity of other and close, chemically cysteine sulfoxide (S-methyl-1-CS and methyl methane-CS) of cabbage also was markedly observed, particularly concentrations of 10, 20 and 50 mg/L (Kyung et al. 1997). In another study, 1% of oregano essential oil incorporated into a calcium caseinate WPI-carboxymethyl cellulose film was found to be effective against *E. coli* O157-H7 and *Pseudomonas* spp on the surface of beef muscle pieces (Oussallah et al. 2004). Previous studies on antimicrobial activity of garlic essential oils in culture media show some inhibitory effect on certain pathogen bacteria (O'Gara et al. 2000).

The mode of action of carvacrol was explained by Burt (2004) that it disintegrates the outer membrane of Gram-negative bacteria, releasing lipopolysaccharides and increasing the permeability of the cytoplasmic membrane to ATP. The result of our present study indicates the antimicrobial activity of two kinds of garlic extract in culture media has shown some inhibitory effect in certain order *S. aureus* ATCC®25923 > *B. subtilis* ATCC®6633 > *E. coli* ATCC®25922 > *P. aeruginosa* ATCC®9027. Alrozeky and Nakahara (2002) reported weak antibacterial activity of extracts from some edible plants commonly consumed in Asia. Combined extracts of *Corni fructus*, cinnamon and Chinese chive (1:6:6, vol/vol/vol) exhibited low inhibitory effect against this bacteria than other combined ratios and against other bacterial species (Hsieh et al. 2001).

Comparative sensitivity of bacteria to garlic extracts and antibiotics

In the present study some bacteria showing resistance to certain antibiotic were sensitive to extract of garlic oil especially Chinese one. *S. aureus* ATCC®25923 was resistant to the antibiotics; penicillin, erthrocin, chloramphenicol, and tetracycline while sensitive to extract of garlic oil. On the other hand, *E. coli* ATCC®25922 was resistant to penicillin, erthrocin, and piperacillin/tazobactam. *B. subtilis* ATCC®6633 was resistant to only two antibiotics Amoxicillin/clavulanate and penicillin. The multi-antibiotics resistant *P. aeruginosa* ATCC®9027 was sensitive to garlic extract as shown in Table 5. It is known that the multi-antibiotic resistant bacteria can cause nosocomial infection which needs not only expensive antibiotic to treatment but also to development of agents with marked antibacterial activity and greater sensitivity and less toxicity. In the present study garlic was more effective in inhibiting multi-antibiotic resistant bacteria when compared with the

Table 3. Sensitivity of bacteria to different concentrations of garlic extract.

Bacteria	Control	Antibacterial activity of Chinese garlic (mm)					Antibacterial activity of Australian garlic (mm)				
		50	100	200	300	500	50	100	200	300	500
P. aeruginosa ATCC®9027	18	15	16	18	19	21	13	13	16	17	20
B. subtilis ATCC®6633	18	18	21	22	23	25	15	17	18	19	22
S. aureus ATCC®25923	18	18	15	17	18	19	14	13	16	17	18
E. coli ATCC®25922	18	18	22	24	26	29	15	17	21	25	28

Table 4. Sensitivity of bacteria into two types of garlic extract.

Bacteria	Antibacterial activity of garlic extracts (mm)					
	C.O.S.	A.R.O.S.	A.W.O.S.	C.W.E.	A.R.W.E.	A.W.W.E.
P. aeruginosa ATCC®9027	28	30	22	28	27	23
B. subtilis ATCC®6633	35	30	22	26	25	24
S. aureus ATCC®25923	36	24	22	25	30	23
E. coli ATCC®25922	27	32	24	28	26	23

C.O.S. = Chinese organic solvent, A.R.O.S. = Australian red organic solvent, A.W.O.S. = Australian white organic solvent, C.W.E. = Chinese water extract, A.R.W.E. = Australian red water extract, A.W.W.E. = Australian white water extract.

Table 5. Sensitivity of bacteria to garlic extracts and antibiotics.

Bacteria	Antibacterial activity of garlic extracts and antibiotics (mm)									
	Pen 10 µg	AK 30 µg	TE 30 µg	AC 30 µg	CIP 30 µg	E 10 µg	C µg 30	TZP 110 µg	C.G. 500 ml/L	A.R.G. 500 ml/L
P. aeruginosa ATCC®9027	None	25	None	None	15	None	None	25	21	20
B. subtilis ATCC®6633	None	30	23	None	25	25	35	28	25	22
S. aureus ATCC®25923	None	28	None	13	35	None	None	30	19	18
E. coli ATCC®25922	None	18	28	23	27	None	25	None	29	28

None = No inhibition zone, Pen = Penicillin, AK = Amikacin, TE = Tetracycline, AC = Amoxicillin/Clavulanate, CIP = Ciprocin, E = Erthrocin, C = Chloramphenicol, TZP = Piperacillin/Tazobactam, C.G. = Chinese Garlic, A.R.G. = Australian Red Garlic.

tested antibiotic. O'Gara et al. (2000) indicated a relationship between lower MICs and number of sulfur atoms/molecules for diallyl sulphides against *H. pylori* and suggested that the number of sulphur atoms/molecules and/or disulphide bonds in the diallyl sulphides was an important factor in determining their antimicrobial activity. Garlic oil and its component possessed in vitro antibacterial activity against multi-antibiotic resistant *P. aeruginosa* and *K. pneumoniae*. Both additive and synergic effects were observed in combinations of ceftzidine, gentamicin, imipenem and meropenem with garlic sulphide agent, therefore, garlic oil and both sulphides DAT and DATS may have the potential to prevent or treat nosocomial infections caused by *P. aeruginosa* and *K. pneumoniae* (Tsao and Yin, 2001).

Conclusion

White Australian garlic is a best type to use due to its high oil content which has potential antibacterial activity and it has the best physical and chemical properties, but it should not be stored to prevent loss on decay. However, the Chinese garlic has higher resistance to decay more than the red Australian garlic.

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