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In vitro antibacterial activities of dietary medicinal ethanolic extracts against pathogenic reference strains of animal origin

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In vitro **antibacterial activities of five extracts from dietary medicinal plants were investigated by agar-well diffusion method (AWD), minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against 13 foodborne pathogenic bacteria reference strains; four Gram positive bacteria including *Staphylococcus aureus* (NCINB 50080), *Bacillus cereus* (NCINB 50014), *Citrobacter freundi* (ATCC 8090) and *Listeria innocua* (ATCC 33090) as well as nine Gram negative bacterial reference strains including *Escherichia coli* (ATCC 11775), *E. coli* O157 (ATCC 700728), *Salmonella typhimurium* (ATCC 13311), *Shigella boydii* (ATCC 9207), *Shigella sonnei (*ATCC 25931), *Shigella flexneri* (ATCC 12022), *Pseudomonas aeruginosa* (NCINB 50067), *Klebsiella pneumoniae* (NCTC 9633) and *Proteus mirabilis* (ATCC 14153). Four ethanolic extracts underwent acetone wash then analyzed for their principal components using gas chromatography-mass spectrometry (GC-MS), Oleamide was the predominant compound in onion, garlic, wheat germ and *Nigella sativa* which have great antibacterial effect. The tested acetone extracts exhibit variable antibacterial activity against foodborne pathogens which differ according to the compounds clarified in the GC-MS analysis. Garlic extract showed the best antibacterial activities, GC-MS analysis showed the presence of five compounds including; tetrasulfide, monosilane, oleamide, stearoylamide and vitamin E. Testing for the presence of 91 pesticides in the tested extracts using GC-MS analysis proved complete absence of pesticides which indicate that the antibacterial activities showed was due to the active components in the tested extracts and not due to the pesticides contaminants. Antimicrobial activities of plant extracts revealed that garlic has greatest inhibitory effect against *S. aureus* NCINB 50080 followed by *S.* Typhimurium ATCC 13311 with zone of inhibition 28 mm, 30 mm for AWDT and and 2.61 µg/ml for MIC, respectively. The best hindrance abilities was shown with garlic extracts with mean zone of inhibition (23.46 mm) followed by onion (18.15 mm), wheat germ extract (17.38 mm), mint (17.15 mm) then *Nigella sativa* (15.69 mm). Results of MIC and MBC confirm the antibacterial activities of the tested extracts.**

Key words: Antibacterial activity, agar well diffusion test (AWDT) minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), seed, bulb, ethanolic extracts.

INTRODUCTION

Food poisoning is still a concern for both consumers and the food industry despite the use of various preservation methods. Food processors, food safety researchers and regulatory agencies are continuously concerned with the

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high and growing number of illness outbreaks caused by some pathogenic and spoilage microorganisms in foods. The increasing antibiotic resistance of some pathogens that are associated with foodborne illness is another concern (Perreten et al*.,* 1998; Stermitz et al*.,* 2000).

The emergence of antimicrobial resistance has its roots in the use of antimicrobials in animals and the subsequent transfer of resistance genes and bacteria among animals and animal products (McEwen and Fedorka-Cray, 2002). Consumers are also concerned about the safety of foods containing synthetic preservatives. Therefore, there has been increasing interest in the development of new types of effective and nontoxic antimicrobial compounds. There is growing interest in using natural antibacterial compounds, such as extracts of spices and herbs, for food preservation (Smid and Gorris, 1999).

Numerous studies have been published on the antimicrobial activities of plant extracts against different types of microbes, including foodborne pathogens (Beuchat, 1994; Lis-Balchin and Deans, 1997; Smith-Palmer et al*.,* 1998; Hara-Kudo et al*.,* 2004). However, the results reported for these different studies are difficult to compare directly, usually because of the low number of plant samples tested, different test methods and diverse bacterial strains and sources of antimicrobial samples used.

Garlic has been used worldwide for many centuries as a spice and herbal medicine and believed to treat and prevent various diseases. It is strong antibacterial against Gram positive and Gram negative bacteria including *Bacillus*, *Brucella*, *Klebsiella*, *Pseudomonas*, *Proteus*, *Shigella*, *Staphylococcus*, *Salmonella* as well as *Helicobacter pylori* (Cellini et al*.,* 1996, Chowdhury et al*.,* 1991). The main component of garlic responsible for its antibacterial activities is allicin (Lixin Xia and Ng, 2005).

Rahman et al. (2012) indicated that garlic has been a favorite additive in food for many years in various cultures as it possesses antimicrobial, antiprotozoal, antimutagenic, antiplatelet and antihyperlipidemic properties. Allicin, a thiosulfinate extract of garlic, has been presumed to be a very strong antioxidant. Garlic contains unique organo-sulfur compounds (Block, 1985), which provide its characteristic flavor and odor and most of its potent biological activity.

The objectives of this study were: (1) to evaluate and compare the *in vitro* antibacterial activity of five plant extracts against 13 highly pathogenic reference strains responsible of food poisoning form food of animal origin (2) to establish the relationship between bacterial inhibition and total ethanolic extract content to confirm whether the ethanolic constituents are responsible for antibacterial activity (3) to ensure that the bactericidal activities are due to the compounds shown by gas chromatography-mass spectrometry (GC-MS) - gas chromatography mass selective detector (GC-MSD) analysis and to ensue the absence of pesticides in the extracts.

MATERIALS AND METHODS

Preparation of ethanolic extracts (Nanasombat and Lohasupthawee, 2005)

Five plant seeds or bulbs Table 1 were cut into small pieces; 20 g of each, were soaked in 100 ml of 95% ethanol, and shaken at 150 rpm for four days at ambient temperature. The mixtures were then filtered. The filtrates were evaporated using vacuum rotary evaporator (BÜCHI Rotavapor R-200/205, Model R205V800), and frozen at -80°C before freeze drying (Labconco, Model Lyph. Lock 6). Stock solutions of crude ethanolic extracts were prepared by diluting the dried extracts with 10% acetone solution to obtain a final concentration of 400 mg/ml.

Gas chromatography mass selective detector (GC-MSD) of acetone extract

GC-MSD operating parameters (Chin-Kai and Bruce, 2005 and Wylie, 2006)

The following parameters were used; gas chromatography (GC): Agilent Technologies 6890N, mass selective detector (MSD): 5975. EPC Split/ Splitless with inlet temperature: 250°C, 1 µl injection. Agilent Technologies column: HP5MS, p/n 19091S-433, stationary phase 5% phenyl methyl siloxane. Dimension 30 m × 0.25 µm ID × 0.25 mm film thickness, UHP Helium gas with 1.3 ml/min flow.

Oven programme: 90ºC (2 min), ramp 20ºC / min, 150 (0 min), ramp 6ºC /min, 270°C (10 min); total run time is 35 min. The used MSD temperature was 290ºC, quad temperature was 150ºC, and ion source temperature was 230ºC. The MSD mode with synchronous scan/SIM (selected ion monitoring); 3 selected ions for each compound. MS library: Wiley7/NIST5 and RTL pesticides mass spectral libraries. Four calibration levels were prepared including 0.01, 0.05, 0.10, and 0.50 µg/ml to construct the multi-level calibration curve. Aldrin was added to each level as internal standard (ISTD) with suggested concentration value of 0.1 µg/ml.

Gas chromatography mass spectrometer detector (GC-MSD) *of ethanolic extract (Chin-Kai and Bruce, 2005 and Wylie, 2006)*

Acetone wash was carried out for the tested ethanolic extract before introduction to Mass Spectrometer Detector. Optimized analytical method, that employing single quadrupole gas chromatograph equipped with mass spectrometer detector (GC-MSD) instrument has been developed for the simultaneous screening of 91 residues of different pesticide types including organophosphorus, organochlorine, pyrethroids and others as shown in Table 2 were monitored in Acetone wash was carried out for the tested ethanolic extracts of garlic, *Nigella sativa*, onion and wheat germ respectively.

Antimicrobial assay

Preparation of bacterial suspensions (NCCLS, 2003)

Antibacterial activities were carried out against thirteen highly pathogenic foodborne pathogenic strains of animal origin including four Gram positive bacterial reference strains; *Staph.aureus* (NCINB 50080), *Bacillus cereus* (NCINB 50014), *Citrobacter freundi* (ATCC 8090) and *Listeria innocua* (ATCC 33090) and nine Gram negative bacterial reference strains including *E. coli* (ATCC 11775), *E. coli* O157 (ATCC 700728), *Salmonella* Typhimurium (ATCC

Table 1. Botanical name of plant extracts and their edible parts.

Common name	Botanical name	The used plant parts in the experiment
Onion	*Allium cepa*	Bulbs
Garlic	*Allium sativum*	Bulbs
Mint	*Mentha canadensis*	Leaves
Wheat germ	*Triticum vulgare*	cereal grain
Black cumin	*Nigella sativa*	Seed

13311), *Shigella bodyii* (ATCC 9207), *Shigella sonnei* (ATCC 25931), *Shigella flexeneri* (ATCC 12022), *Pseudomonas aeruginosa* (NCINB 50067), *Klebsiella pneumoniae* (NCTC 9633) and *Proteus mirabilis* (ATCC 14153). Agar well diffusion test (qualitative method) and minimum inhibitory concentration (MIC) as well as minimum bactericidal concentration (MBC) (quantitative method) were used in this study. Wherein a suspension of bacterial strains were freshly prepared by inoculating fresh stock culture from each strain into separate broth tubes, each containing 7 ml of Muller Hinton Broth. The inoculated tubes were incubated at 37°C for 24 h. Serial dilutions were carried out for each strain, dilution matching with 0.5 Mc-Farland scale standard was selected for screening of antimicrobial activities. Ciprofloxacin 100 µg/ml was used as reference drugs.

Agar well diffusion method

The antimicrobial activity of 5 ethanolic extracts; onion, garlic, mint, wheat germ and *Nigella sativa* against bacterial strains were evaluated by using agar-well diffusion test (Katirciolu and Mercan, 2006). Hundred µl of cell culture suspension matching with 0.5 McFarland of target strains were spread onto the plates. For the investigation of the antibacterial activity, 100 µl of extracts (400 mg/ml), ciprofloxacin (100 µg/ml) as control positive and DMSO as control negative were added into wells of agar plates directly. Plates were left for 1 h at 25°C to allow a period of pre-incubation diffusion in order to minimize the effects of variation in time between the applications of different solutions. The plates were re-incubated at 37°C for 24 h. After incubation, plates were observed for antimicrobial activities by determining the diameters of the zones of inhibition for each of the strains. For an accurate analysis, tests were run in triplicate for each strain to avoid any error.

Determination of the minimum inhibitory concentration using micro broth dilution test (Jorgensen et al., 1999)

The dilution test was performed to determine minimum inhibitory concentrations (MICs). One hundred microliters of Mueller-Hinton broth (MHB) were added in each well of the 96-well sterile micro-titer plate. The 100-µl aliquot of stock solution of crude ethanolic extract (400 mg/ml) was added in the first well, and subsequently two- fold serially diluted with MHB. The inoculum suspension (20 µl) of each bacterial reference strains (0.5 McFarland, ~1 × 10^8 cfu/ml) were then added in each well containing crude ethanolic extract and MHB. The final concentrations of the extract were 166.7, 83.3, 41.7, 20.8, 10.4, 5.2, and 2.6 mg/ml. The negative and positive controls were also performed using DMSO and Ciprofloxacin, respectively. Duplicate wells were run for each concentration of herbal extracts. The plates were incubated at 37°C for 24 h. The lowest concentration that inhibited visible growth of the tested organisms was recorded as the MIC.

Determination of minimum bactericidal concentration (MBC) (Alade and Irobi, 1995)

After culturing the test organisms separately in nutrient broth containing various concentrations of the active ingredients, the broth was inoculated onto freshly prepared agar plates to assay for the bactericidal effect. The culture was incubated at 37°C for 24 h. The lowest concentration of extracts that does not yield any colony growth on the solid medium after the incubation period was regarded as minimum bactericidal concentration.

RESULTS

Gas chromatography mass spectrometer detector (GC-MSD) analysis

GC-MSD was carried out for four out of the five tested extracts; *Nigella sativa*, garlic, onion and wheat germ. Mint extract was not included as it contains heavy matrix which affect the column and interfere with a chromatographic analysis. Results reveal that the tested extracts vary in their compounds. The extract active compounds showed different antibacterial effects. 9-octadecenamide (oleamide) was found in the four tested extracts. Palmitic acid was found in *Nigella sativa*, Onion and Wheat germ extracts. Compounds found in each extracts play an important role in their antibacterial activities. Results revealed that eleven compounds were analyzed in Onion extract, nine compounds in *Nigella sativa* extract, eight compounds in Wheat germ extract and five compounds in garlic extract as shown in Table 3.

Results clearly indicate that no residues of the 91 tested pesticides were found in any of these four tested extracts Table 2. Results indicate that the pesticide residues have no roles in the antibacterial activities of the studied extract and results confirm that the active ingredients found in the extracts have bactericidal activities against the tested strains.

Antimicrobial studies

Results of agar well diffusion test (AWDT) Table 4, Figures 1, 2 and 3 reveal that garlic extract showed the highest antibacterial activities with mean zone of inhibition equals 23.46 mm. On the contrary, *Nigella sativa*

Table 2. List of pesticides tested in the portion of the extracted materials.

No.	Pesticide	No.	Pesticide	No.	Pesticide
1	Alachlor	32	Deltamethrin	63	Imazalil
2	Amitraz	33	Dichlobenil	64	Iprodione
3	Atraton	34	Dicloran	65	Isophenphos
4	Bifenthrin	35	Dicofol	66	Isophenphos-methyl
5	Biphenyl	36	Dieldrin	67	Methoxychlor
6	Bromophos-ethyl	37	Diniconazole	68	Metribuzin
7	Bromophos-methyl	38	Diphenylamine (DPA)	69	Mirex
8	Bromopropylate	39	Ditalimfos	70	Orthophenylphenol (OPP)
9	Cadusafos	40	Endosulfan-alpha	71	Oxadiaxyl
10	Captafol	41	Endosulfan-beta	72	Oxadiazon
11	Captan	42	Endosulfan-sulfate	73	Oxyfluorfen
12	Chlordane-cis	43	Endrin	74	Pentachloroanisole (PCA)
13	Chlordane-trans	44	Ethoxyquin	75	Pentachlorobenzene
14	Chlorfenapyr	45	Etofenprox	76	Permethrin
15	Chlorobenzilate	46	Etridiazole	77	Procymidone
16	Chlorpyrifos	47	Fenarimol	78	Profluralin
17	Chlorothalonil	48	Fenazaquin	79	Propiconazol
18	Chlorpropham	49	Fenitrothion	80	Prothiofos
19	Chlorthal-dimethyl	50	Fenpropathrin	81	Quintozene
20	Chlozolinate	51	Fenvalerate	82	Spiromesifen
21	Cinmethylin	52	Flucythrinate	83	Sulfur
22	Cyanophos	53	Fludioxonil	84	Tecnazene
23	Cyfluthrin	54	Folpet	85	Tefluthrin
24	Cyhalothrin-lambda	55	HCH-alpha	86	Tetradifon
25	Cypermethrin	56	HCH-beta	87	Thiometon
26	Dazomet (Basomid)	57	HCH-delta	88	Triadimefon
27	DDD o.p`-	58	HCH-gamma (Lindane)	89	Triadimenol
28	DDD p,p`-	59	Heptachlor	90	Trifluralin
29	DDE p.p`-	60	Heptachlor-endo-Epoxide	91	Vinclozolin
30	DDT o,p`-	61	Heptachlor-exo-Epoxide		
31	DDT p,p`-	62	Hexachlorobenzene (HCB)		

Table 3. The GC-MSD analysis of the tested extracts.

Onion extract	Garlic extract	Wheat germ extract	*Nigella sativa* extract
Bisabolol oxide A	Tetrasulfide	Tetradecanoic acid (**Myristic acid)**	Tetradecanoic acid (**Myristic acid)**
n-difluoro phosphine dimethyl hydroxyl amide	**Silane** (**monosilane**)	Palmitic Acid -methyl ester	**Palmitic Acid**
3-Eicosene	9-Octadecenamide (**Oleamide)**	**Palmitic Acid**	Ethyl palmitate
Palmitic Acid	Stearoylamide	9,12-Octadecadienoic acid	9,12-Octadecadienoic acid
9,12-Octadecadienoic acid	Vitamine E	9-Octadecenamide **(Oleamide)**	Ethyl linoleate
9,17-Octadecadienal		1,2-Benzenediccaroxylic acid	Ethyl Oleate
Linoleic acid, ethyl ester		Beta-Tecopherol	Stearic acid ethyl ester
Palmitic acid amide		Campesterol	9-Octadecenamide (**Oleamide)**
9-Octadecenamide **(Oleamide)**			Ethyl nonadecanoate
Tetradecanamide			
Cholesteryl alcohol			

Table 4. Antibacterial activities of plant extracts against bacterial reference strains using Agar well diffusion method, results given in (mm).

Bacterial Ref. strain	Extract						
	Onion	Garlic	Mint	Wheat germ	*Nigella sativa*	Mean	CIP
S. aureus NCINB 50080	11	30	10	12	18	16.20	40
B. cereus NCINB 50014	20	23	14	12	10	15.80	34
C. freundi ATCC 8090	12	24	18	18	16	17.60	50
L. innocua ATCC 33090	22	25	20	20	18	21.00	40
E. coli ATCC 11775	12	20	12	18	12	14.80	40
E. coli O157 ATCC 700728	12	26	16	10	14	15.60	40
S. Typhimurium ATCC 13311	20	28	10	30	24	22.40	40
Shigella bodyii ATCC 9207	28	26	30	20	14	23.60	36
Shigella sonnei ATCC 25931	28	20	20	14	22	20.80	40
Shigella flexeneri ATCC 12022	25	22	24	30	20	24.20	30
Ps. aeruginosa NCINB 50067	14	12	20	20	10	15.20	42
K. pneumoniae NCTC 9633	18	25	14	12	14	16.60	26
Proteus mirabilis ATCC 14153	14	24	15	10	12	15.00	30
Mean	18.15	23.46	17.15	17.38	15.69	---	37.54

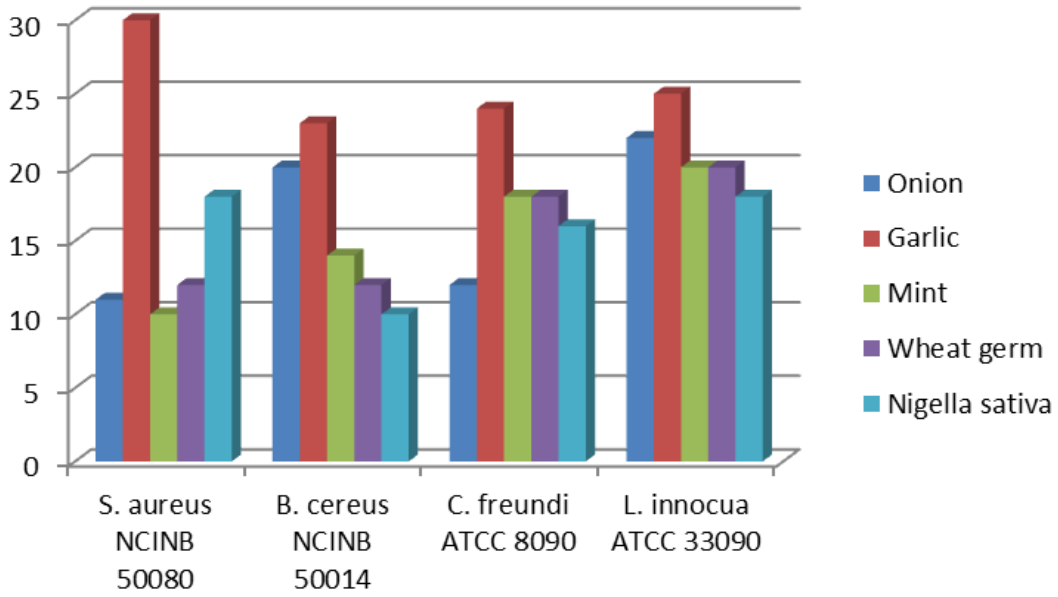

Figure 1. The Agar Well Diffusion Test of the tested extracts against Gram positive reference strains.

showed the least zone inhibition (15.69mm). Among the tested strains, the best results was shown against *Shigella flexeneri* ATCC 12022 (24.2mm), then *Shigella bodyii* ATCC 9207 (23.6mm) followed by *S.* Typhimurium ATCC 13311 (22..4mm) then *L. innocua* ATCC 33090 (21mm).On the other hand *E. coli* ATCC 11775showed the least hindrance of abilities with zone of inhibition 14.8mm then *Proteus mirabilis* ATCC 14153 (15mm) then *Ps. aeruginosa* NCINB 50067 (15.20mm) then *E. coli* O157 ATCC 700728 (15.60mm). Results were confirmed by the results of MIC shown in Table 5 and Figure 4 the best MIC was given against *Shigella flexeneri* ATCC 12022 (2.61 to 10.42 µg/ml). On the other hand, garlic showed the best MIC results range from (2.61 to 5.21 µg/ml).

Results of MIC Table 5 confirm the previous finding of AWDT showing the efficiency of garlic for hindrance of the tested strains with the highest dilution 2.61µg/ml for hindrance of *S. aureus* NCINB 50080 and *S.* Typhimurium ATCC 13311. MIC was 5.21 µg/ml against *B. cereus* NCINB 50014, *C. freundi* ATCC 8090, *L. innocua* ATCC 33090, *E. coli* O157 ATCC 700728,

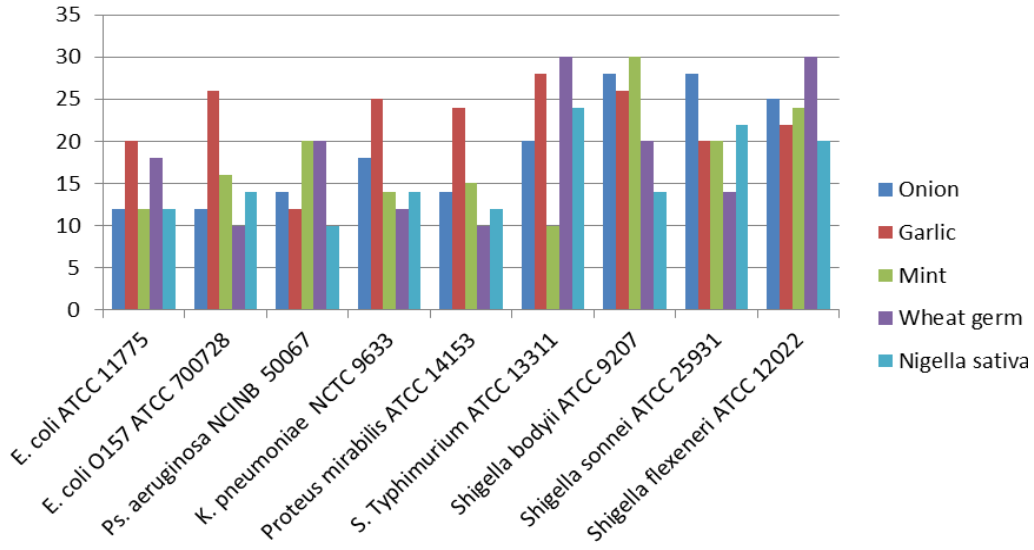

Figure 2. The Agar Well Diffusion Test of the tested extracts against Gram negative reference strains.

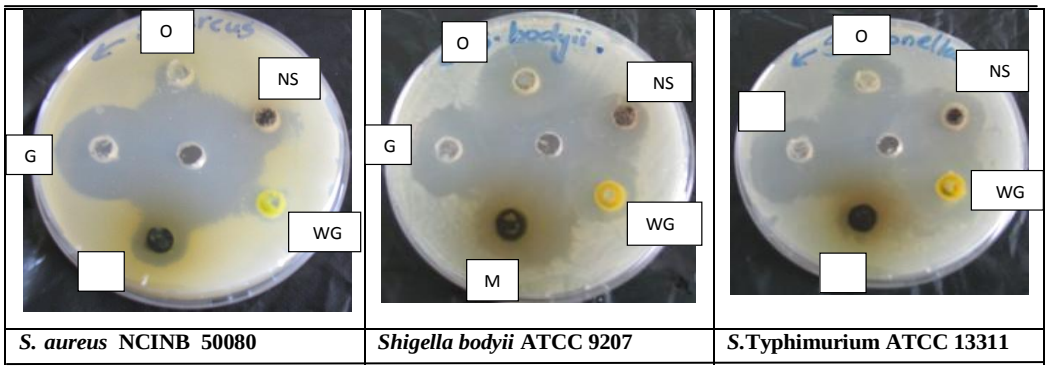

Figure 3. Showing the antimicrobial activities of some of the tested extracts against some of the tested reference strains. O=onion, G=garlic, M=mint, WG=wheat germ, NS=*Nigella sativa, CIP at the center.*

Shigella bodyii ATCC 9207, *Shigella flexeneri* ATCC 12022, *K. pneumoniae* NCTC 9633, *Proteus mirabilis* ATCC 14153.

On the other hand, Onion showed great hindrance capability against *Shigella bodyii* ATCC 9207 and *Shigella sonnei* ATCC 25931 with concentration 2.61 µg/ml followed by *Shigella flexeneri* ATCC 12022 and *L. innocua* ATCC 33090 with concentration 5.21µg/ml. Wheat germ and *Nigella sativa* were effective against *S.* Typhimurium ATCC 13311 with concentration 2.61 and 5.21 µg/ml, respectively. On the other hand wheat germ and mint were effective against *Shigella flexeneri* ATCC 12022 with concentration 2.61 and 5.21 µg/ml.

Results of AWDT and MIC were confirmed by MBC shown in Table 6 and Figures 5; results reveal that garlic extract showed bactericidal effect using high dilution among the five tested extracts, with MBC 5.21µg/ml against *S.aureus* NCINB 50080 and *S.* Typhimurium ATCC 13311. MBC was 10.42 µg/ml against *B. cereus* NCINB 50014, *C. freundi* ATCC 8090, *L. innocua* ATCC 33090, *E. coli* O157 ATCC 700728, *Shigella bodyii* ATCC 9207, *Shigella flexeneri* ATCC 12022, *K. pneumoniae* NCTC 9633, *P. mirabilis* ATCC 14153.

On the other hand, Onion and Mint showed the highest bactericidal activities against *Shigella bodyii* ATCC 9207. Onion was effective against and *Shigella sonnei* ATCC 25931 and Wheat germ showed bactericidal effect against *S.* Typhimurium ATCC 13311 and *Shigella flexeneri* ATCC 12022 with concentration 5.21 µg/ml.

DISCUSSION

Screening of the antibacterial activities of the tested

Table 5. Minimum Inhibitory Conc. of plant extracts against bacterial reference strains compared with Ciprofloxacin.

Strain	Extract					
	Onion	Garlic	Mint	Wheat germ	Nigella sativa	CIP
S. aureus NCINB 50080	166.7	2.61	166.7	166.7	20.84	3.125
B. cereus NCINB 50014	10.42	5.21	83.35	166.7	166.7	1.56
C. freundi ATCC 8090	166.7	5.21	20.84	20.84	41.68	1.56
L. innocua ATCC 33090	5.21	5.21	10.42	10.42	20.84	3.125
E. coli ATCC 11775	166.7	10.42	83.35	20.84	166.7	3.125
E. coli O157 ATCC 700728	166.7	5.21	41.68	166.7	83.35	3.125
S. Typhimurium ATCC 13311	10.42	2.61	166.7	2.61	5.21	3.125
Shigella bodyii ATCC 9207	2.61	5.21	2.61	10.42	83.35	1.56
Shigella sonnei ATCC25931	2.61	10.42	10.42	83.35	5.21	3.125
Shigella flexeneri ATCC 12022	5.21	5.21	5.21	2.61	10.42	3.125
Ps. aeruginosa NCINB 50067	83.35	166.7	10.42	10.42	166.7	3.125
K. pneumoniae NCTC 9633	20.84	5.21	83.35	166.7	83.35	6.25
P. mirabilis ATCC 14153	83.35	5.21	83.35	166.7	166.7	3.125

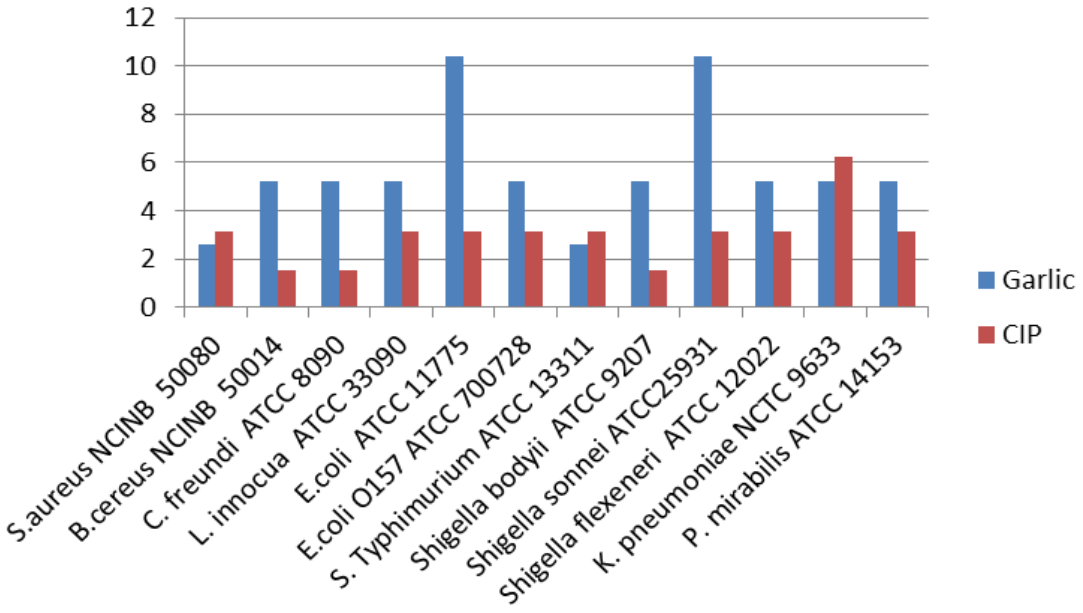

Figure 4. The Minimum Inhibitory Concentration of the garlic extract and Ciprofloxacin against reference strains.

extracts using AWDT, MIC and MBC revealed that garlic extract showed the highest hindrance capability AWDT Table 4, Figures 1, 2 and 3 with mean zone of inhibition equals 23.46 mm. On the contrary, *Nigella sativa* showed the least zone inhibition (15.69 mm). Among the tested strains, the best results was shown against *Shigella flexeneri* ATCC 12022 (24.2 mm), *Shigella bodyii* ATCC 9207 (23.6 mm), *S.* Typhimurium ATCC 13311 (22.4 mm) then *L. innocua* ATCC 33090 (21 mm). On the other hand, *E. coli* ATCC 11775, *Proteus mirabilis* ATCC 14153, *Ps. aeruginosa* NCINB 50067 and *E. coli* O157 ATCC 700728 showed the least hindrance abilities. Results agree with Suree and Pana (2005) who indicated that crude ethanolic herbal extracts showed different degrees of growth inhibition, depending on the tested strains. They added that *E. aerogenes* and *E. coli* were resistant to most of the ethanolic extracts. The present study showed that *E. coli* was one of the most resistant strains among the tested once.

MIC results Table 5 and Figure 4 and MBC (Table 6) and Figures 5 confirmed AWDT results; the best MIC was shown against *Shigella flexeneri* ATCC 12022 with

Table 6. Determination of Minimum Bactericidal Concentration (MBC) of extracts against bacterial reference strains compared with reference drugs.

Strain	Extract					
	Onion	Garlic	Mint	Wheat germ	*Nigella sativa*	CIP
S. aureus NCINB 50080	166.70	5.21	166.70	166.70	41.68	6.25
B. cereus NCINB 50014	20.84	10.42	166.70	166.70	166.70	3.125
C. freundi ATCC 8090	166.70	10.42	41.68	41.68	83.35	3.125
L. innocua ATCC 33090	10.42	10.42	20.84	20.84	41.68	6.25
E.coli ATCC 11775	166.70	20.84	166.70	41.68	166.70	6.25
E.coli O157 ATCC 700728	166.70	10.42	83.35	166.70	166.70	6.25
S. Typhimurium ATCC 13311	20.84	5.21	166.70	5.21	10.42	6.25
S. bodyii ATCC 9207	5.21	10.42	5.21	20.84	166.70	3.125
S. sonnei ATCC25931	5.21	20.84	20.84	166.70	10.42	6.25
S. flexeneri ATCC 12022	10.42	10.42	10.42	5.21	20.84	6.25
Ps. aeruginosa NCINB 50067	166.70	166.70	20.84	20.84	166.70	6.25
K. pneumoniae NCTC 9633	41.68	10.42	166.70	166.70	166.70	12.5
P. mirabilis ATCC 14153	166.70	10.42	166.70	166.70	166.70	6.25

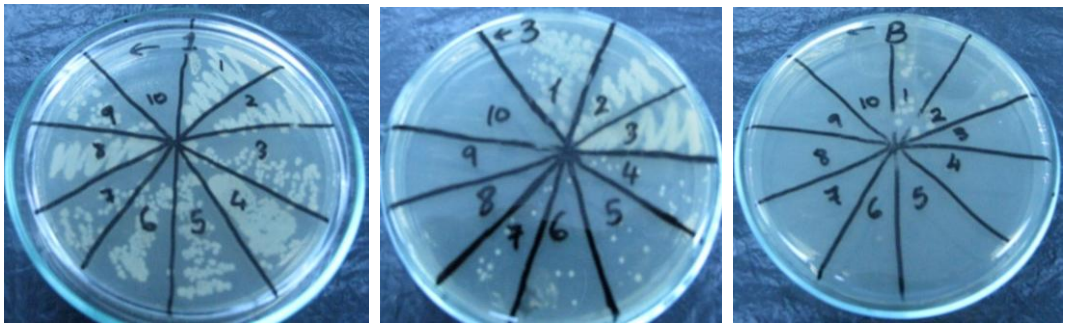

Figure 5. Minimum Bactericidal concentration using onion (a), *Nigella sativa* (b) and garlic (c), respectively against *S. aureus* NCINB 50080.

concentration range from 2.61-10.42 µg/ml. Garlic showed the best MIC results range from 2.61-5.21 µg/ml among the tested strains with exception of *Ps. aeruginosa* NCINB 50067 which was the most resistant strain with MIC 166.70 µg/ml. *E. coli* ATCC 11775 and *S. sonnei* ATCC 25931 were highly resistant to garlic with MIC 20.84 µg/ml. *E. coli* ATCC 11775 was resistant to onion and mint with conc. 166.70 µg/ml. Results agree with Ziarlarimi et al. (2011) who indicated that MIC of the garlic aqueous extract was 5%, but *E. coli* was resistant to the aqueous extracts of onion and mint. Results agree with Bin Shan et al. (2005) who found that *E. coli* was the most resistant strain to the 46 tested extracts.

Suree and Pana (2005) proved that the MIC values of garlic and ginger oils varied depending on the bacterial strains. Garlic extracts have been found to possess antibacterial property against several bacteria including *S. Typhimurium, S. Typhi, E. coli, Bacillus cereus*, *S. epidermidis*, and *S. aureus* (Arora and Kaur, 1999; Johnson and Vaughn, 1969; Saleem and Al-Delaimy, 1982).

The present study shows that highest hindrance abilities of the tested extracts using MIC was shown using concentration 2.61 µg/ml, given with garlic extract against *S. aureus* NCINB 50080 and *S. Typhimurium* ATCC 13311, onion extract against *Shigella bodyii* ATCC 9207 and *Shigella sonnei* ATCC 25931 and wheat germ extract against *S. Typhimurium* ATCC 13311 and *Shigella flexeneri* ATCC 12022. Results match with the findings of Suree and Pana (2005) who indicated that MIC results showed that *S. Typhimurium* was the most susceptible strain to most of the ethanolic extracts. Also, results agree with Bin Shan et al., 2005 who found that *S. aureus* was the most sensitive to the 46 extracts. The highest sensitivity of *S. aureus* may be due to its cell wall structure and outer membrane (Zaika, 1988).

GC-MS analyzed indicated that the number of compounds analyzed from each extract have no role in the antibacterial activities as garlic contains only five compounds but it was the most effective extract against the tested strains. While onion contains eleven compounds, *Nigella sativa* (nine compounds) and wheat germ extract (eight compounds) showed lower bactericidal activities. The present work shows that antibacterial activity is closely related to the type of ethanolic extracts. Other researchers have reported that compounds from different plant sources could inhibit various foodborne pathogens (Nychas, 1995; Prashanth et al*.,* 2001; Kim et al*.,* 2005). Results clearly indicated that no residues of the 91 tested pesticides were found in any of these 4 tested extracts (Table 2). Thus the pesticide residues have no roles in the antibacterial activities of the studied extract which confirm that the active ingredients found in the extracts have bactericidal activities against the tested strains. Sheikh et al*.* (2013) showed that onion samples were contaminated with profenofos, and enosulfan. Recent study showed that mint and onion were contaminated with pesticide residues with an incidence reach 100 and 33.3%, respectively. The pesticides detected in all samples were chlorpyrifos, chlorpyrifos-methyl, malathion, cypermethrin, l-cyhalothrin and sulfur Farag et al*.* (2011).

Results of AWDT and MIC were confirmed by MBC shown in Table 6 and Figures 5. The highest bactericidal effect with concentration 5.21 µg/ml was given with garlic against *S. aureus* NCINB 50080 and *S. Typhimurium* ATCC 13311, onion against *Shigella bodyii* ATCC 9207 and *Shigella sonnei* ATCC 25931, wheat germ against *S. Typhimurium* ATCC 13311 and *Shigella flexeneri* ATCC 12022 and mint against *Shigella bodyii* ATCC 9207. GC-MS revealed that Oleamide is the predominant compound found in the four tested extracts, which must have an important role in bacterial inhibition activities. Palmitic acid found in *Nigella sativa*, onion and wheat germ extracts may play an important role in the antibacterial activities of the tested extracts. Crude ethanolic herbal extracts showed different degrees of growth inhibition, depending on the tested strains. The mechanisms of action of each compound against various bacteria are very complicated. Results agree with Ultee et al*.* (1999) and Lambert et al*.* (2001) who proved that the antimicrobial activities of compounds may involve multiple modes of action as degrading the cell wall, interacting with the composition and disrupting cytoplasmic membrane. Raccach (1984) found that compounds may cause damaging of membrane protein, interfering with membrane integrated enzymes, causing leakage of cellular components, coagulating cytoplasm, depleting the proton motive force, changing fatty acid and phospholipid constituents, impairing enzymatic mechanisms for energy production and metabolism, altering nutrient uptake and electron transport (Taniguchi et al*.*, 1988). All of these mechanisms are not separate targets; some are affected as a consequence of another mechanism being targeted. The mode of action of antimicrobial agents depends on the type of microorganisms and is mainly related to their cell wall structure and the outer membrane arrangement. Plants including spices and herbs contain complex compounds. The mechanisms of action of each compound against various bacteria are very complicated (Kalemba and Kunicka, 2003; Burt, 2004). The main component of garlic responsible for its antibacterial activities is allicin. The enzyme allicinase converts allicin into these volatile compounds, once garlic is damaged by crushing or cutting, most antimicrobial agents are able to modify bacterial cell membranes and this leads to leakage and autolysis, thereby preventing growth and causing cell death (Lixin Xia and Ng, 2005). Garlic has been found to have a morphological effect on various bacterial cells, resulting in changes to the outer surfaces, internal properties as well as behavior of the cells (Ankri and Mirelman, 1999).

Conclusions

The degree of antibacterial property of tested ethanolic extracts can be put in the following order: garlic > wheat germ > mint > onion > *Nigella sativa*. These spices may be selected for use as potentially useful anti-bacterial agents in fermented meat products and other foods, depending upon the desired flavor of the products. *S. flexeneri* ATCC 12022, *S. bodyii* ATCC 9207, *S. Typhimurium* ATCC 13311 and *L. innocua* ATCC 33090 are the most vulnerable to crude ethanolic extracts, while *E. coli* ATCC 11775, *Pr. mirabilis* ATCC 14153 and *Ps. aeruginosa* NCINB 50067 were the most resistant. This study reported a highly positive relationship between antibacterial activity and total ethanolic content in a large number of herb extracts. This suggested that the ethanolic compounds might significantly contribute to their antibacterial activity. The present study also demonstrated that garlic among many of the ethanolic extracts tested possessed strong antibacterial activity. They could be a potential source for inhibitory substances against some foodborne pathogens. Garlic, wheat germ and mint extracts showing high antibacterial activity may be subjected to future studies of synergism, compatibility and activity in food-processing systems against specific pathogens of animal origin.

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