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Screening for the Antimicrobial Activities of Alcoholic and Aqueous Extracts of Some Common Spices in Egypt

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Abstract: The extracts of 17 spices commonly used in Egypt as food condiments were screened for their antimicrobial activities against 7 standard microbial strains (*S. aureus, B. subtilis, E. coli, P. aeruginosa*, *S. typhi*, *C. albicans* and *A. niger*). Among the tested alcoholic extracts only 3 (clove, thyme and cinnamon) showed broad spectrum antimicrobial activities. Garlic juice too showed broad spectrum antimicrobial activities. Only 4 alcoholic extracts (black pepper, safflower, coriander and myrrh) had no antimicrobial activities. The remaining extrcts (53%) showed only anti-Gram positive bacteria activities. None of all tested aqueous extracts showed antifungal activity with lower antibacterial activities compared to their alcoholic ones. The antibacterial effect of alcoholic extracts was not concentration dependant, however their antifungal effect was concentration dependant. Interestingly, both alcoholic and aqueous extracts of cinnamon and alcoholic extract of clove showed the highest antiviral activity where the $TCID_{50}$ was reduced by about 1 log cycle. This was followed by the aqueous extract of clove, garlic juice then aqueous extract of thyme, while both alcoholic and aqueous extracts of liquorice had no antiviral activity.

Key words: Foodborne Diseases · Spices · Antimicrobial Activity · Antiviral Activity

INTRODUCTION

Foodborne diseases are currently one of the main emerging problems worldwide. For instance, Acute diarrheal illness is very common worldwide and estimated to account for 1.8 million childhood deaths annually, predominantly in developing countries [1]. Moreover, acute foodborne illnesses cost a developed country such as United States about 152 $ billion per year in healthcare, workplace and other economic losses [2].

No doubt that change in the life style and the consequent changes in dietary habits increased the demand for ready-to-eat products, also, cold distribution of perishable food can help, but it cannot guarantee the overall safety and quality of the product. Moreover, the expansion in the industry of fruits and vegetables trade have been followed by increasing reports of food borne pathogens because of the presence of pathogens in raw materials [3, 4].

Recently several methods have been attempted to overcome these microbial threats. However, most of these methods still suffer from many drawbacks. For instance, food processing technologies such as chemical preservatives cannot eliminate food pathogens such as *Listeria monocytogenes* or delay microbial spoilage totally in addition to its questionable effect on the health [5].

Hence, aromatic plants that have been used in folk medicine as antimicrobial agents since ancient times [6, 7] could represent a promising solution and wise tool. Interestingly, in addition to their flavoring effects, some spices and herbs have antimicrobial effects on plant and human pathogens [8]. Spices and herbs were originally added for improving taste and also can naturally and safely improve shelf life of food products [9]. Studies done previously has confirmed that garlic, onion, cinnamon, cloves, thyme, sage and other spices inhibit the growth of both Gram positive and Gram negative food borne pathogens or spoilage bacteria, yeast and molds [10, 11].

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In this prospective the main aim of this study was to investigate the antimicrobial activities of extracts of some selected herbs and spices that commonly used in Egypt. A comparison between 2 practically possible extracts (alcoholic and aqueous) was attempted. The antimicrobial activities were studied against a wide range of relevant microbial markers and evaluation of their antimicrobial spectrum including the antiviral activities was done.

MATERIALS AND METHODS

Preparation of the Spices Extracts: A total of 17 common spices in Egypt were selected based on previous literature or the publicity in the Egyptian market (Table 1). The alcoholic extracts were prepared by double maceration in 95% ethanol for 4 d. then for another 2 d. with frequent shaking [12]. The collected extracts were concentrated using Rota vapor device (BUCHI 011) at 65 °C then the concentrated extracts were air-dried. From each extract, stock solutions were then prepared by dissolving in 95% ethanol.

Aqueous extracts were prepared by maceration in distilled water for 4 d. at 4 °C with intermediate shaking [13]. Then the extracts were filtered through cotton then kept at 4 °C till use. The yielded extracts were lyophilized then reconstituted with sterilized distilled water when needed for testing. Garlic bulbs (*Allium sativum*), was only prepared as fresh juice under aseptic conditions by crushing and squeezing the fresh bulbs through gauze. The juice was prepared at a concentration of 30 % (v/v).

Table 1: A list with the Latin names, common names and used part of the tested 17 spices

Latin name	Common name	Part used
Thymus vulgaris	Thyme	dried leaves
Cinnamomum cassia	Cinnamon	dried bark
Syzigium aromaticum	Clove	dried buds
Allium sativum	Garlic	fresh bulbs
Glycyrrhiza glabra	Liquorice	dried roots
Eucalyptus globules	Eucalyptus	dried leaves
Curcuma longa	Curcuma	dried rhizomes
Coriandrum sativum	Coriander	dried fruits
Nigella sativa	Nigella (Black seed)	dried seeds
Zingeber officinale	Ginger	dried rhizome
Peperium nigrum	Black pepper	dried fruits
Cuminum cyminum	Cumin	dried fruits
Rosemarinus officinale	Rosemary	dried leaves
Origanum majorana	Marjoram	dried leaves
Capsicum annum	Capsicum	dried pods
Commiphora myhrra	Myhrr	Dried oleo gum resin
Catharansus tinctorius	Safflower	dried leaves

Prior to testing, all prepared extracts (alcoholic and aqueous) were sterilized by filtration with 0.2 µm syringe filter.

Microorganisms: Seven standard microbial strains were used as follow, 2 Gram positive (*S. aureus ATCC 6538, B. subtilis ATCC CC33)*, 3 Gram negative bacteria (*E. coli ATCC 5087, P. aeruginosa ATCC 35664*, *S. typhi ATCC 9027*), one yeast strain (*C. albicans ATCC 60193*) and one mold strain (*A. niger ATCC 1718109*). All of these strains were maintained using seed-lot culture maintenance technique with no more than five passages. Bacterial strains were cultivated and tested on nutrient agar medium at 37 °C for 24 h, while yeast and mold strains were cultivated and tested on Sabouraud's 's dextrose agar medium at 25°C for 2 d. and 4-7 d. respectively.

Besides, a reference viral strain (Adenovirus serotype-7, AdV-7) was used and cultivated in Vero cell line (derived from the kidney of a normal, adult, African green monkey) maintained in medium-199 with Earle's salts (E-199 medium) and heat-inactivated foetal bovine serum at 37 °C for 24-72 h at 5% CO_2 atmosphere [14]. All these standard strains were kindly provided by VACSERA Company, Dokki, Giza.

Determination of the Cytotoxic Effect of the Extracts: Cytotoxicity testing was performed using 96-well flat bottom tissue culture plates (Corning® Costar® cell culture plates CLS3595-96 well, flat bottom, tissue-culture treated, Sigma Aldrich) according to Meager [14] using Vero cell line and E-199 medium supplemented with 10% heat inactivated foetal bovine serum (FBS). Controls; (cells with no extract) and (cells with the solvent, 95% ethanol) were conducted in quadruplicates. All plates were then incubated at 37 °C in a 5% CO_2 atmosphere. After 24 h., the cytotoxic effect was determined microscopically (loss of cell monolayer's, rounding). The safe concentration was estimated as the highest concentrations of the extracts which had no effect on the integrity of the monolayer's of the cells.

Screening of the Spices Extracts for Antimicrobial Activity: *Determination of antibacterial and antifungal activities:* Preliminary antimicrobial spectrum was determined using disc diffusion Kirby-Bauer method [15]. Each alcoholic extract was tested at 3 concentrations (100, 200 and 300 mg/ml). The inoculums sizes used from each of tested standard microbial strain were (10^5-10^6 CFU/10 µl). Positive controls were conducted

using references antimicrobials (Amoxycillin (AM) 25µg for Gram positive bacteria, Cephradine (CV) 25µg for *E. coli* and *S. typhi*, Gentamicin (G) 10µg for *P. aeruginosa* and Amphotericin B (Ap) 100 units for fungal strains). Negative control was conducted using discs loaded with 20 µl of 95% ethanol alone.

Determination of the Minimum Inhibitory Concentration (MIC): The MIC of the relevant extracts was determined using agar dilution technique [16]. Two-fold serial dilutions ranging from 100 mg/ml to 100 µg/ml, inoculums size of 10^5-10^6 CFU/ 10 µl) and incubation at 37°C for 24 h for bacteria and at 25-30°C for 2 d. for yeast and mold. MIC was determined as the lowest concentration inhibited the growth on nutrient agar plates for bacteria or on Sabouraud's dextrose agar for yeast and fungi.

Determination of the Antiviral Activities: The protective effect of the tested relevant extracts was tested against adenovirus type 7 (AdV-7). The assay was performed according to Meager [14] and similar to the cytotoxicity testing. Vero cell line was prepared as in case of cytotoxicity testing and prior to antiviral testing, plates which were pre-checked and only those having integral cell monolayer's were initially treated with the highest non-toxic concentrations of the extracts and incubated for 24 h at 37°C in a 5% CO_2 atmosphere. These plates were further treated with 10-fold serially-diluted AdV-7 (initial concentration of $10^{6.3}$ $TCID_{50}$ "Tissue culture infective dose that cause CPE for 50 % of cells"/0.1 ml) in E-199 medium according to Del Barrio and Parra [17]. The virus cytopathic effect was determined and expressed in $TCID_{50}$ / 0.1 ml. Negative controls (cells only) and positive control (cells and AdV-7 with no extracts) were conducted. All plates were incubated at 37°C in a 5% CO_2 atmosphere for 24-72 h.

The CPE was checked at different time intervals (24-72h.); microscopically (loss of cell monolayer's, rounding) and with MTT dye according to Javed *et al.* [18] using microtiter plate reader (stat fax 2100 automatic micro plate reader) at 545 nm. The results were expressed as reduction in % infectivity and compared to that of the virus control (with no extract). Blank was conducted using commercially available interferon reference (Interferon alfa 2b-Pegintron120 µg/ml Schering-plough). All experiments were conducted in quadruplicates under aseptic conditions. Accordingly, the recorded CPE from different viral dilutions, ($TCID_{50}$) was calculated using Reed-Muench formula [19].

RESULTS

Screening of the Antibacterial and Antifungal Activities: In general, among the tested 16 alcoholic extracts only 3 (19%) (clove, thyme and cinnamon) showed broad spectrum antimicrobial activities against all tested microbial markers (Table 2). Similarly garlic juice showed braod spectrum antimicrobial actitivities. On the other hand 4 alcoholic extracts (black pepper, safflower,

Table 2: The recorded relative activity % and MICs of the tested of alcoholic and aqueous extracts of spices and garlic juice against 7 tested microbial markers

		S. aureus ATCC 6538		*subtilis* B. ATCC CC33		*E. coli* ATCC 5087		*S. typhi* ATCC 9027		*P. aeruginosa* ATCC 35664		*C. albicans* ATCC 60193		*A. niger* ATCC 1718109	
		RA%[a]	MIC[b]	RA%[a]	MIC[b]	RA%[a]	MIC[b]	RA%[a]	MIC[b]	RA%[a]	MIC[b]	RA%[a]	MIC[b]	RA%[a]	MIC[b]
Alcoholic	Clove	40±1	0.8	62±4	0.9	31±2	1.5	28±0	2	29±0	3	63±5	0.7	87±7	0.7
Extracts	Cinnamon	25±1	2	34±2	2	31±2	3	NS	3	29±1	4	76±7	3	63±7	3
	Thyme	43±3	0.3	57±3	0.35	30±0	0.8	31±0	0.9	27±0	2.5	48±7	0.6	50±3	0.7
	Liquorice	33±4	ND	36±2	ND	NS	ND	NS	ND	NS	ND	NS	ND	NS	ND
Garlic Juice[c]	29±0	2.5	31±2	5	38±2	5	37±2	5	29±0	15	98±7	5	107±5	10	
Aqueous	Clove[d]	40±0.5	ND	48±1	ND	43±0.5	ND	34±0	ND	41±0.5	ND	NS	ND	NS	ND
Extracts	Cinnamon[e]	43±0.5	ND	48±2	ND	39±0.5	ND	45±1	ND	52±0.5	ND	NS	ND	NS	ND
	Thyme[e]	37±0.5	ND	NS	ND	NS	ND	NS	ND	NS	ND	NS	ND	NS	ND
	Liquorice[e]	37±0.5	ND	62±1	ND	NS	ND	NS	ND	41±0.5	ND	NS	ND	NS	ND

(a) RA% = Relative activity %, which has been calculated relative to the applied reference antimicrobial agent as follow:

[Mean of zones of inhibition diameters (mm) of extract / Mean of zones of inhibition diameters (mm) of reference antimicrobial))*100

(a) MIC is the determined minimum inhibitory concentration (mg/ml) using agar dilution technique

(b)Garlic was just prepared as fresh juice (neither alcoholic nor aqueous extracts of this spice) and the tested concentration was 50 mg/ml

(c)The aqueous extract of clove was tested at 100 mg/ml

(d)These aqueous extracts were tested at 200 mg/ml

(e)NS means not sensitive (no antimicrobial activity was recorded)

(f)ND means not determined

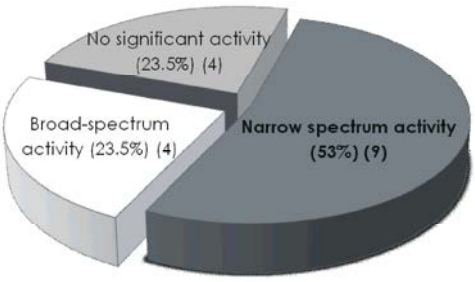

Fig. 1: The recorded number and % of the alcoholic extracts of the tested spices and garlic juice showing different spectra of antimicrobial activities against the tested 7 microbial markers.

coriander and myrrh) had no or slight activity against any of the tested strains (data not shown). The remaining extracts (53%) were only active against G+ bacterial strains at all tested concentrations (Fig. 1). Interestingly, the 4 extracts possessing broad spectrum activities showed relatively better activities against tested yeast and mold strains (Table 2). Almost all alcoholic extracts and garlic juice had almost the same order of magnitude of relative activities againts tested bacterial markers (Table 2). However, clove and thyme showed slightly better inhibitory activity against tested Gram positive bacteria.

Thyme alcoholic extract had the lowest recorded MIC followed by clove extract. The highest MIC recorded for alcoholic extracts was this of cinnamon (2-4 mg/ml). Garlic juice had higher MIC than all tested alcolic extracts (Table 2). Interestingly, *P.aeruginosa* showed the highest resistance to all extracts, while Gram positive strains were the most sensitive. Based on the previous data, extracts showed only broad spectrum were tested as aquoeous extracts forms. None of all tested aqueous extracts (clove, cinnamon, thyme and liquorice) showed antifungal activity (Table 2). Additionally the recorded antibacterial activities were lower than those recorded for the alcoholic extracts of these spices (Table 2).

Interestingly, increasing the concentration of the tested extracts had no remarkable effect on the recorded antibacterial activities for all extracts (Table 2). For instance increasing the extract concentrations from 100 to 300 mg/ml had no impact on the antibacterial activity against *P.aeruginosa* (Fig. 2a). On the other hand, increasing the concentrations of alcoholic extracts of clove, cinnamon and thyme showed a remarkable increase in antifungal effect on the tested yeast and mold strains

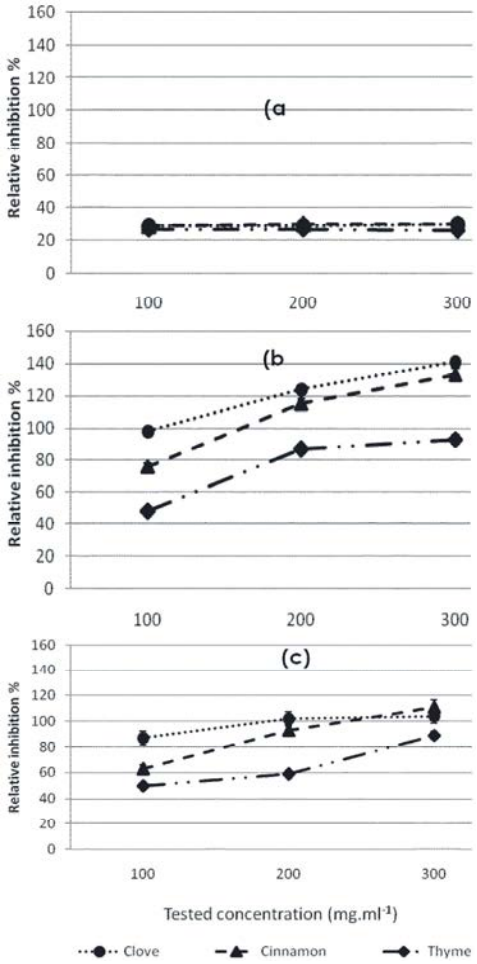

Fig. 2: The recorded impact of increasing the concentrations of the tested alcoholic extracts on the relative activity % against *Pseudomonas aeruginosa* (●); *Candida albicans* (♦) and *Aspergillus niger* (▲).

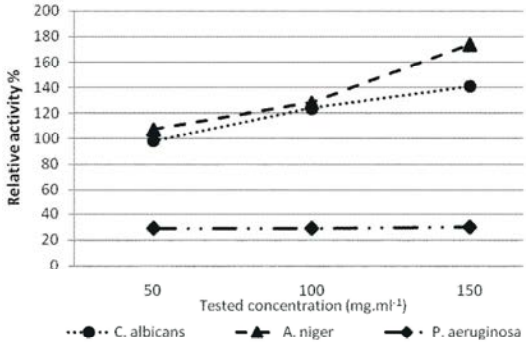

Fig. 3: The recorded impact of increasing the concentrations of the tested garlic juice on the relative activity % against *Pseudomonas aeruginosa* (●); *Candida albicans* (♦) and *Aspergillus niger* (▲).

Table 3: The recorded highest non-toxic concentrations and the change in viral infectivity in terms of log cycle reduction or increase compared to the viral control infectivity of alcoholic and aqueous extracts and garlic juice on Vero cell line

	Highest safe concentration (mg/ml)				
Extract type	Thyme	Clove	Cinnamon	Liquorice	Garlic juice
Alcoholic	0.25	0.063	0.125	0.218	NA[a]
Aqueous	0.218	0.156	0.078	1	0.39[b]
	[c] Number of log cycle changed in the TCID50 / 0.1 ml^{-1}				
Alcoholic	+ 0.2	-1.1	-1.3	0	ND
Aqueous	-0.3	-0.7	-1	0	-0.5

(a) NA means not applicable.

(b) Garlic was perpared as a juice not as aqueous extract.

(c) (+) means increased infectivity, (-) means reduced infectivity

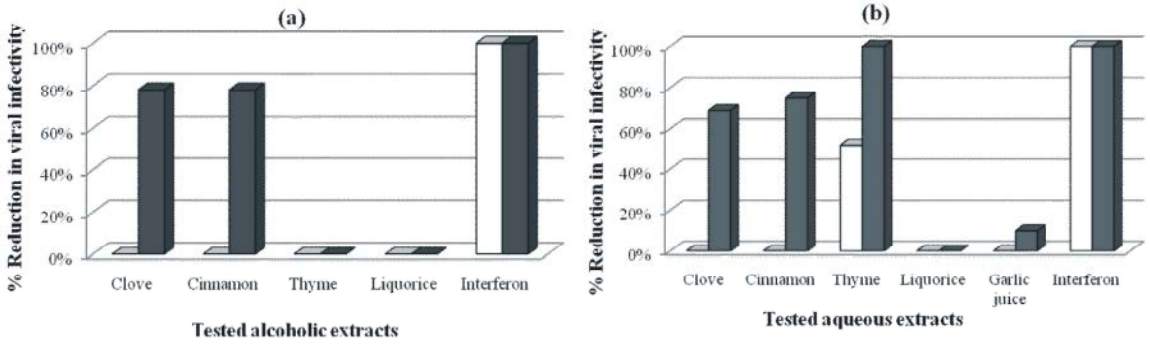

Fig. 4: The reduction % in viral infectivity caused by tested alcoholic extracts and interferon (a) and aqueous extracts and garlic juice and interferon (b) at both tested dilutions of adv-7 virus 10^4 (white bars) and 10^5 (grey bars) of the initial virus titer ($10^{6.3}$ $TCID_{50}$ /0.1 ml)

(Fig. 2b,c). Similarly, increasing the concentration of the tested garlic juice from 50-150 mg/ml1 had no effect on the antibacterial activities but increased the antifungal effect (Fig. 3).

Determination of the Antiviral Activity: Initially the cytotoxic effect of all prepared extracts were tested and estimated. Generally, Aqueous extracts of both clove and liquorice were safer than their corresponding alcoholic extracts (Table 3). On the contrary, the highest safe concentration of alcoholic extract of cinnamon was 5 times higher than this for aqueous one (Table 3). Garlic juice had the second highest safe concentration (Table 3). There was no big difference between the safe concentrations of alcoholic and aqueous extracts of thyme (Table 3).

Initially, interferon showed the best antiviral activity where the $TCID_{50}$ was reduced by about one log cycle at virus dilution of 10^4 (data not shown). The highest protective effect against the tested viral infection was shared by 3 extracts (alcoholic and aqueous extracts of cinnamon and alcoholic extract of clove) where these extracts were able to reduce the $TCID_{50}$ by almost 1 log cycle (Table 3). This was followed by the aqueous extract of clove and garlic juice where the $TCID_{50}$ was reduced by 0.7 and 0.5 log cycle respectively. Both alcoholic and aqueous extracts of liquorice showed negligible antiviral activity. Although aqueous extract of thyme reduced the $TCID_{50}$ by 0.3 log cycle, its alcoholic extract increased $TCID_{50}$ by 0.2 log cycle (Table 3).

When these recorded data was compared to those collected using MTT dye method, interferon still showed the best antiviral activity at both tested viral titers (Fig. 4). Although alcoholic extract of thyme had no antiviral activities (Fig. 4a), the aqueous extract of thyme was the only one showed protective activity when the virus was used at a dilution of 10^4 and this effect was increased to more than 90% at virus dilution of 10^5 (Fig. 4b). Both alcoholic and aqueous extracts of cinnamon and alcoholic extract of clove showed almost the same order of magnitude of protective activity where the reduction of infecetivity was around 80% using virus dilution of 10^5 (Fig. 4a,b). Yet, alcoholic and aqueous extracts of liquorice in addition to garlic juice had no antiviral activities (Fig. 4a,b).

Hence and in general, alcoholic extracts of cinnamon and clove showed the best antiviral activity followed by their aqueous extracts, garlic juice and aqueous extracts of thyme were less active while both aqueous and alcoholic extracts of liquorice were inactive.

DISCUSSION

Antimicrobial Activites of Alcoholic Extracts: Most of the tested extract (75 %) showed antibacterial activities against Gram positive bacteria, however, only 4 extracts had broad spectrum antibacterial activities. Indeed, Gram negative bacteria known to be more resistant to antibiotics than Gram positives ones. For instance, the resistance could be due to the permeability barrier provided by the cell wall or to the membrane accumulation mechanism [20].

In particular, clove, thyme and cinammon alcoholic extracts had broad spectrum antimicrobial activies especially against Gram positive bacteria and tested fungi. Several previous stuidies [21-24] have reported broad spectrum activities for these extracts. Morover, Ponce *et al.* [25] reported that the main antimicrobial activities of thyme, clove and cinnamon are due to the phenolic compounds (mainly, Eugenol, carvacrol and thymol). Interestingly, Gupta *et al.,* [22] have reported that Pseudomonas aeruginosa is the least sensitive to the tested clove extract, which is in agreement with the results recorded in the present study.

In the present study, garlic was prepared as fresh juice, it was reported that antimicrobial activity of garlic is attributed to its key component allicin, which is a volatile and unstable molecule [26]. Garlic juice showed broad spectrum antimicrobial activities but the strongest effect was as antifungal against the tested fungal strains and this effect was concentration dependant (antifungal effect increased by increasing the concentration). This is in agreement with shams-Ghahfarokhi *et al*. [27] who have reported strong antifungal activity and that garlic aqueous extract was found to be able to inhibit the growth of all fungi tested in a dose-dependent manner. Garlic juice also showed good activity against tested Gram positive bacteria. Similarly, Shin *et al*. [28] have reported similar spectrum when evaluated the antimicrobial activity of garlic juice powder.

Antimicrobial Activities of Aqueous Extracts: None of the tested aqueous extracts showed antifungal activity. This is in disagreement with Thanaboripat *et al.* [29] who reported antifungal activity of aqueous extract of clove on *A. flavus* growth. However, this variation may be attributed to differences in concentration of the active chemical inhibitors and in their relative solubility in water [30]. On the other hand, clove and thyme aqueous extracts were active against all bacterial strains as their alcoholic ones. It was reported that clove aqueous extract showed broad spectrum antibacterial activity against all tested bacterial markers [31].

Although alcoholic extract of cinnamon showed broad spectrum antimicrobial activities, its aqueous extract had no antimicrobial activities except aganist *S.aureus*. This may be attributed to what have been reported by Cowan [7] who reported that the main activity of cinnamon is mainly due to its content of eugenol and cinnamaldehyde. These compounds are preferably dissolved in ethyl alcohol more than in water [32].

In general, aqueous extracts showed less activity than ethanol extracts, which may be explained in the light of what have been reported by De Boer *et al*. [33] that the same active substances are present in water extracts, but in lower concentrations and/or that some active substances were more soluble in organic solvents and, therefore, not present in water extracts.

The Antiviral Activity: Alcoholic extracts of cinnamon and clove showed the highest antiviral activity among the tested extracts followed by their aqueous extracts. Yet these activities were lower than the tested reference antiviral (interferon). Previosu studies have reported an antiviral effect of cinnamon extracts and these studies attributed this activity to its content of cinnamaldehyde [34].

Tragoolpua and Jatisatieur [35] have reported that clove oil had antiviral effect and attributed this effect to its content of eugenol. Interestingly, alcoholic extracts of both cinnamon and clove were better antiviral than their corresponding aqueous ones to some extent which may be attributed to the principal active compounds of cinnamon and clove: cinnamaldehyde and eugenol respectively and which are preferably dissolved in ethyl alcohol more than in water [32]. On the other hand, garlic juice showed weak antiviral activity (reduced the viral infectivity by only 0.5 log cycle) which is in agreement with Chen *et al*. [36] who reported that lyophilized powder solution of garlic and onions showed little activity against adenoviruses type-41 and type-3.

Although Reichling [37] have reported that essential oils, derived from aromatic medicinal plants such as thyme, have been reported to be active against viruses, in the present study aqueous extract of thyme was less

active and its alcoholic extract had no activity at all. Similarly, Unexpected results (no antiviral activity) were recorded for both aqueous and alcoholic extracts of liquorice, although Fiore *et al.* [38] have reported that liquorice extract inhibited several DNA and RNA viruses. These may be attributed to difference in extraction methods.

Concluseively, 4 extracts (3 alcoholic; thyme, cinammon and clove in addition to garlic juice) showed broad spectrum antimicribial activities. These activities were concentration depnedant against tested yeast and mold but not against tested bacterial indicators. Additionally, both alcoholic and aqueous extracts of cinnamon and alcoholic extract of clove showed good antiviral activity. These extracts shall provide an effective and safe alternative for food presenvation.

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REFERENCES

1. WHO, 2008. Foodborne disease outbreaks: Guidelines for investigation and Control. ISBN 9789241547222.
2. CDC (Centre for Diseases Control and Prevention), 2012. Budget Changes for Environmental Public Health (http://www.cdc.gov/nceh/information/2012budget.htm)
3. Li, A., Y. Zhu, X. He, X. Tian, L. Xu, W. Ni and P. Jing, 2008. Evaluation of antimicrobial activity of certain Chinese plants used in folkloric medicine. World Journal of Microbiology and Biotechnology, 24: 569-572.
4. Li, H., M. Tajkarimi and B.I. Osburn, 2008. Impact of vacuum cooling on Escherichia coli O157:H7 infiltration into lettuce tissue. Applied and Environmental Microbiology, 74: 3138-3142.
5. Gutierrez, J., C. Barry-Ryan and P. Bourke, 2009. Antimicrobial activity of plant essential oils using food model media: Efficacy; synergistic potential and interactions with food components. Food Microbiology, 26: 142-150.
6. Grayer, R.J. and J.B. Harborne, 1994. A survey of antifungal compounds from higher plants; 1982-1993. Phytochemistry, 37: 19-42.
7. Cowan, M.M., 1999. Plant products as antimicrobial agents. Clinical Microbiology Review, 12: 564-582.
8. Brandi, G., G. Amagliani, G.F. Schiavano, M. De Santi and M. Sisti, 2006. Activity of Brassica oleracea leaf juice on food borne pathogenic bacteria. Journal of Food Protection, 69: 2274-2279.
9. Holley, R.A. and D. Patel, 2005. Improvement in shelf-life and safety of perishable foods by plant essential oils and smoke antimicrobials. Food Microbiology, 22: 273-292.
10. Paster, N., M. Menasherov, U. Ravid and B. Juven, 1995. Antifungal activity of oregano and thyme essential oils applied as fumigants against fungi attacking stored grain. Journal of Food Protection, 58: 81-85.
11. Snyder, O.P., 1997. Antimicrobial effects of spices and herbs. Hospitality Institute of Technology and Management. St. Paul; Minnaesota. http: //www. hitm.com/Documents/Spices.html.
12. Nanasombat, S. and P. Lohasupthawee, 2005. Antibacterial activity of crude ethanolic extracts and essential oils of spices against Salmonellae and other enterobacteria, kmitl Science Technology Journal, 5: 527-538.
13. Ekpo, M.A. and P.C. Etim, 2009. Antimicrobial activity of ethanolic and aqueous extracts of Sida acuta on microorganisms from skin infections. Journal of Medicinal Plants Research, 3: 621-624.
14. Meager, A., 2010. Assays for Antiviral Activity. In: Methods in Molecular Biology; 249: Cytokine Protocols Ed., M. De Ley. Humana Press Inc.; Totowa; NJ., pp: 121-134.
15. Bauer, A.W., W.M.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic sensitivity testing by a standardized single disk method. American Journal of Clinical Pathology, 45: 493-496.
16. CLSI (Clinical and Laboratory Standards Institute), 2011. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational Supplement. Wayne; Pennsylvania 19087 USA.
17. Del Barrio, G. and F. Parra, 2000. Evaluation of the antiviral activity of an aqueous extract from Phyllanthus orbicularis (Short communication) Journal of Ethnopharmacology, 72: 317-322.
18. Javed, T., U. Ashfaq, S. Riaz, S. Rehman and S. Riazuddin, 2011. *In vitro* antiviral activity of Solanum nigrum against Hepatitis C Virus. Virology Journal, 19: 8-26.
19. Reed, L.J. and H. Muench, 1938. A simple method of estimating fifty percent endpoints. American Journal of Hygiene, 27: 493-497.

20. Ceylan, E. and D.Y.C. Fung, 2004. Antimicrobial activity of spices. Journal of Rapid Methods and Automation in Microbiology, 12: 1-55.
21. Braga, P.C., M. Dal-Sasso, M. Culici and M. Alfieri, 2007. Eugenol and thymol; alone or in combination; induce morphological alterations in the envelope of Candida albicans. Fitoterapia, 78: 396-400.
22. Gupta, C., A.P. Garg, R.C. Uniyal and S. Gupta, 2009. Comparison Of Antimicrobial Activities Of Clove Oil and Its Extract On Some Food Borne Microbes. The Internet Journal of Microbiology, pp: 7.
23. Tayel, A.A. and W.F. El-Tras, 2009. Possibility of Fighting Food Borne Bacteria by Egyptian Folk Medicinal Herbs and Spices Extracts. Journal of Egypt Public Health Association, 84: 21-32.
24. Bayoub, K., T. Baibai, D. Mountassif, A. Retmane and A. Soukri, 2010. Antibacterial activities of the crude ethanol extracts of medicinal plants against Listeria monocytogenes and some other pathogenic strains African Journal of Biotechnology, 9: 4251-4258.
25. Ponce, A.G., R. Fritz, C. del-Valle and S.I. Roura, 2003. Antimicrobial activity of essential oils on the native microflora of organic Swiss chard. Lebensm. Wiss. u. Technol., 36: 679-684.
26. Jabar, M.A. and A. Al-Mossawi, 2007. Susceptibility of some multiple resistant bacteria to garlic extract. African Journal of Biotechnology, 6: 771-776.
27. Shams-Ghahfarokhi, M., M. Shokoohamiri, N. Amirrajab, B. Moghadasi, A. Ghajari, F. Zeini, G. Sadeghi and M. Razzaghi-Abyaneh, 2006. *In vitro* antifungal activities of Allium cepa; Allium sativum and ketoconazole against some pathogenic yeasts and dermatophytes. Fitoterapia, 77: 321-323.
28. Shin, D.B., Y.S. Kim and Y.C. Lee, 1999. Effect of dehydration methods on the antimicrobial activity of garlic juice powder. IFT (Institute of food technology) Annual Meeting, pp: 98.
29. Thanaboripat, D., K. Nontabenjawan, K. Leesin, D. Teerapiannont, O. Sukcharoen and V. Ruangrattanametee, 1997. Inhibitory effect of garlic; clove and carrot on growth of Aspergillus flavus and aflatoxin production. Journal of Forestry Research, 8: 39-42.
30. Qasem, J.R. and H.A. Abu-Blan, 1995. Antifungal activity of aqueous extracts from some common weed species. Annual Applied Biology, 127: 215-219.
31. Nzeako, B.C., S.N. Zahra, Al-Kharousi and Z. Al-Mahrooqui, 2006. Antimicrobial Activities of Clove and Thyme Extracts. Sultan Qaboos University Medical Journal, 6: 33-39.
32. Peter, A.G.M. and De Smet, 2002. Herbal remedies. New England Journal of Medicine, 347: 2046-2056.
33. De Boer, H.J., A. Kool, A. Broberg, W.R. Mziray, I. Hedberg and J.J. Levenfors, 2005. Antifungal and antibacterial activity of some herbal remedies from Tanzania. Journal of Ethnopharmacology, 96: 461-469.
34. Lei, L., W. Feng-xiang, Q. Zhang-yi, W. Shu-qiu, C. Guang, G. Hong, Z. Hong-yan, S. Lei, Y. Xiao-hui and W. Ying-chen, 2009. The Antiadenovirus Activities of Cinnamaldehyde *In vitro*. Science, 40: 669-674.
35. Tragoolpua, Y. and A. Jatisatieur, 2007. Anti-herpes simplex virus activities of Eugenia caryophyllus (Spreng.) Bullock and S. G. Harrison and essential oil; eugenol. Phytotherapy Research, 21: 1153-1158.
36. Chen, C., T. Chou, L. Cheng and C. Ho, 2011. *In vitro* anti-adenoviral activity of five Allium plants. Journal of the Taiwan Institute of Chemical Engineers, 42: 228-232.
37. Reichling, J., 1999. Plant-microbe interaction and secondary metabolites with antiviral; antibacterial and antifungal properties. In: Functions of Plant Secondary Metabolites and Their Exploitation in Biotechnology. Ed., M. Wink, Sheffield; Sheffield Academic Press, pp: 187-273.
38. Fiore, C., M. Eisenhut, R. Krausse, E. Ragazzi, D. Pellati, D. Armanini1 and J. Bielenberg, 2008. Antiviral Effects of Glycyrrhiza species. Journal of Phytotherapy Research, 22: 141-148.