Asian Journal of Biological and Life Sciences | Original Research

Effect of extraction methods on the antibacterial activity of spices (garlic, cinnamon, turmeric, clove) against Escherichia coli K12 (JM109)

Talal Abdullah, Kahlan Albeshari, Idris Adewale Ahmed , Aida Baharuddin*

Department of Biotechnology, Faculty of Science, Lincoln University College, Malaysia.
Kelana Jaya Campus, No 2, Jalan Stadium SS7/15, Kelana Jaya, 47301, Selangor, Malaysia.

E-mail : envelope910@gmail.com Contact No. : Tel: +603-78063478, Fax: +603-78063479

Submitted : 12.05.2017 Accepted : 28.07.2017 Published : 30.08.2017

Abstract

In this study, simple extraction methods were used to determine the antibacterial activity of crude extracts of garlic, cloves, cinnamon and turmeric against *Escherichia coli* K12 (JM109). These extraction methods include ethanolic extraction, aqueous extraction, soaking, boiling and juicing. These methods were applied to spices in different forms, for example the soaking method was used for crushed spices while the boiling method was employed for whole, crushed and powdered forms. The disc diffusion method was utilised to determine antibacterial activity. In the case of garlic, the results revealed that soaking for 3 hours in a sterile aqueous solution (using the crushed form), boiling (using crushed and powdered forms) and using juice extraction caused antibacterial activity to occur against *E. coli* K12 (JM109). Fifteen grams of crushed garlic soaked in 20 ml of sterile aqueous solution exhibited the best inhibition zone of 19.6 ± 0.64 mm. Cloves extract acquired from using ethanolic extraction, aqueous extraction, soaking and boiling extraction methods displayed antibacterial activity against *E. coli* K12 (JM109). In the case of the soaking method, these results were obtained when crushed cloves were soaked for 3 hours. While in the case of the boiling method, both crushed and powdered cloves were used. The ethanolic extract of cloves at 0.40 g/ml produced an inhibition zone of 10.0 ± 1.0 mm, while the aqueous extract produced an inhibition zone of 13.3 ± 0.58 at 0.72 g/ml. The antibacterial activities from crude extracts of cinnamon and turmeric were not detected against *E. coli* K12 (JM109).

Key words : crude extract, E. coli K12 (JM109), medicinal spices, antibacterial activities

INTRODUCTION

Herbs and spices have been used in conventional medicine since ancient times as natural antimicrobial substances for the treatment of infectious diseases. Thus, much attention has been given to medicinal plants as alternative antimicrobial sources and as promoters of good health with no side effects[1] [1]. Garlic is a member of the *Alliaceae* family[2] [2] and is one of the best herbal medications. It was also studied in various levels of the medical field. Garlic's attributes culminate a mixture of different substances that are biologically active and which are responsible for its medicinal effects[3] [3]. The constituents of garlic are divided into two groups: sulphur-containing compounds, including alliin which is considered the main active compound, and non-sulphur containing compounds[4] [4].

Cinnamon, the evergreen tree of the tropical area, is a member of the *Lauraceae* family. It consists of essential oils and other compounds like cinnamic acid, cinnamaldehyde (which is considered the main compound) and cinnamate[567] [5-7]. Turmeric is a spice derived from the rhizomes of the plant *Curcuma longa* which belongs to the ginger family (*Zingiberaceae*) [8-10]8910. It has a wide spectrum of uses in the medical fie ld as it contains anti-inflammatory, antioxidant, anti-carcinogenic, anti-mutagenic, anticoagulant, anti-diabetic, antibacterial and antifungal properties[11] [11]. Turmeric contains 29% curcuminoids which are composed of a group of compounds. This group contains curcumin (diferuloylmethane) which constitutes the major bioactive components, demethoxycurcumin and bis-demethoxycurcumin[12] [12]. It also contains volatile oils (tumerone, atlantone and zingiberone), sugars, proteins and resins [10].

Cloves are the aromatic dried flower buds of a tree in the *Myrtaceae* family. It is one of the most valuable spices due to its various characteristics. It represents a functional ingredient in numerous products and has applications in the pharmaceutical, agricultural, fragrance, flavour and cosmetic industries, as well as various others. Cloves are considered the best source of phenolic compounds such as eugenol. Eugenol is the main bioactive component in cloves and shows antimicrobial activity against both Gram-positive and Gram-negative bacteria[13] [8, 13-17]14151617.

The general techniques of medicinal plant extraction are maceration, infusion, percolation, digestion, decoction, hot continuous extraction (Soxhlet), aqueous-alcoholic extraction by fermentation, counter-current extraction, microwave-assisted extraction, ultrasound extraction (sonication), supercritical fluid extraction and phytonic extraction (with hydrofluorocarbon solvents). Some of the current extraction methods for aromatic plants include microdistillation, thermomicrodistillation, molecular distillation headspace trapping, solid phase micro-extraction and protoplast extraction[18] [18].

E. coli K12 (JM109) is Gram-negative bacteria which was used in this study as it is an opportunistic bacterium that can be cultured in a Biosafety Level 1 Microbiology Laboratory (for low risk microbes). The objective of this study is to determine the antibacterial activity of crude extracts of these spices against *E. coli* K12 (JM109) using simple extraction methods.

MATERIALS AND METHODS

Bacterial strain

Escherichia coli K12 JM109 [*sup*E44, *end*A1, *hsd*R17, *gyr*A96, *rel*A1, *thi*Δ (*lacpro*AB), *rec*A1, F' [*tra*D36*pro* AB^{+}*lac*I^{q}*lac*ZΔM15] was used in this study. The bacterial culture was maintained at 4°C on a nutrient agar plate. Isolated single colonies of bacterial cultures on the nutrient agar (NA) plate were prepared by incubation at 37°C for 12-14 hours.

E. coli K12 (JM109) standardised suspension for spices extract susceptibility testing

Three to four colonies from an overnight bacterial culture were transferred to a tube of sterile normal saline (Klean & Kare) to make the inoculum suspension. The inoculum suspension was emulsified to avoid lumps. The inoculum turbidity was compared to the 0.5 McFarland standard using paper with black lines[19] [19]. A visible spectrophotometer at 625 nm wavelength was employed to determine the optical density (OD = 0.08-0.10) of the McFarland 0.5 turbidity standard.

Medicinal spices used in this study

Four medicinal spices consisting of garlic (*Allium sativum*), turmeric (*Curcuma longa*), cinnamon (*Cinnamon zeylanicum*) and cloves (*Syzgium aromaticum*) were employed in this study. The spices were purchased from the Giant hypermarket in Kelana Jaya, Selangor, Malaysia. The extracts were immediately tested for antibacterial activity after preparation. Garlic and turmeric skins were peeled off before treatment. The spices (garlic, turmeric, cinnamon and cloves) were then cleaned with distilled water and finally rinsed with 95% denatured ethyl alcohol and dried at room temperature approximately one hour prior to treatment.

Preparation of spices' powder forms

The garlic and turmeric were cut into small slices. The spices were dried in a hot air oven at 50-55°C. The garlic was dried for 2-3 days. The turmeric, cloves and cinnamon were dried for 8-10 days. The dried spices were crushed using a dry blender[20] [20].

Ethanolic extracts

Fifty grams of the dried powdered spices were soaked in 100 ml solution of 95% denatured ethyl alcohol and were kept at room temperature for 24 hours, followed by filtration using Whatman no. 1 filter paper. The filtered solutions were allowed to evaporate at 40-50°C using a water bath until they formed a thick paste. The paste extracts were weighed and stored at 4°C until they were applied [20].

To determine the stock concentration of the paste extract, 1 ml of sterile distilled water was added. The volume of the sterile distilled water was increased until all the extract had dissolved. The stock concentrations of the ethanolic extracts were as follows: garlic - 0.5 g/7 ml (0.07 g/ml), cloves - 2.36 g/6ml (0.4 g/ml), cinnamon - 1.78 g/9 ml (0.2 g/ml) and turmeric - 2 g/10 ml (0.2 g/ml). The antibacterial activity of the ethanolic extracts against *E. coli* K12 (JM109) was determined using the stock solutions.

Aqueous extracts

The preparation of the aqueous extracts was the same as that of the ethanolic extracts but instead of using 95% denatured ethyl alcohol, sterile distilled water was added to each powdered spice. Initially, 50 g of the dried powdered spices were soaked in 100 ml of sterile distilled water, however, the saturation point was reached at this volume. The final volume of sterile distilled water used for soaking 50 g of dried powdered cinnamon, garlic and cloves was 200 ml. For turmeric it was 400 ml. As in the case of ethanolic extracts, the soaked powder was kept at room temperature for 24 hours, followed by filtration using Whatman no. 1 filter paper. The filtered solutions were allowed to evaporate at 40-50°C using a water bath until they formed a thick paste. The stock concentrations of the aqueous extracts were as follows: garlic - 4.28 g/4 ml (1.07 g/ml), cloves - 2.16 g/3 ml (0.72 g/ml), cinnamon - 1.4 g/10.4 ml (0.13 g/ml) and turmeric - 2.1 g/3 ml (0.7 g/ml). The spices' stock concentrations were used to determine the antibacterial activity against *E. coli* K12 (JM109).

Aqueous soaking extraction (crushed form)

The garlic and turmeric were cut into small pieces. The spices were crushed using the mortar and pestle. Table 1 indicated the amount of crushed spices and volume of sterile distilled water used for soaking. Crushed spices were soaked for 3 hours in sterile distilled water. These extracts were then ready to use for antibacterial activity testing. The spices were soaked for 3 hours since soaking at 30 minutes and 1 hour had initially yielded negative results.

Boiling extracts

The whole, crushed and powdered forms of the 4 spices were weighed to be 2 g and 10 g. Next, 20 ml of distilled water was added to the spices then heated gently and brought to a boil using a hot plate. Once boiled, the spices were removed from the hot plate and cooled at room temperature prior to the evaluation of antibacterial activity.

Garlic and turmeric juice extracts

Two grams of garlic and 10 grams of turmeric were minced. Twenty millilitres of sterile distilled water were added to the minced spices. Next, the minced spices were ground using a mortar and pestle for 5 to 10 minutes. The garlic and turmeric were pureed then strained through a strainer and the juices were collected.

Antibacterial activity determination by disc diffusion method

Several 6 mm diameter discs of Whatman no. 3 filter paper

Table 1: Amount of crushed cinnamon, garlic, cloves, turmeric and volume of sterile distilled water used for soaking.

Weight (g)	Volume of sterile distilled water (mL)	
20	10	20
15	10	20
10	10	20
2	10	20

were prepared using a paper puncher and sterilised by dry-heating at 120°C for 60 minutes. The Mueller-Hinton agar (MHA) plate was swabbed with the *E. coli* K12 (JM109) standard suspension. The disc was then immersed in the extract solutions for 5 minutes and applied to the agar surface with sterilised forceps. The sterile distilled water was applied as the negative control (no inhibition zone), and 10 µg/ml ampicillin and 10 µg/ml gentamicin were used as the positive controls (produced inhibition zone). The disc diffusion agar plates were incubated at 37°C for 12-16 hours. The clear zones were checked and the diameters of the inhibition zones were measured. The disc diffusion experiment of each extract was performed in triplicate.

Calculation of the mean and standard deviation

The mean and standard deviation (SD) were calculated using Microsoft Excel 2013.

RESULTS

The lowest amount of spices chosen in this study was 2 g and the highest amount was 20 g. The amount of sterile distilled water used to soak and boil was 10 ml and 20 ml, respectively. For the preparation of juice extracts, only 20 ml of sterile water was used. The amount of water utilised was based on our initial study where at 5 ml of aqueous solution used for soaking and boiling, no antibacterial activity was detected against *E. coli* K12 (JM109). The ampicilin (10 µg/ml) and gentamicin (10 µg/ml) were employed as positive controls and the means of the inhibition zones (mm) were 16 0.10 and 30 0.18 mm (Fig. 1A). Sterile distilled water was used as the negative control and no inhibition zone (clear zone) was detected.

Garlic extract

The inhibition zones on *E. coli* K12 (JM109) were produced by crude extracts of crushed garlic soaked in an aqueous solution for 3 hours, boiled garlic (powdered and crushed) and juice extracts of garlic (Tables 2 and 3). The soaking and boiling of whole garlic extracts did not exhibit clear inhibition zones. The diameter of the inhibition zones increased with the increase in the amount of crushed garlic (2 g, 10 g and 15 g) soaked in 10 ml and 20 ml of a sterile aqueous solution. Fifteen grams of crushed garlic soaked in 20 ml of an aqueous solution produced 19.6 0.64 mm inhibition zone, while 20 g of crushed garlic soaked in the same amount of water produced 16.5 1.0 mm inhibition zone (Fig. 1B). The reduction in the inhibition zones could be because at 20 ml of aqueous solution, the volume is already limited to extracting the bioactive compounds of the 20 g of crushed garlic. No antibacterial activity was observed when whole garlic was soaked in an aqueous solution for 3 hours.

Table 2: Antibacterial activity of crude garlic extract from 3 hours soaking extraction method in aqueous on *E. coli* K12 (JM109)

Weight (g)	Sterile distilled water (mL)	Mean of inhibition zone (mm) ± SD	
		Crushed	Whole
20	10	16.5± 1.0	negative
15		19.0 ± 1.0	negative
10		17.3 ± 0.58	negative
2		10.1± 0.47	negative
20	20	16.5± 1.0	negative
15		19.6 ± 0.64	negative
10		17.0 ± 1.0	negative
2		9.3 ± 0.58	negative
Positive control	10 µg/mL Ampicillin	16 ± 0.10	
	10 µg/mL Gentamicin	30 ± 0.18	
Negative control	Sterile distilled water	negative	

When 10 g of powdered and crushed garlic were boiled in 20 ml of aqueous solution, inhibition of *E. coli* K12 (JM109) resulted with inhibition zones of 16.3 0.58 mm and 18.0 1.0 mm, respectively. Two grams of powdered and crushed garlic boiled in 20 ml of aqueous solution produced extracts with inhibition zones of 7.5 0.8 mm and 9.6 0.58 mm, respectively. Garlic juice extracts of 2 g and 10 g in 20 ml of sterile distilled water exhibited inhibition zones of 12.3 0.58 mm and 17.0 1.0 mm, respectively.

Garlic extracts from the ethanolic and aqueous extraction methods did not show any inhibition zones against *E. coli* K12 (JM109). These results were consistent with several studies where garlic extract discs did not present any inhibition zones[2122][21, 22] On the other hand, studies conducted by[2324][20, 23, 24] showed antibacterial activity against *E coli.* In our study, 95% denatured ethyl alcohol was used instead of non-denatured ethyl alcohol. However, by using the same type of ethyl alcohol, ethanolic extracts of cloves exhibited antibacterial activity against *E. coli* K12 (JM109). Our result further indicated that the type of ethanol used for soaking may affect the extraction process.

Cloves extract

The crude extracts of cloves from the ethanolic extraction, aqueous extraction, soaking and boiling extraction methods possessed inhibition zones (Table 4). Ethanolic extracts at a stock concentration of 0.40 g/ml exhibited inhibition zones of 10.0 1.0 mm, while stock concentration of aqueous extract at 0.72 g/ml exhibited 13.3 ± 0.58 mm (Fig. 2). As in Table 4, crushed cloves, when soaked for 3 hours in 20 ml of sterile aqueous solution, produced a slightly higher amount of antibacterial activity compared to when soaked in 10 ml. Two grams of crushed cloves soaked in 20 ml of a sterile aqueous solution produced an 11.5 0.57 mm inhibition zone. An increase in the inhibition zone to 18.5 0.68 mm was observed when 20 mg of crushed cloves were soaked in 20 ml of sterile aqueous solution for 3 hours.

Similar to garlic, whole cloves from the soaking and boiling methods did not produce any antibacterial activity since no inhibition zones were observed. This is because the cell walls were not broken down so the constituents were not extracted. Prolonged soaking of 12 hours up to 1 week at 4ºC in a tight container may produce different results. Several studies have indicated that important bioactive compounds with antibacterial activity are released by crushing the spices25 [25].

Turmeric and cinnamon extracts

In this study, the crude extracts of turmeric and cinnamon using ethanolic extraction, aqueous extraction, 3 hours soaking in sterile distilled water, boiling and juice extraction methods did not possess antimicrobial activity against *E. coli* K12 (JM109). The antibacterial activity of cinnamon and turmeric against other *E. coli* strains were reported[26] [9, 20, 26] The differences between the results obtained in our study and others may be due to the quality and age of the spices used, as well as the extraction methods. These methods include the processes of drying, soaking and evaporating. The quality of the ethanol applied may have also played a role in creating these differences.

DISCUSSION

Numerous studies showed that the plant extract from herbs as well as spices possesses antibacterial activities against many types of pathogenic bacteria at different variable degrees [27, 28]. This is because the plant has many bioactive compounds that exhibit antibacterial activities [27]. However, the active part of the plant that is useful is contained within the cells of the plant. To make use of these, the cell walls must be broken down and the constituents must be extracted in a form that can be used by a human. Unfortunately transforming the cocktail of biochemicals in the cell is a difficult task. The bioactive compounds vary in their solubility in water and sensitivity to heat. Although solubility increases with temperature, some delicate chemicals may be degraded by exposure to heat. For this reason, most herbalists prefer to use cold extraction methods, which, if done properly, involve minimal pharmaceutical processing and therefore might faithfully reflect the chemical characteristics of the plant.

In this study, the crude extracts from the boiled whole garlic and the whole garlic soaked in sterile distilled water for 3 hours demonstrated no antibacterial activity. This could be because the cell walls of the spices were possibly not broken down as a result of non-optimal boiling and soaking time. Thus the bioactive compounds were not released. However, Sah et al. demonstrated that pieces of cut garlic when boiled at 100ºC for 30 minutes

Table 3: Antibacterial activity of juice and boiling garlic extracts on *E. coli* K12 (JM109)

	Weight (g)	Sterile distilled water (mL)	Mean of inhibition zone (mm) ± SD			
				Boiling extracts		
			Juice	Powder	Crushed	Whole
Boiling extracts	2	20	-	7.5 ±.0.80	9.6 ± 0.58	negative
	10	20	-	16.3 ± 0.58	18.0 ± 1.0	negative
Juice extracts	2	20	12.3 ± 0.58	-	-	-
	10	20	17.0 ± 1.0	-	-	-

Table 4: Antibacterial activity of crude cloves extract from the ethanolic, aqueous, soaking and boiling extraction methods on *E. coli* K12 (JM109)

Extraction methods	Concentration		Mean of inhibition zone (mm) ± SD		
Ethanolic extract	0.40 g/mL		10.0 ± 1.0		
Aqueous extract	0.72 g/mL		13.3 ± 0.58		
Soaking extract for 3 hours in sterile distilled water	Weight (g)	Sterile distilled water (mL)	Mean of inhibition zone (mm) ± SD		
			Powder	Crushed	Whole
	20	10	-	17.4 ± 0.12	negative
	15		-	15.4 ± 0.28	negative
	10		-	13.7 ± 0.25	negative
	2		-	8.8 ± 0.52	negative
	20	20	-	18.5 ± 0.68	negative
	15		-	17.0 ± 0.65	negative
	10		-	15.0 ± 0.48	negative
	2		-	11.5 ± 0.57	negative
Boiling extract	2	20	6.0 ± 0.58	5.8 ± 0.18	negative
	10		10.4 ± 0.60	9.6 ± 0.35	negative

showed antibacterial activity against *E. coli* [29]. In our case, the whole garlic was soaked and then heated gently up to boiling. After that, the garlic was removed form the hot plate. Perhaps the boiling temperature and the varying amount of boiling time could have affected the antibacterial activity of the whole garlic. The boiled powdered and crushed garlic prepared in this study, however, possess antibacterial activity against *E. coli.* This is in line with the literature on the potential of garlic as natural drugs. Garlic has also been used in natural food preservatives for fishes at room temperature and deep-fried fishes, since it retains its antibacterial activity up to 120 ºC [30]. Concentrated fresh garlic juice extract was known to exhibit antibacterial activity against common pathogenic bacteria (*Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Staphylococcus aureus*) [31]. Our preparation of diluted fresh garlic juice and crushed garlic crude extracts from soaking in sterile distilled water for 3 hours also showed inhibition against *E. coli.* According to [32], antibacterial activity was detected when garlic was crushed and soaked in a different amount of sterile distilled water for various soaking hours. The antibacterial activity of crushed and powdered boiling garlic, as well as crushed garlic, soak in sterile distilled water can be explained because when garlic is crushed, cut, chewed or dehydrated, pulverized and then exposed to water, the vacuolar alliinase enzyme rapidly lyse the cytosolic cysteine sulfoxides (allicin) to form diallyl disulphide (allicin) [33]. This process is essential for garlic potency. Allicin is a

highly unstable molecule, which rapidly transforms into sulfides, including ajoene and dithiins. It has been reported that allicin is responsible for garlic antibacterial activity [34]. Although it has been known that allicin acts as an effective antimicrobial agent *in vitro*, its effects as an antimicrobial agent i*n vivo* is questionable since the bioavailability of allicin is reported to be poor [35]. Ethanolic and aqueous garlic extracts, on the other hand, showed no antibacterial activity against *E. coli.* Our results are consistent with the literature [21, 22]. The use of old garlic and/or denatured ethyl alcohol in the ethanolic extracts preparation might be responsible for its lack of antibacterial activity. In fact, Sasaki et al. [36] observed that the use of powder from fresh garlic was more effective for antibacterial activity than that from old garlic, though, several other studies [21,24,25] indicated otherwise.

Cloves have been used in Ayurveda, Chinese medicine and Western herbalism [37]. In this study both ethanolic and aqueous extracts of clove showed satisfactory inhibitory activity in comparison to the selected antibiotics (10 µg/ml of ampicillin and gentamycin). The ethanolic extract of clove was more efficient in its antibacterial activity as compared to the aqueous extract. At a concentration of 40 µg/ml of ethanolic extract the zone of inhibition was 10.0 ± 1.0 mm while at 72 µg/ml of aqueous extract the zone of inhibition was 13.3 ± 0.58 mm. This is because the antimicrobial components of the clove are more soluble in ethanol than water resulting in the release of a greater amount of active components. Our study agreed with the literature [38, 39]. The ethanolic extract and aqueous extract of cloves gave 18 mm [38] and 7 mm [39] as zones of inhibition, respectively against *E. coli*. Although, the initial amount of clove and water used for the extractions were not similar in each of the studies. Eugenol and carvacrol are phenolic compounds found in cloves known for the antibacterial activity. These compounds are also responsible for aroma and flavor as well as can act as mold inhibitors [38]. On the other hand, ethanol and aqueous extracts of clove by Hoque et al. [40] did not inhibit the *E. coli* O15:H7. Clove kill and inhibit microorganisms by destroying cell walls and membranes of the microorganism, then enter the cells and inhibit the normal synthesis of DNA and protein [41].

Soaking of the crushed clove for 3 hours and boiling extraction methods on cloves that were crushed and powdered showed significant inhibition zone against *E. coli.* In the 3 hours soaking method, a slight increase in the inhibition zones was detected when the amount of water increased from 10 mL to 20 mL while the amount of crushed clove was fixed to 2 g. The reason could be that when the aqueous amount increases, it helps to release more soluble active components. Interestingly, our aqueous infusion from the 3 hours soaking at room temperature produced inhibition zone of 8.8 ± 0.52 mm while aqueous infusion left for 24 hours produced similar inhibition zone against *E. coli*, 8.73 ± 1.18 mm [42]. Besides the soaking duration, our method used 2 g of crushed clove soaked in 10 mL of sterile distilled water and left at room temperature while Saeed and Tariq [42] used 10 g of the whole clove soaked in 100 mL of distilled water with occasional shaking. One possibility could be that the infusion rate of the active components into the water are much faster when crushed clove was used instead of the whole clove. According to Saeed and Tariq [42], the aqueous decoction of 10 g clove boiled in 100 mL of distilled water for 20 minutes inhibition zone is 9.07 ± 1.46 mm against *E. coli.* Instead of prolonged boiling, we used gradual gentle heating which was brought to a boil using a hot plate. The inhibition zone of 10 g of crushed and powdered clove in 20 mL which was gently heated up until boiling was 10.4 ± 0.60 mm. We observed quite similar inhibition zone value comparable with the author [42], although the amount of water and form of clove used were different. This can be explained by the fact that longer boiling time was needed to break the whole clove cell walls in the report [42], but the cell walls of the crushed and powdered form was already broken and further breaking by heating gently until boiling will help to speed up the process of releasing the active components. However, our whole boiling and soaking clove for 3 hours produces no inhibition zone against *E.coli.* The reason was that the soaking time and the boiling duration were not optimal to break the cell walls. Prolong incubation time and boiling time might produce positive results.

CONCLUSION

In this study, garlic and cloves displayed antibacterial activity against *E. coli* K12 (JM109) while no antibacterial activity was observed from cinnamon and turmeric. The processes of soaking crushed garlic for 3 hours in a sterile aqueous solution, boiling crushed garlic and boiling powdered garlic demonstrated antibacterial activity against *E. coli* K12 (JM109). Juice extracts from garlic also showed such activity. The best inhibition zone of 19.6 ± 0.64 mm was observed when 15 g of crushed garlic was soaked in 20 ml of sterile aqueous solution. The antibacterial activity of cloves extract was observed when ethanolic extraction, aqueous extraction, soaking (for 3 hours) and boiling extraction methods were employed on cloves that were either crushed or powdered. Our study further indicated that one of the crucial factors that determined the success of the extraction process was the type of ethanol used for extracting the bioactive ingredients. The ethanolic extract from garlic displayed negative results, however, the cloves extract from ethanol exhibited antibacterial activity against *E. coli* K12 (JM109). An inhibition zone of 10.0 ± 1.0 mm was obtained from 0.40 g/ml of cloves ethanolic extract, while cloves in an aqueous extract produced an inhibition zone of 13.3 ± 0.58 at 0.72 g/ml.

ACKNOWLEDGEMENT

This study was supported by the Lincoln University College MALAYSIA. Mr Talal Abdullah and Mr Kahlan Albeshari fellowship were provided by the Ministry of Public Health and Population, Yemen. The author would like to thank to Prof Razip Samian from School of Biological Sciences, Universiti Sains Malaysia Penang for providing the *Escherichia coli* K12 (JM109).

REFERENCES

1. Nabavi S, Di Lorenzo A, Izadi M, Sobarzo-Sánchez E, Daglia M, Nabavi S. Antibacterial Effects of Cinnamon: From Farm to Food, Cosmetic and Pharmaceutical Industries. Nutrients. 2015:7(9):7729-7748.

2. Londhe VP, Gavasane AT, Nipate SS, Bandawane DD, Chaudhari PD. Review Role of Garlic (*Allium Sativum*) in Various Diseases : an Overview. J Pharm Res Opin. 2011:4:129-134.

3. Nasri H, Nematbakhsh M, Rafieian-kopaei M. Ethanolic Extract of Garlic for Attenuation of Gentamicin- induced Nephrotoxicity in Wistar Rats. ijkd Iran J Kidney Dis. 2013:7(5):376-382.

4. Majewski M. *Allium sativum*: facts and myths regarding human health. Rocz Państwowego Zakładu Hig. 2014:65(1):1-8.

5. Jakhetia V, Patel R, Khatri P, Pahuja N, Garg S, Pandey A,

Sharma S. Cinnamon: A Pharmacological Review. J Adv Sci Res. 2010:1(2):19-23.

6. Rao PV, Gan SH. Cinnamon: a multifaceted medicinal plant. Evidence-based Complement Altern Med. 2014:2014:1-12.

7. Vangalapati M, N SS, DV SP, Avanigadda S. A Review on Pharmacological Activities and Clinical effects of Cinnamon Species. Res J Pharm Biol Chem Sci. 2012:3(1):653-663.

8. Aggarwal BB, Kunnumakkara AB, eds. Molecular Targets and Therapeutic Uses of Spices. Singapore, SG: World Scientific Publishing Co. Pte. Ltd. 2009. p.201.

9. Mari selvam R, Kalirajan K, Ranjit Singh a. J a. Anti-microbial activity of turmeric natural dye against different bacterial strains. J Appl Pharm Sci. 2012:2(6):210-212.

10. Jurenka JS. Anti-inflammatory properties of curcumin, a major constituent of Curcuma longa: A review of preclinical and clinical research. Altern Med Rev. 2009:14(2):141-153.

11. Suvarna R, Bhat SS, Hegde KS. Antibacterial Activity of Turmeric against *Enterococcus faecalis* An In vitro Study. Int J Curr Microbiol Applide Sci. 2014:3(2):498-504.

12. Priyadarsini K. The Chemistry of Curcumin: From Extraction to Therapeutic Agent. Molecules. 2014:19(12):20091-20112.

13. Cortés-Rojas DF, de Souza CRF, Oliveira WP. Clove (*Syzygium aromaticum*): a precious spice. Asian Pac J Trop Biomed. 2014:4(2):90-96.

14. Alitonou G, Tchobo F, Avlesse F, Yehouenou B, Yedomonhan P, Koudoro AY, Menut C, Sohounhloue DK. Chemical and biological investigations of *Syzygium aromaticum* L. essential oil from Benin. Int J Biol Chem Sci. 2012:6(3):1360-1367.

15. Sohilait HJ. Chemical Composition of the Essential Oils in *Eugenia caryophylata* , Thunb from Amboina Island. Sci J Chem. 2015:3(6):95-99. .

16. Gaylor R, Michel J, Thierry D, Panja R, Fanja FPD. Bud, leaf and stem essential oil composition of clove (*Syzygium aromaticum* L.) from Indonesia, Madagascar and Zanzibar. Int J Basic Appl Sci. 2014:3(3):224-233.

17. Bhowmik D, Kumar KPS, Yadav A, Srivastava S, Paswan S, Dutta AS. Recent Trends in Indian Traditional Herbs *Syzygium aromaticum* and its Health Benefits. J Pharmacogn Phytochem. 2012:1(1):13-22.

18. Sukhdev SH, Suman PSK, Gennaro L. Extraction Technologies for Medicinal and Aromatic Plants. Trieste: International Center for Science and High Technology, 2008. Available from:10.1017/CBO9781107415324.004.

19. Cheesbrough M. District Laboratory Practice in Tropical Countries. Vol Part 2. 2nd Ed.. New York, NY: Cambridge University Press, 2006. p.9.

20. Mukhtar S and, Ghori ifra. Antibacterial Activity of Aqueous and Ethanolic Extracts of Garlic, Cinnamon and Turmeric against *Escherichia Coli* ATCC 25922 and *Bacillus Subtilis* DSM 3256. Int J Appl Biol Pharm Technol. 2012:3(2):131-136.

21. Onyeagba RA, Ugbogu OC, Okeke CU, Iroakasi O. Studies on the antimicrobial effects of garlic (*Allium sativum Linn*), ginger (*Zingiber officinale Roscoe*) and lime (*Citrus aurantifolia Linn*). African J Biotechnol. 2004:3(10):552-554.

22. Mohsenipour Z, Hassanshahian M. The Effects of *Allium sativum* Extracts on Biofilm Formation and Activities of Six Pathogenic Bacteria. *J*undishapur J Microbiol. 2015:8(8).

23. Karuppiah P, Rajaram S. Antibacterial effect of *Allium sativum* cloves and *Zingiber officinale* rhizomes against multiple-drug resistant clinical pathogens. Asian Pac J Trop Biomed. 2012:2(8):597-601.

24. Khalid N, Ahmed I, Shah M, Latif Z, Rafique T, Fawad SA. Comparison of Antimicrobial Activity, Phytochemical Profile and Minerals Composition of Garlic *Allium sativum* and *Allium tuberosum*. J Korean Soc Appl Biol Chem. 2014:57(3):311-317.

25. Borlinghaus J, Albrecht F, Gruhlke MCH, Nwachukwu ID, Slusarenko AJ. Allicin: chemistry and biological properties. Molecules. 2014:19(8):12591-12618.

26. Usha M, Ragini S, S.M.A N. Antibacterial Activity of Acetone and Ethanol Extracts of Cinnamon (*Cinnamomum zeylanicum*) and Ajowan (*Trachyspermum ammi*) on four Food Spoilage Bacteria. Int Res J Biol Sci. 2012:1(4):7-11.

27. Djeussi DE, Noumedem JA, Seukep JA, Fankam AG, Voukeng IK, Tankeo SB, Nkuete AH, Kuete . Antibacterial activities of selected edible plants extracts against multidrug-resistant Gram-negative bacteria. BMC Complement Altern Med. 2013:13(1):164.

28. Sellami M, Ghariani B, Louati H, Miled N, Gargouri Y. Biological Activities of Extracts of Different Spices and Plants. Int J Curr Eng Technol. 2013:3:1051-1060.

29. Sah P, Al-tamimi B, Al-nassri N, Al-mamari R. Effect of temperature on antibiotic properties of garlic (*Allium sativum L* .) and ginger (*Zingiber officinale Rosc*). African J Biotechnol. 2012:11(95):16192-16195..

30. Ranjan S, Dasgupta N, Saha P, Rakshit M, Ramalingam C. Comparative study of antibacterial activity of garlic and cinnamon at different temperature and its application on preservation of fish. Adv Appl Sci Res. 2012:3(1):495-501. Effect of temperature on antibiotic properties of garlic and ginger.pdf.

31. Yadav S, Trivedi NA, Bhatt JD. Antimicrobial activity of fresh garlic juice: An in vitro study. Ayu. 2015:36(2):203-207.

32. Foster S, Chongxi Y. Herbal Emissaries: Bringing Herbal Emissaries: Bringing Chinese Herbs to the West: A Guide to Gardening, Herbal Wisdom, and Well-Being. 3rd ed. Rochester,NY: Healing Art Press, 1992. pg 87.

33. Amagase H, Petesch BL, Matsuura H, Kasuga S, Itakura Y. Intake of Garlic and Its Bioactive Components. Jounral Nutr. 2001:131(3):955S-962S.

34. Avato P, Tursi F, Vitali C, Miccolis V, Candido V. Allylsulfide constituents of garlic volatile oil as antimicrobial agents. Phytomedicine. 2000:7(3):239-243.

35. Lawson LD, Ransom DK, Hughes BG. Inhibition of whole blood platelet-aggregation by compounds in garlic clove extracts and commercial garlic products. Thromb Res. 1992:65(2):141-156.

36. Sasaki J, Kita T, Ishita K, Uchisawa H, Matsue H. Antibacterial activity of garlic powder against *Escherichia coli*

O-157. J Nutr Sci Vitaminol (Tokyo). 1999:45(6):785-790.

37. Balch PA, Balch JF. Prescription for Nutitional Healing. 3rd ed. USA: AVERYA. pg94.

38. Pandey A, Singh P. Antibacterial activity of *Syzygium aromaticum* (clove) with metal ion effect against food borne pathogens. Asian J Plant Sci Res. 2011:1(2):69-80.

39. Sewani S, Qureshi M. Antimicrobial activity of Neem, Clove, Curry leaves, Cardamom, Tulsi stem and Tulsi leaves. Int Res J Biol Sci. 2016:5(1):2278-3202.

40. Hoque MM, Bari ML, Juneja VK, Kawamoto S. Antimicrobial activity of cloves and cinnamon extracts against food borne pathogens and spoilage bacteria, and inactivation of *Listeria monocytogenes* in ground chicken meat with their essential oils. Rep Nat'l Food Res Inst. 2008:72:9-21.

41. Xu J-G, Liu T, Hu Q-P, Cao X-M. Chemical Composition, Antibacterial Properties and Mechanism of Action of Essential Oil from Clove Buds against *Staphylococcus aureus*. Molecules. 2016:21(9):1194.

42. Saeed S, Tariq P. In vitro antibacterial activity of clove against gram negative bacteria. Pakistan J Bot. 2008:40(5):2157-2160.