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ORIGINAL STUDY

Antimicrobial Activities of Clove and Thyme Extracts

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الفعاليات المضادة للجراثيم لمشتقات القرنفل والسعتر

الملخص: المقدمة: كانت هناك نظرية مؤداها أن المناطق الجغرافية للأعشاب تؤثر على مكونات زيوتها الجوهرية و هذا بدوره يؤثر على درجة فعاليتها المضادة للجراثيم. **الهدف:** اختبار عينتين من القرنفل من كل من سري لانكا وزنجبار، وعينتين من السعتر من إيران وعمان لمعرفة القابلية المضادة للجراثيم في زيوتها المستخلصة. **الطريقة:** استخلصت العناصر الفعالة من كل نبات بواسطة التقطير والغليان. وحددت الفعاليات المضادة للجراثيم للمستخلص بواسطة تخفيف المحلول الثنائي في تقنية انتشار آجر (*agar*) باستعمال (*Staphylococcus aureus Pseudomonas aeruginosa, Escherichia coli, Streptococcus pyogenes, Corynebacterium species, Salmonella species, Bacteroides fragilis and Candida albicans*). **النتائج:** كل المستخلصات تحتوي على فعالية مضادة لكل البكتريا والخمائر التي كانت قيد الدراسة. المستخلصات المائية كان لديها فعالية أقل ضد الجراثيم. أما مستخلص السعتر المائي فكان لديه فعالية ضد جراثيم العنقودية الذهبية فقط. وجدت أقل فعالية ضد الجراثيم (٠,١٪ أي ١:١٠٢٤) في المشتق الزيتي للسعتر باستخدام (*Candida albicans*). لا يوجد فرق معنوي في الفعالية المضادة للجراثيم بين القرنفل السري لانكي أو الزنجباري وبين السعتر المأخوذ من ايران أو عمان. **الخلاصة:** أوضحت تجربتنا أن المنطقة الجغرافية التي تعيش فيها الأعشاب لا تؤثر على فعاليتها المضادة للجراثيم. على كل حال، من الضروري القيام بأعمال أخرى لمعرفة لماذا تكون (*Candida albicans*) ذو حساسية ملموسة للمستخلصات أكثر من كل الأحياء الدقيقة التي تم اختبارها.

ABSTRACT ***Objective:*** It has been postulated that geographical locations of the herbs affect the constituents of their essential oils and thus the degree of their antimicrobial action. This study examine two samples of clove obtained from Sri Lanka and Zanzibar and two samples of thyme from Iran and Oman to determine the antimicrobial potential of their extracted oils. ***Method:*** The active agents in each plant were extracted by steam distillation and by boiling. The antimicrobial activities of the extracts were determined at neat and by two-fold dilutions in well agar diffusion technique using *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Streptococcus pyogenes, Corynebacterium species, Salmonella species, Bacteroides fragilis* and *Candida albicans*. ***Results:*** All oil extracts possessed antimicrobial activity against all bacteria and yeast tested. Their water extracts exhibited lower antimicrobial activity, though thyme aqueous extract was active only against *S. aureus.* The lowest concentration of antimicrobial activity (0.1% i.e., 1:1024) was obtained with thyme oil extract using *Candida albicans*. There was no significant difference in antimicrobial activity between clove obtained from Sri Lanka or Zanzibar or thyme obtained from Iran or Oman. ***Conclusion:*** Our experiment showed that the country of origin of the herbs has no effect on their antimicrobial activity. However, further work is necessary to ascertain why *Candida albicans* displayed remarkable degree of sensitivity with the extracts than all the other organisms test.

Keywords: Thyme, Clove, Oil, Extract, Antimicrobial, Oman, Iran, Sri Lanka, Zanzibar.

The essential oil of thyme (Thymus vulgaris) is utilized as a flavour enhancer in a wide variety of foods, beverages, confectionery products and in perfumery for the scenting of soaps and lotions.[1] It possesses some antiseptic, bronchiolytic, antispasmodic and antimicrobial properties that make it popular as a medicinal herb and as a preservative for foods.[2,3]

The therapeutic potential of thyme rests on its contents of flavonoids, thymol, eugenol, aliphatic phenols as well as saponins, luteolin and tetramethoxylated flavones.[4,5]

Clove (*Syzygium aromaticum*) on the other hand, is a plant widely cultivated in Spice Islands, Indonesia, Pemba and Zanzibar, though earlier production of the plant was in China. Like thyme, it is used in the seasoning of food.[4,6] Its antimicrobial potential was established when its essential oil extracts killed many Gram

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positive and Gram negative organisms including some fungi.[6,7] The antimicrobial activity of clove is attributable to eugenol, oleic acids and lipids found in its essential oils.[8]

Thyme oil extract failed to kill *Staphylococcus aureus*, *Salmonella choleraesuis* or *Klebsilela pneumoniae* but stopped the growth of *Pseudomonas aeruginosa* and *Candida albicans*. While clove extracts were found to inhibit the growth of *Pseudomonas aeruginosa*, *Candida albicans*, *Staphylococcus aureus*, *Salmonella choleraesuis*, *Klebsiella pneumoniae,*[3] Arora and Kaur[8] found the aqueous extracts of clove to possess no antimicrobial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus* or *Escherichia coli* but possessed some activity against *Shigella flexneri*.

Dorman and Deans[7] assessed the antibacterial activity of thyme, clove and other medicinal plants against 25 different genera of bacteria including animal and plant pathogens, food poisoning and spoilage bacteria using Iso-sensitest agar. They found all the organisms tested against clove and thyme to be sensitive, with thyme exhibiting more antibacterial effect than clove.

From the work of other investigators, it is observed that oil and water extracts of both clove and thyme kill many microorganisms. However, it is said that differences in major oil constituents and thus in antimicrobial effect is affected by differences in the geographical areas from where the plants were grown.[9-11]

Thyme and clove are routinely used as food additives in Omani food menus.

The aim of this project is to assess the antimicrobial activity of water and oil extracts of thyme from Oman and Iran and clove from Sri Lanka and Zanzibar using standard laboratory procedures. We hope to establish if differences exist in their respective abilities to exhibit some antimicrobial action since they come from various parts of the world.

METHOD

SOURCES OF THYME AND CLOVE

Thyme (Thymus vulgaris)

Thyme used in this experiment came from Oman and Iran. The parts of the plant employed for the distillation of essential oils were leaves, stems and flowers.

Clove (Syzygium aromaticum)

The buds were used and they came from Zanzibar and Sri Lanka.

Extraction of essential oils

About 20g of each herb soaked in distilled water was distilled in a stove still (steam distiller, locally fabricated). The volatile vapour that condensed at water temperature of 80°C was called essential oils. The clove buds were distilled in the same way as the thyme. The distilled oils were labeled and placed in a fridge until ready for use.

Extraction of essential oils by boiling

About 20g of each herb were soaked in 50ml of distilled water and were subsequently boiled at 100°C for 15 minutes. The boiled extracts were allowed to cool at room temperature after which they were left at fridge temperature overnight to allow the solute particles to settle. The aqueous phases were pipetted, labeled and called aqueous extracts. They were kept in the fridge until ready for use.

Media

Diagnostic sensitivity test (DST, Oxoid, UK) agar plates were used for the determination of zones of inhibition produced by each extract on the control and test organisms. Blood agar were used for the preparation of pure cultures of bacteria while Sabouraud plates (Oxoid, UK) were used for *C. albicans*.

Control organisms

The control organisms used for screening the extracts for the presence of antimicrobial activities were: *Staphylococcus aureua* (NCTC 6571), *Pseudomonas aeruginosa* (NCTC 10662) and *Escherichia coli* (NCTC 10418). These bacteria are routinely used in our laboratory as control organisms for antibiotic sensitivity testing of bacterial isolates from clinical specimens and they are usually sensitive to most antibiotics.

Test organisms

The test bacteria were organisms kept as stock cultures in our laboratory and was made of:

- *Streptococcus pyogenes*
- *Corynebacterium spp*
- *Salmonella spp*
- *Bacteroides fragilis*
- *Candida albicans*

INOCULUM PREPARATION

The control and test organisms were each plated on blood agar and incubated overnight at 37°C except *C. albicans* which was plated on Sabouraud medium and incubated for 48 hours. Suspensions of pure colonies in broth at 106 CFU/ml from growth on the plates

were made using Mcfarland's turbidity standards (tube 0.5).

screening of oil and aqueous extracts

Oil extracts

One hundred microlitres (100µL) of each oil extract were added to 100µL of 5% dimethylsulphoxide (DMSO) to solubilize the extracts.[3,9]

The aqueous extracts were used without solubilization. Each control organism (*S. aureus* (NCTC 6571), *P. aeruginosa* (NCTC 10662), *E. coli* (NCTC 10418) was spread on DST agar using an inoculating rotator (BBL, USA). Wells of 4 mm were bored into each plate with sterile un-drawn. Pasteur pipette and plugs were removed with sterile tips.[5] One hundred microlitres (100µL) of each extract were put in triplicates into each plate of media. The plates were kept at room temperature for 15 minutes to allow the extracts to seep into the media. They were subsequently incubated at 37°C overnight. The concentration of organisms used for screening the extracts were cross checked using visible count method in which 3.3µL (3mm diameter loop) was plated on plates of blood agar in triplicates. Counts were made after incubation at 37°C overnight. Control for DMSO made up of equal volumes of DMSO and saline was put up as in the test. Zones of inhibition or no inhibition produced by each extract were measured and average zone size was taken.

Determination of the antimicrobial concentration of each oil and water extracts

The concentration end-points of the activity of the extracts were done using the organisms listed above. Two-fold dilutions of the extracts except water extracts were first made in 5% (DMSO) and were subsequently diluted in sterile saline using equal volumes of the extracts and saline to 0.1% (1:1024) dilution. Two-fold dilutions of aqueous extracts were made in saline. All dilutions were prepared fresh each time they were to be used.

Wells of 4 mm diameter were made on DSTs which were swabbed with the appropriate organism at 10^6 CFU/mL as previously described. Each well was separately filled with 100µL of each dilution in triplicates. Controls for DMSO were put up as previously described. The plates were incubated aerobically at 37°C overnight except for *B. fragilis* which were incubated anaerobicaly.

Zones of inhibition were measured and the average zone size of each dilution was taken.

The end-point of activity for each extract was defined as the lowest dilution of the extract showing growth inhibition of the organisms.

RESULTS

screening of oil and aqueous extracts

DMSO

The DMSO control showed no growth inhibition with any of the microorganism including *C. albicans*.

Control bacteria

The zones of inhibition produced by the oil extracts with the control organisms varied from 14.7mm (*P. aeruginosa*) to 33.7mm (*S. aureus*). Their water extracts produced zones between 10mm (*E. coli*) and 34.3mm (*S. aureus*). Zone of 15.7mm was obtained with *P. aeruginosa*.

determination of the antimicrobial concentration oil and water extracts

Figures 1 represents the zones of inhibition produced by Omani thyme oil (anticlockwise direction) at 0.78% (128) and 0.39% (256) against *Escherichia coli* while figure 2 represents that of Zanzibar clove oil at 1.56% (64) and 0.78% (128) against *Staphylococcus aureus*.

Figure 3 shows zones of inhibition produced against *Pseudomonas aeruginosa* by Zanzibar (right) and Sri Lanka (left) clove water extracts at no dilutions.

Table 1 and Figure 4 represent the concentration end-points of the antimicrobial activities of thyme and clove extracts using various organisms. All essential oils possessed antimicrobial activity against all bacteria and yeast tested. The Sri lankan clove oils had concentration end points at 0.2% (512) with *C. albicans* while Omani and Iranian thymes oils had 0.1% (1024) respectively. However, the concentration end-points for the oils for various bacteria varied from 0.39% (256) to 1.56% (64) with *Corynebacteria spp*. 0.39% (256), *E. coli* 0.78% (128), *S. pyogenes* 0.78% (128), *P. aeruginosa* 1.56% (64) to *B. fragilis* 1.56% (64) respectively.

Sri Lankan and Zanzibar clove oils had concentration end points of 1.56% (64) and 0.78% (128) respectively with *S. aureus* and *Salmonella spp* while Omani and Iranian thymes had end-points of 0.39% (256) respectively.

Table 2, figure 5 show the antibacterial and antifungal activities of clove and thyme water extracts which were demonstrable only at neat dilutions for

Table 1. *The end point of antimicrobial activity of clove and thyme oils at % v/v dilution.*

Plant extract	*Staphylococcus aureus*	*Streptococcus pyogenes*	*Corynebacterium spp*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Salmonella spp*	*Bacteroides fragilis*	*Candida albicans*
Clove (Sri Lanka)	1.56 (64)	0.78 (128)	0.39 (256)	0.78 (128)	1.56 (64)	0.78 (128)	1.56 (64)	0.20 (512)
Clove (Zanzibar)	1.56 (64)	0.78 (128)	0.39 (256)	0.78 (128)	1.56 (64)	0.78 (128)	1.56 (64)	0.20 (512)
Thyme (Oman)	0.39 (256)	0.78 (128)	0.39 (256)	0.78 (128)	1.56 (64)	0.39 (256)	1.56 (64)	0.10 (1024)
Thyme (Iran)	0.39 (256)	0.78 (128)	0.39 (256)	0.78 (128)	1.56 (64)	0.39 (256)	1.56 (64)	0.10 (1024)

( ) Double dilution end-points

Corynebacterium spp., *E. coli* and *Salmonella spp*. No inhibition zones were demonstrable with *C. albicans*, *S. pyogenes* or *B. fragilis*. However, Omani and Iranian thyme water extracts each showed of some least activities at 25% (4) with *S. aureus* while Sri Lanka and Zanzibar clove water extracts showed activities each at 6.25% (16) dilution.

DISCUSSION

Thyme and clove oil extracts are known to possess some antimicrobial activities and are used in various food preparations as flavour enhancers and in herbal medicine.[1-3] Though the modes of action of the extracts are not known, their antimicrobial agents include thymol, terpenes, eugenol, flavones, glycosides of phenolic monoterpenoids and aliphatic alcohols among other elements.[4,5,8] These substances acting alone or in combination may result in a broad spectrum of antimicrobial activity exhibited for both bacteria and fungi. Thyme and clove oils possess antimicrobial activity against *S. aureus*, *E. coli*, *P. aeruginosa* as well as against *S. pyogenes*, *Corynebacterium*, *Salmonella*, *Bacteroides* and *C. albicans* at various dilu-

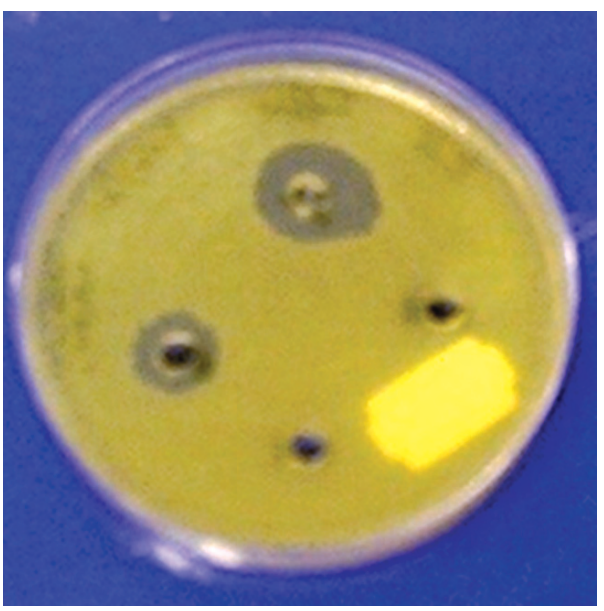


Figure 1. *Zones of inhibition produced by Omani thyme oil (anticlockwise direction) against E. coli at 0.78% (128) and 0.39% (256) dilutions*

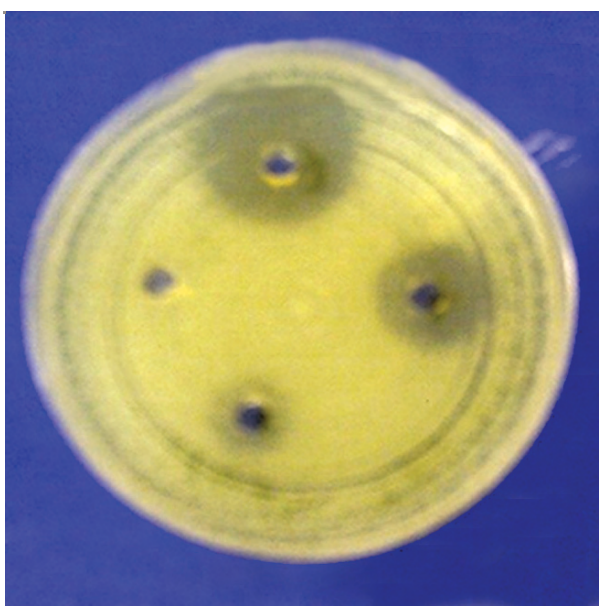
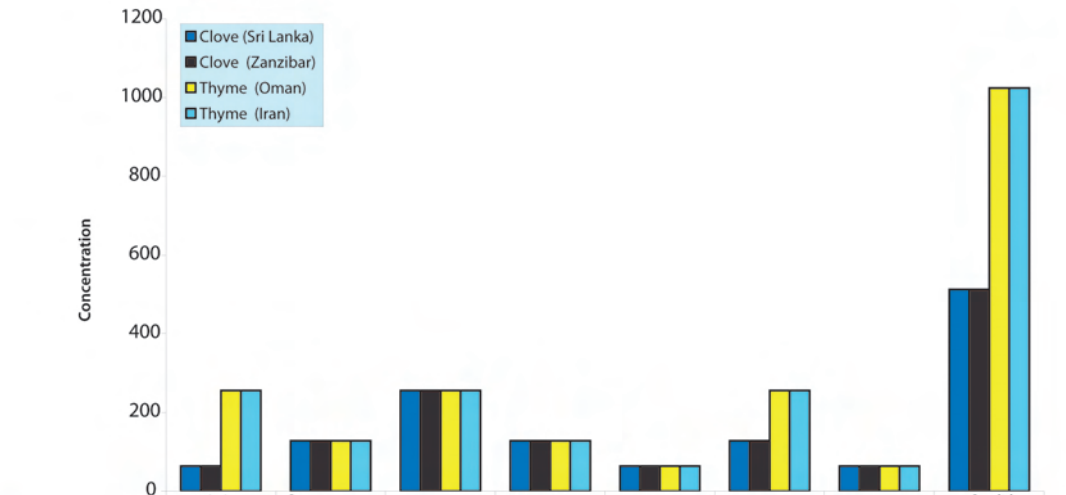


Figure 2. *Zones of inhibition produced by clove oil (clockwise direction) against S. aureus, 1.56% (64) and 0.78% (128) dilutions*



	Staphyloccus aureus	Streptococcus pyogenes	Corynebacteium	Escerichia coli	Pseudomonas aeruginosa	Salmonella spp	Bacteroides Fragilis	Candida albicans
Clove (Sri Lanka)	64	128	256	128	64	128	64	512
Clove Zanzibar	64	128	256	128	64	128	64	512
Thyme (Oman	256	128	256	128	64	256	64	1024
Thyme (Iran)	256	128	256	128	64	256	64	1024

Figure 4. ***The end point of antimicrobial activity of clove and thyme oils at v/v dilution***

tions of the extracts [Table 1, Figure 4]. These findings agree with the work of other workers.[7-9]

In this study, *C. albicans* was found to be the most susceptible to thyme and clove oil with concentration end-points of 0.1% (1024) and 0.2% (512) respectively. Similar findings were reported by other workers.[9,12,13] Therapeutic agents with high killing propensity for fungi are few. The reason why yeast is more susceptible to the extracts than bacteria is unclear but it may be that at any given time, these oils may break up the structural integrity of *C. albicans* faster than they dissociate bacteria. In this study, the incubation time and temperature were the same for bacteria and fungi.

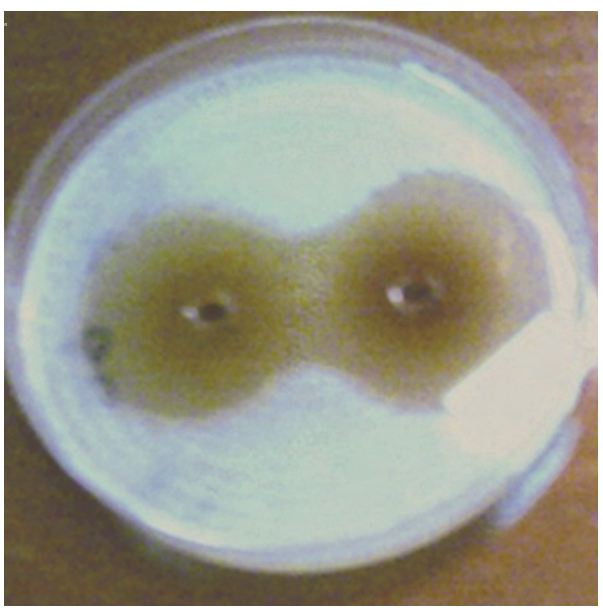


Figure 3. ***Zones of inhibition produced by Zanzibar (right) and Sri Lanka (left) clove water extracts against Pseudomonas aeruginosa***

The inhibition zones obtained with thyme oil against *P. aeruginosa*, *Salmonella spp* and *S. aureus* were greater than those obtained by other workers[3,14]. The differences may be due to the methods used in solubilizing the oils to obtain hydrophilic molecules. We observed that dissolving the extracts in DMSO produced higher zones than using tween 20, though Hili *et.al.*[9] obtained faster killing of bacteria in the absence of DMSO.

The study, using t-test at 95% level, demonstrates that there is no significant difference ($p < 0.05$) in antimicrobial activities of cloves obtained from Sri Lanka and Zanzibar or between thyme from Oman and Iran [Table 1, Figure 4].

The activities of water extracts differed from that of oil extracts where all the organisms tested produced zones of inhibition [Table 2, Figure 5]. Clove water extract showed inhibitions with all the organisms tested except with *S. pyogenes*, *B. fragilis* and *C. albicans*. Thyme water extract possessed antimicrobial activity only against *S. aureus*. It is therefore an extract with

Table 2. ***The end point of antimicrobial activity of clove and thyme water extracts at % v/v dilution***

Plant extract	*Staphylococcus aureus*	*Streptococcus pyogenes*	*Corynebacterium spp*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Salmonella spp*	*Bacteroides fragilis*	*Candida albicans*
Clove (Sri Lanka)	6.25% (16)	-	neat	neat	6.25 (16)	neat	-	-
Clove (Zanzibar)	6.25% (16)	-	neat	neat	6.25 (16)	neat	-	-
Thyme (Oman)	25.00 %(4)	-	-	-	-	-	-	-
Thyme (Iran)	25.00 %(4)	-	-	-	-	-	-	-

( ) Double dilution end-points

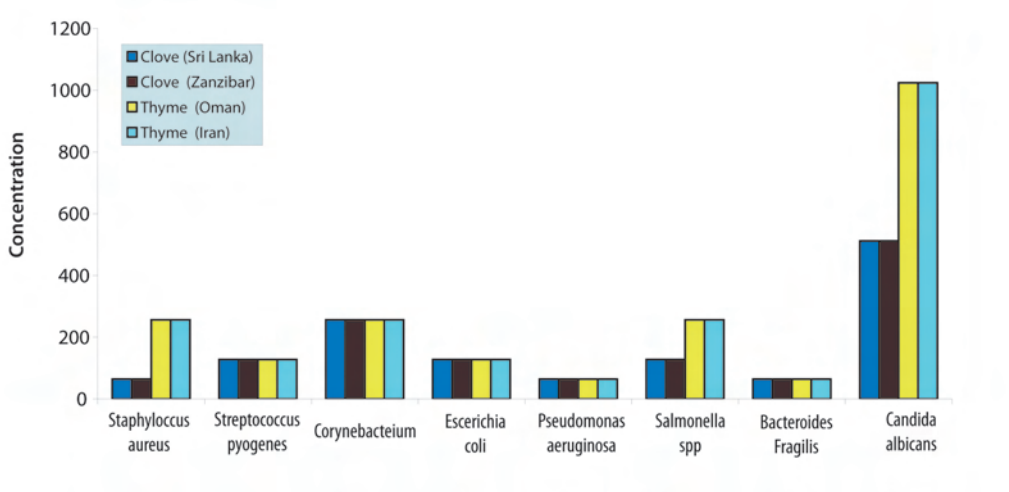


Figure 5. ***The end point of antimicrobial activity of clove and thyme water extract at v/v dilution***

limited or narrowed antimicrobial activity. This observation is in contrast with the finding of Gislene[15] where extracted materials contained agents that killed *Salmonella*, *C. albicans*, *P. aeruginosa* and *E. coli.* The difference in the individual findings may be due to the differences in the methods of extraction. In our study, water was used for the extraction whereas in Gislene's[15] study, ethanol was used. Moreover, the water extraction method in our finding was not as good as extraction by distillation method [Table 1, Figure 4] where the extracted oils contained antimicrobial agents for all the organisms tested. The reason for poor antimicrobial activity exhibited by the water extracts is attributable to the evaporation of the essential active agents during boiling.

There was no significant difference using student t-test at 95% level ($p<0.05$) between clove water extract from Sri Lanka and Zanzibar or thyme water extract from Oman and Iran [Table 2, Figure 5].

In summary, our work showed that differences in antimicrobial activities do not exist between thyme *(Thymus vulgaris)* obtained from Oman and Iran or clove *(Syzygium aromaticum)* obtained from Sri Lanka and Zanzibar. However, our work does not rule out differences in the constituents of oils within species. Besides possession of antimicrobial action, essential oils of herbs function as phytoprotective agents, defending the plant from herbivores and lethal pathogens.[16]

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