IAA Journal of Applied Sciences 6(1):52-63, 2020. ISSN: 2636-7246

The Antimicrobial Effects of *Thymus vulgaris* on *Staphylococcus aureus* and *Escherichia coli*

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ABSTRACT

Antibacterial potency of thyme spices were studied. Crude extracts of the thyme powder were obtained after soaking 50g of the thyme powder in methanol, ethanol, and distilled water for 48h. This study was carried out using Disc diffusion, Agar-well and pour plate methods. The extracts were screened against *Escherichia coli* and *Staphylococcus aureus*. The crude extracts of ethanol and methanol were sensitive to *Eschericha coli* and *Staphyloccus aureus* with zone diameter inhibition of 17.00mm – 21.00mm and 14.00mm-16.00mm respectively where as the organisms were resistant to aqueous extract. The pour plate method of ethanol and methanol extracts of thyme was sensitive after 24-72h while the aqueous extract did not have any effect after 24 hours. Minimum inhibitory concentration of ethanolic and methanolic extract of 0.5mg/ml and 2.4mg/ml for *Escherichia coli* and 0.6mg/ml and 1.25mg/ml for *Staphylococcus aureus* were determined. The phytochemical analysis of thyme spice revealed the presence of alkaloids, tannins, steroids, saponins and flavonoids. The significance of thyme in ready to eat meals were also discussed. Statistical differences were carried out using Analysis of Variance at 95% confident level. Thyme spice extracts have antimicrobial activities against food borne bacterial species and should be considered as potential antibacterial agents for addition to ready to eat meals.

Keywords: Antimicrobial, thymus, vulgaris, staphylococcus aureus, Escherichia coli.

INTRODUCTION

The continuous evolution of bacterial resistance to current available antibiotics has necessitated the search for novel and effective antimicrobial compound [1]. For the past two decades, there has been an increasing interest in the investigation of different extracts obtained from traditional medicinal plants as potential sources of new antimicrobial agents. Plant derived medicine have been reported to have large contribution towards human health and well being. Medicinal plants have contributed tremendously in the management of diseases and maintenance of plants. This could be as a result of their availability, higher safer margin and cheaper cost. Several herbal preparations have reportedly been employed in under treatment. Different parts of *Thymus vulgaris* such as leaves, roots and stalks have been reported to possess haemolytic effects [2]. The purposes of this study are to evaluate the antimicrobial properties of *Thymus vulgaris* extracts against the test organisms, (*Staphylococcus aureus* and *Escherichia coli*. The continuous evolution of bacterial resistance to current available antibiotics has necessitated the search for novel and effective antimicrobial compounds. Herbs and spices contain products of secondary metabolism such as phenolics, phenolic acid, quinines, flavonoids, tannins, which are rich sources of antioxidants and provide defense mechanisms to plants against predation by infectious organisms and insects.

Aim and Specific Objectives

Aim: The aim of the study is to determine the antimicrobial activities of *Thymus vulgaris* on *Staphylococlus aureus* and *Escherichia coli* and to determine the phytochemicals present in *thymus*

vulgaris responsible for the antimicrobial activities of sensitive.

Objectives: The specific objectives of this study is to identify the active phytochemicals in *thymus vulgaris and t*o determine the antibacterial activities of the phytochemicals

MATERIALS AND METHODS

Media used: Nutrient agar, MacConkey agar, Saboraud dextrose agar and Peptone broth.

Reagents: Ethanol, Methanol and Distilled Water

Test Organisms: *Staphylococcus aureus* and *Escherichia coli.*

Methods

Sterilization of Glass Wares

All glass wares used were washed, allowed to dry. Wrapped in foil paper and sterilized in hot air oven pre-heated at 100^{0}C for 1h then the oven was switched off and allowed to cool.

Preparation of Aqueous Extracts

About 50g of different thyme spices were weighed out using mechanical weighing balance and dispensed into sterile conical flask [3]. Then 500ml of sterile distilled water was measured out and dispensed into the flask with the ground thyme spice then covered with sterile foil paper. The mixture was agitated intermittently and left to soak for 48h at room temperature. Thereafter, filtered using whatman filter paper into sterile beaker. It was concentrated using water bath at 37^{0}C for 6h and then allowed to cool and stored in the refrigerator until when needed.

Preparation of Ethanolic Extract

About 50g of different thyme powder were weighed out using mechanical weighing balance and dispensed into sterile conical flask. Then 500ml of ethanol was measured out and dispensed into the flask with the ground thyme. Then it was covered with sterile foil paper. The mixture was agitated intermittently and left to soak for 48h. Thereafter, the mixtures was filtered through what man filter paper into sterile beaker and covered with perforated sterile foil paper [4]. The filterate was concentrated using rotary evaporator and stored in the refrigerator until when needed.

Preparation of Methanolic Extract

Total of 50g of different thyme powder was weighed out using mechanical weighing balance and dispensed into sterile conical flask. Then 500ml of methanol was measured out and dispensed into the flask with the ground thyme spice. Then it was covered with sterile foil paper [5]. The mixture was agitated intermittently and left to soak for 48h. Thereafter, the mixture was filtered using sterile whatman filter paper into sterile beaker and covered with perforated sterile foil paper. The filterate was concentrated using rotary evaporator and stored in the refrigerator until when needed.

Antimicrobial Disc Preparation

A perforator was used to perforate about 6mm of whatman filter paper, the disc were wrapped in a foil paper and placed in a beaker covered with foil and then sterilized using hot air oven at 80^{0}C for one hour and sterile disc were stored in the refrigerator until when needed.

Preparation of Sensitivity Disc

The sterilized filter discs were introduced with 20ml of each extract, left for 24h then harvested and dried in a hot air oven at 37^{0}C for few minutes

Media Preparation

Nutrient Agar

Total of 10g of nutrient agar power was weighed out and dispensed into 250ml of distilled water in a conical flask. Allowed to dissolve completely in distilled water for 10minutes with continuous agitation. Thereafter, sterilized using autoclave at 121^{0}C for 15 minutes. The sterilized media was allowed to cool to 48^0-50^{0}C and then dispensed into sterile petri dishes 20ml each. The plates were left to solidity and stored for use at 4^{0}C.

Preparation of Peptone Broth

Total of 1.9g of peptone was weighed into conical flask. Then dissolved in 125ml of distilled water, it was properly shaken to have a uniform mixture. Then 10ml each was aseptically dispensed into test tubes. Each test tube was properly sealed with cotton wool and foil paper and arranged into a beaker and sterilized

using autoclave at 121^{0}C for 15 minutes [6]. The sterilized media was allowed to cool, then stored in the refrigerator at 4^{0}C until when needed.

Microorganisms and Growth Condition

Bacterial Cultures of *Escherichia coli* and *Staphylococcus aureus* used in the present studies were kindly provided by the Department of Life sciences Culture Collection Center, University of Nigeria Teaching Hospital, Ituku Ozalla, Enugu State. Stock cultures were maintained at 4^{o}C and sub-cultured twice onto Nutrient agar followed by incubation at 37^{0}C under Optional Conditions for each microorganism.

Preparation of Broth Culture of the Test Organisms for Sensitivity Testing

The test organisms *Staphylococcis aureus* and *Escherichia coli* were inoculated into different already sterile peptone broth and incubated at 37^{0}C for 24 hours.

Sensitivity Test

The antibacterial assay of thyme spices were carried using three methods: pour plate, agar- well and disc diffusion methods.

Pour Plate Method

Total of 0.1ml of broth cultures containing *Staphylococcus aureus* and *Escherichia coli* was inoculated into 9.9ml of various extract; methanol, ethanol and aqueous extract of thyme spice using sterile micro pipette and incubated at 37^{0}C for 24h. Thereafter, 0.1ml of the preparation of the respective extracts and test organisms was inoculated into sterile petri dishes and overlaid with sterile molten nutrient agar [7]. Rotated clockwise and anticlockwise to mix properly. Then incubated for 72h at 37^{0}C with observation taken every 24 hours for activities.

Agar-Well Method

Serially diluted broth culture (10^{-4}) of the test organisms were inoculated into sterile nutrient agar plate and spread using a sterile swab stick. Sterile cork borer was used make 3 wells on each plate [8]. Thereafter, the wells were filled with each extract carefully into the wells. The preparations were incubated for 24-48hours at 37^{0}C. Then the preparation was observed for zone of inhibition and measured and recorded

Disc Diffusion Method

Serially diluted broth culture (10^{-4}) of the test organisms were inoculated into ready prepared nutrient agar plates, sterile swab stick was in spreading [9]. The already prepared sensitivity disc were placed at different locations on the plates and incubated for 24h at 37^{0}C. The zones of inhibition were observed and measured and recorded.

Determination of Minimum Inhibitory Concentration

The Minimum Inhibitory Concentration (MIC) was determined using agar dilution technique, two fold dilution was done. Total of 0.3ml of the diluted methanolic and ethanolic extracts were incorporated at varying concentration into the nutrient agar plate containing the test organism (*Staphylococcus aureus* and *Escherichia coli*. The plates were incubated at 37^{0}C for 24 hours. The lowest concentration of extracts that did not allow bacterial growth within the incubation period was taken to be the minimum inhibitory concentration (MIC). And diameter of zones of inhibition were read and recorded accordingly [10].

Total Aerobic Bacterial Count Serial Dilution

Nine milliliter (9ml) of sterile distilled water was placed in ten sterile test tubes. One milliliter (1ml) of the sample was placed in the first test tube with a sterile pipette. Thereafter, 1ml was transferred to the next test tube and serially equivalent quantities were transferred to all the test tube until the sixth tube make 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} dilutions.

Minimum Inhibitory Concentration (MIC) and 50% Growth Reduction

Minimum inhibitory concentration were determined for extracts displaying antimicrobial properties in screening studies, using a modified microdilution broth method in 96-well microplates [11] Briefly, the extracts were first dissolved in DMSO, then diluted in sterile water and tested over a range of concentrations against overnight broth cultures of *Escherichia coli* and *Staphylococcus aureus* grown in Nutrient agar. DMSO concentration never exceeded 5%V/V. Microplates was incubated at optimum growth

temperature for each bacterial strain and growth was monitor by measuring absorbance using microplate reader. The minimum inhibitory concentration was defined as the lowest concentration of the extract which completely inhibited the growth of a particular microorganism and the concentration which inhibited 50% of growth respectively. To determine the MIC, a spot on agar test was performed by transferring 5ml aliquots to nutrient agar plates followed by incubation at appropriate temperatures for 24 hours. Controls were set up with DMSO in amounts corresponding to the highest quality present in the test solution where appropriate. The experiments were replicated three times on different occasions with duplicate samples per replicate [12].

DETERMINATION OF PHYTOCHEMICAL ELEMENTS

Total Phenolic Content

Total phenolics content of the extract and the fractions were determined using the method of [13] with slight modifications. Calibration was prepared by mixing ethanol solution of thyme (1ml; 0025-0.400mgml-1 with 5ml folin – ciocalteu reagent (diluted tenfold) and sodium carbonate (4ml, 0.7m). Absorbance values were measured at 765nm using an UV-VIS spectrophotometer and the standard curve was plotted using thyme exract. One milliliter of each of the extract solution in methanol (5gl-1) was also mixed with the reagents above and after 30minutes, the absorbance was measured to determined the total phenolic contents. The total phenolic components in the extracts and fractures in thyme equivalent were calculated by the following formular; T = C.V/M where calculated by the following

Where T = Total phenolic content

Milligram per gram of sample extract, of thyme

Where C = Concentration of gallic acid established from the calibration curve, mgMl-1.

Where V = volume of extract, milliliter.

M = the weight of sample extract (g).

Total Flavonoids Content

Total flavoniod content was determined following a method by Park *et al.*, 2008. In 10ml test tube, 0.3ml of extract 3.4ml of 30% methanol, o.15ml of $NaNO_3$ (0.5m) and 0.15ml of $ALCL_3.6H_20$ (0.3m) were mixed. After 5 minutes, 1ml of NaOH was added. The solution was mixed well and the absorbance was measured against the reagent blank at 506nm. The standard solution (Oto 100mg.l) under the same procedure as earlier described. The total flavonoids were expressed as milligrams of rutin equivalents per gram of dried fraction.

Determination of Alkaloids

Total of 5g of the sample was weighed into a 250ml conical flask and 299ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 24 hours. This was filtered and the extract was concentrated on water both to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete [14]. The whole solution was allowed to settle and the precipitated was washed with dilute ammonium hydroxide and then filtered. The residue is the collected and alkaloid which was dried and weighed.

Determination of Total Saponins

The samples were grounded and 20g of each were put into a 250ml conical flask and 100ml of 20% aqueous ethanol were added. The samples were heated over a hot water both for 24 hours with continuous stirring at 55^0C. The mixture was filtered and the residue re-extracted with another 200ml of 20% ethanol. The combined extracted were reduced to 40ml over water both at about 90^0C. The concentration was transferred into a 250ml separating funnel and 20ml of diethyl other was added and shaken vigorously, the aqueous layer was recovered while other layer was discarded. The purification process was repeated. 60ml of n-butanol was added, the n-butanol extract were washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution as heated in a water bath after evaporation, the samples was dried in an oven to a

constant weight, and the saponin content was calculated [15] [16].

Determination of Glycosides

A total of 5g of the sample was weighed into a beaker and added 100ml of distilled water. Soak for 3 hours and filter to get the filterate [17]. Then pipette 1ml of filtrate into a test tube, followed by 2ml of 3, 5-Dinitro-salicylic acid and boil in a water bath for 10-15minutes, then cool the test tubes and add 10ml of distilled water and then read the absorbance at 540nm.

Test for Tannins

A quantity (50mg) of extract was boiled in 20ml of distilled H_20 and filtered. A few drops of 0.16 $Fecl_3$ was added in the filterate and observed for colour charge; brownish green or a blue black colouration was taken as evidence for the presence of tannins [18].

Test for Resins

A quality 0.12g of the extract was extracted with chloroforms and the extract concentrated to dryness. The residue was re-dissolved in 3ml acetone and 3ml concentrated HCL added. This mixture was heated in a water bath for 30 minutes. A pink colour that changes to magnets red indicates the presence of resins [19] [20].

Test for Oil

About 0.1 g of the extracts was passed pressed between filter paper and filter paper was observed [21]. Translucency of the filter paper indicates the presence of the oils.

Test for Terpenoids

Presence of terpenoids in extracts was carried out by taking 5ml (1mg/ml) of extracts and mixed with 2ml of chloroform, followed by 3ml of conc. H_2SO_4. A reddish brown colouration at the interface confirmed the presence of terpenoids.

Test for Steroids

A volume of (5 drops) of conc. H_2SO_4 was added to 1ml of the extract in a test tube [22]. A red coloration indicates the presence of steroid.

EXTRACTION OF PHYTOCHEMICALS

Tannins Extraction

The extract was poured into a column containing sephadex LH_20 (15X45cm) that had been equilibrated in 95% ethanol. The column was eluted with 95% ethanol until UV-absorbance (280nm) indicated that no material was eluting [23] [24]. The ethanol fraction that contained other polyphenolics was determined to have no enzyme inhibiting ability and was discarded. The column was then eluted with 50% aqueous acetone until absorbance at 420nm. The eluted material was exposed to warm air current to remove the acetone, after which the remaining solution was freeze dried. The resulting fluffy brown powder was stored at room temperature until when needed [25].

Saponin Extraction

20grams of powdered sample was added to a conical flask and 100ml of 20% ethanol was added to it. The sample was heated at 55^0C on the water bath for 4 hours with continuous shaking. The sample was filtered 4hours and the residue obtained was re-extracted with 200ml of 20% ethanol [26] [27].

The sample obtained was heated until the volume was reduced to 40ml. The concentrated volume obtained was transferred into the separating funnel and after addition of 20ml of diethyl ether, the whole volume was vigorously shaken in the separating funnel, to obtain two layers. The separating funnel was put calm for a while. The lower aqueous layer was collected while the upper layer was discarded. The 60ml n-butanol was added into the aqueous portion and the combined n-butanol extract was washed with 10ml of 5% sodium chloride solution. The final solution obtained was kept in a hot water bath until the solvent was fully evaporated and the saponins obtained were dried using oven at low temperature and kept until when needed [28].

Alkaloids Extraction

The extract was concentrated using rotary evaporated till a concentrated mass is formed. The P^H was adjusted to 9 by the addition of concentrated ammonium hydroxide solution. It was then extracted with chloroform (50ml) three times. During extraction, the contents were subjected to vigorous shaking [29]. The chloroform layer was

separated using separating funnel. The chloroform portion was concentrated using rotary evaporator and the eluate was collected and kept until when needed.

Terpenoids Extraction

The extract was concentrated under a nitrogen stream without drying the samples. Then the concentrated sample was loaded into a small silica column. The sample was allowed to be absorbed into the column, then about 1-3ml of hexane was added and the column eluate was collected [30]. The eluate was dried under a nitrogen stream and stored until when needed.

Sensitivity Testing of Phytochemicals

The assay was conducted using agar well technique. Serially diluted broth culture (10^{-4}) of test microorganism was inoculated into sterile nutrient agar and spread using a sterile swab stick. Sterile cork borer was used to make three wells on each plate. Thereafter 0.1ml of phytochemicals were carefully pipetted into the wells [31] [32]. The dissolution of phytochemicals was aided by 1% (v/v) DMSO. The preparations were incubated for 24 hours at 37^0C. Then antibacterial activities were evaluated by measuring zones and recording the zones of inhibition.

Statistical Analysis

Data were analyzed using Microsoft Excel 2000 (Microsoft Coporation, Redmund, WA, USA) and Graphpad Prism Version 4.03 statistical software (Graphpad Software, San Diego California USA). Analysis of Variance (ANOVA) was carried out, and statistical differences ($P< 0.05$) between means of pairs were resolved by means of confidence intervals using Tukey's tests.

RESULTS

Tables 1, 2 and 3 showed the results of the antimicrobial sensitivity of different concentrations of methanolic, ethanolic and aqueous extracts of thyme on *Escherichia coli*. The ethanolic and methanolic extracts were the most effective on *Escherichia coli* at 100mg/ml concentration with the zone of inhibition of diameter 26mm using Disc diffusion method, 21mm using Agar-well method and aqueous extract did not show any zone of inhibition in both the Agar-well method and Disc diffusion method on two tested microorganisms [33]. The pour plate method of the aqueous extract recorded 217 and 197 colonies of *Escherichia* coli and *Staphylococcus aureus* respectively.

Tables 4, 5 and 6 showed the results on the antimicrobial sensitivity of different concentration of methanolic, ethanolic and aqueous extracts of thyme on *Staphylococcus aurous.* The ethanolic and methanolic extracts of thyme have antimicrobial properties. The methanolic extracts was also the effective on *Staphylococcus aureus* at 100mg/ml concentration with the zone of inhibition of diameter 24mm using Disc diffusion method, 16mm using Agar-well method, and no colony formed using pour plate method. While the aqueous extract did not show any zone of inhibition both the Agar-well method, Disc diffusion method and pour plate method respectively. The pour plate method recorded numerous colony [34].

Table 7 showed the results of the minimum inhibition concentration of thyme on *Staphylococcus aureus*. The ethanolic extract of thyme has a minimum inhibitory concentration of 0.78mg/ml while the methanolic extract recorded 0.78mg/ml.

Table 8 showed the results of the minimum inhibition concentration of thyme o *Escherichia coli.* The ethanolic extract of thyme has a minimum inhibition concentration of 0.78mg/ml while the methanolic extract recorded 0.78mg/ml respectively.

Table 9 showed the results of the quantitative phytochemical screening of thyme.

Table 10 showed the results of the quantitative phytochemical screening of thyme. The result revealed the presence of tannins, alkaloids, terpenoids, saponins, phenols, flavonoids, steroids and glycosides.

Table 11: Shows the results of the sensitivity testing of phytochemicals on test organisms

Table 1: Antibacterial Activities of *Thymus vulgaris* on *Escherichia coli* Using Disc Diffusion Method

Test Organisms	Extract	Zone of Inhibition	Remarks
Escherichia coli	Ethanol	14.00mm	Intermediate
	Methanol	26.00mm	Sensitive
	Aqueous	-	Resistant

Key: - Resistant

Table 2: Antibacterial Activities of *Thymus vulgaris* on *Staphylococcus aureus* Using Disc Diffusion Method

Test Organisms	Extract	Zone of Inhibition	Remarks
Staphylococcus aureus	Ethanol	14.00mm	Intermediate
	Methanol	24.00mm	Sensitive
	Aqueous	-	Resistant

Key: - Resistant

Table 3: Antibacterial Activities of *Thymus vulgaris* on *Escherichia coli* Using Agar-well Method

Test Organisms	Extract	Zone of Inhibition	Remarks
Escherichia coli	Ethanol	17.00mm	Intermediate
	Methanol	21.00mm	Sensitive
	Aqueous	-	Resistant

Key: - Resistant

Table 4: Antibacterial Activities of *Thymus vulgaris* on *Staphylococcus aureus* Using Agar-well Method

Test Organisms	Extract	Zone of Inhibition	Remarks
Staphylococcus aureus	Ethanol	14.00mm	Intermediate
	Methanol	16.00mm	Intermediate
	Aqueous	-	Resistant

Key: - Resistance

Table 5: Antibacterial Activities of *Thymus vulgaris* on *Escherichia coli* Using Pour Plate Method

Test Organisms	Extract	CFU/ML	Remarks
Escherichia coli	Ethanol	No growth	Sensitive
	Methanol	No growth	Sensitive
	Aqueous	219	Resistant

Key:
UCF Uncountable colonies formed
CFU/ML Colony Forming Units Per ML

Table 6: Antibacterial Activities of *Thymus vulgaris* on *Staphylococcus aureus* Using Pour Plate Method

Test Organisms	Extract	CFU/ML	Remarks
Staphylococcus aureus	Ethanol	No growth	Sensitive
	Methanol	No growth	Sensitive
	Aqueous	197	Resistant

Key:
UCF Uncountable Colonies Formed
CFU/ML Colony Forming Units Per ML

Table 7: Results on Minimum Inhibition Concentration of *Thymus vulgaris* on *Escherichia coli*

Test Organisms	Extract	MIC (Mg/ML)	Zone of Inhibition	Remarks
Escherichia coli	Ethanol	0.78	20.00	Sensitive
	Methanol	0.78	18.00	Sensitive

Table 8: Results on Minimum Inhibition Concentration of *Thymus vulgaris* on *Staphylococcus aureus*

Test Organisms	Extract	MIC (Mg/ML)	Zone of Inhibition	Remarks
Staphylococcus aureus	Ethanol	0.78	22.00	Sensitive
	Methanol	0.78	20.00	Sensitive

Table 9: Results on Quantitative Phytochemical screening of *Thymus vulgaris*

Phytochemicals	Weight of sample(g)	% Phytochemicals	Remarks
Glycosides	5.000	0.0150	Intermediate
Alkanoids	5.000	2.852	Intermediate
Saponins	5.000	479	High
Tannins	5.000	1.010	Intermediate
Flavoniods	5.000	0.126	Low
Phenol	5.000	0.10672	High
Moisture Content	1013.9	10.49	Intermediate
Ash	1016.8	4.58	Intermediate
Protein Content	0.504	10.50	Intermediate
Oil Content	5.0439	54.579	High
Crude Fibre	2.048	12.82	Intermediate

Table 10: Results on Qualitative Phytochemical Screening of *Thymus vulgaris*

Phytochemical Screening	Results	Remarks
Saponins	++	Intermediate
Tanin	+++	High Concentration
Flavonoid	+	Trace Concentration
Glycosides	++	Intermediate
Alkaloids	++	Intermediate
Steroids	+	Trace Concentration
Terpeniods	+++	High Concentration
Phenols	+	Low
Resin	-	Absent

Table 11: Results of the Sensitivity Testing of Phytochemicals on Test Organisms

	Zone of Inhibition(mm)			
Phytochemical	*E.coli*	*S. aureus*	Remarks	
Saponins	---	---		Resistant
Alkaloids	---	---		Resistant
Tannin	19.00	21.00	Sensitive	
Terpenoids	17.00	22.00	Sensitive	

KEY: -= No zone of inhibition

DISCUSSION

The type of solvent used to extract herbs and spices appeared to have major impact on their antimicrobial activity. Generally, extraction of herbs and spices with ethanol and methanol resulted in a product with greater over all antimicrobial activity than extraction with water as aqueous extracts of all the herbs and spices displayed little or no antimicrobial activity against any of the bacteria tested [35] [36]. This is probably due to the fact that although some solvent were removed from extracts by evaporation, different chemical compounds where extracted using various solvents and most of the components with antimicrobial proportion are aromatic or saturated organic compounds which are generally more soluble in solvents such as ethanol or methanol [37]. In another study, on antimicrobial activity of Australian Spices, water, ethanol and methanol were also the solvents used for extraction and was observed that the aqueous extracts displayed little of no antimicrobial activity [38]. In the present study, water was evaporated at 70^0C, therefore it is possible that heating may have inactivated some compounds displaying antimicrobial properties resulting in the extracts with lower activities. [39] studied the antimicrobial properties of several thyme species and found despite a common botanical origin, the chemical composition and antimicrobial activity varied considerably. Moreover, antimicrobial properties an differ significant within the same herb or spices as the proportions of individual components in essential oil of plants are affected by genotype.

The total phenolic data were difficult to compare directly due to differences in extraction methods used. According to [40], phenolic substances present in spices may be responsible for antimicrobial activity and [41] said that antibacterial activity of selected dietary spice and medicinal extracts was closely related to the concentration of the phenolic compounds hence extracts with high phenolic contents has a profound antimicrobial properties against all bacteria tested such as the *Staphylococcus aureus* and *Escherichia coli* .

In the light of trends towards selection of natural antimicrobial compounds, some of these extracts have the potential to extend the shelf-life or improve the safety of foods including ready meals with reduced salt content [42] [43]. However, antimicrobial activity of spice derived preparations has been reported to diminish within food systems [44]. Therefore, further studies on the efficacy of these extracts in a range of ready meal products as well as evaluation of potential interactions of antimicrobial compounds with components of food matrices, such as fats, carbohydrates, proteins, pH are warranted.

CONCLUSION

The commercially essential oils from thyme exhibit promising antimicrobial effects against selected food borne and food spoilage bacteria which can be attributed to the presence of the principle bioactive constituents especially thymol. These investigated essential oils and their main active components could be potential candidates to be used as natural alternatives for further application in food preservation to retard or inhibit the bacterial growth and for safety and to extend the shelf life of the food products. However, the confirmation of thee essential oils in food stuffs need to be evaluated.

RECOMMENDATION

Thyme essential oil strongly inhibited the growth of the clinical strains of bacteria tested. The use of phytochemicals based on an investigated essential oil from thyme in the prevention and treatment of various human infections may be reasonable. Further research on the application in the food sector to improve food safety by the total elimination of *Escherichia coli* and *Staphylococcus aureus* and particularly, interactions with other food

ingredient necessitate more thorough investigation.

I therefore recommend that *Thymus valgaris* should be used for therapeutic purposes and alternative medicine.

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