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IN VITRO ANTIMICROBIAL, ANTIBIOFILM AND ANTIPHAGE ACTIVITY OF THYME (*THYMUS VULGARIS*)

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Abstract

The present study was conducted to estimate the antimicrobial activity of *Thymus vulgaris* water extract and essential oil against multidrug resistant clinical isolates (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*). Antiphage activity of thyme water extract was studied by phage inhibition assay. Thyme extract was prepared with water while oil was extracted from dried thyme plant using steam distillation. The antimicrobial activity and minimum inhibitory concentration (MIC) were evaluated by agar well diffusion method. The mechanism of action of thyme water extract against bacterial cells and biofilm formation was studied by scanning electron microscopy. Thyme oil was fractionated by column chromatography (normal phase chromatography) and thin layer chromatography (TLC). Bioactivity of oil fractions against bacteria was also studied. Thyme in both forms (oil and water extract) was effective against all the tested isolates however, Gram positive bacterial and *Candida* strains were found more sensitive compared to Gram negative bacterial strains. Minimum inhibitory concentration of thyme oil for *Candida albicans* and *Staphylococcus aureus* was recorded to be 0.2 mg/ml. Scanning electron microscopy results revealed disruptive properties of thyme against biofilm formation and significant distortion of bacterial cell morphology. Reduction in phage (in terms of plaque forming units percentile i.e. pfu) showed thyme water extract possessed antiviral potential.

Key words: Antimicrobial, *Thymus vulgaris*, Biofilm, Multidrug resistance, Antiphage activity.

Introduction

Drug resistance enables microbes to become resistant (to which they were once sensitive) to antimicrobials. It is a natural phenomenon that usually occurs due to extensive use of drug (s) against the pathogens. There are many mechanisms that cause drug resistance including membrane impermeability (via efflux mechanisms), mutation, horizontal resistance gene transfer, enzymatic degradation or alteration of drug target. One of the most common mechanisms of drug resistance is the ability of planktonic cells to form biofilm. Biofilms (formed by adsorption of dividing cells to different biotic and abiotic surfaces) play an important role acquiring resistance to antimicrobials and immune defense systems. Biofilm constitutes the resistant structures called extracellular polymeric substances (EPS) of microbial communities produce thick layer of extracellular polymeric substance containing nucleic acid, protein, lipids, and polysaccharides (Salimena *et al*., 2014). This property also helps pathogens to cause chronic infections as they resist the action of antibiotics, disinfectant chemicals, and phagocytosis. According to National Institutes of Health, 60 % of microbial infections are caused by biofilms (Lewis, 2001). Biofilm associated infections include urinary tract infection caused by *E. coli*, catheter related infections by *S. epidermidis*, middle ear infection in children by *H. influenzae*, pulmonary infections by *P. aeruginosa* (Stephens, 2002) and tooth decay by *S. mutans* (Nakano, 2018).

Drug resistance has been an issue of concern to public health globally because of the massive use of antibiotics. The need for cost effective alternative therapy (with fewer side effects), drugs with strong bioactivity against drug resistant microbes has increased. Medicinal plants are considered cheap (Majeed *et al*., 2019) and effective alternative to antibiotics to combat drug resistant pathogenic organisms**.** Researchers have been studying different medicinal plants to explore their effect on pathogens, toxicity potential and ease of availability. Thyme (*Thymus vulgaris*) is one of the plants with magnificent medicinal properties.

Thymus vulgaris (commonly called Thyme) is a perennial herb belonging to the Lamiaceae family that usually grows in Mediterranean region (Alkowni *et al.,* 2017). It has varied health benefits. It possesses antiseptic properties, soothes the skin by easing out skin rashes, scar wounds, sores, relief in burns and used to treats acne and eczema. It is also used in cooking (for seasoning), biopesticide (Panezai *et al.,* 2019), preservation (Khalili *et al.*, 2015) and making tea. It also stabilizes the oil with its antioxidant property by preventing lipid oxidation (Zaborowska *et al.*, 2012). It has been used as embalming agent, flavoring agent for cheese and beverages, cures melancholic conditions, skin lesions and respiratory disease (from ancient times). Thyme has many health benefits because of its action against harmful microbes. It had also been reported to be bioactive against fungi (Šegvić *et al*., 2007), viruses (Nolkemper *et al*., 2006), bacteria and tumors (Fayad *et al*., 2013).

This research highlights the antimicrobial potential of thyme (*Thymus vulgaris*) water extract and oil against MDR bacterial strains (and biofilm formation) and *Candida sp* isolated from clinical specimens.

Materials and Method

Sample collection: Cultures (MDR) of *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans* were procured from Dr. Essa Laboratory and Diagnostic Center, Karachi. Isolates were identified on the basis of cultural characteristics, microscopy, and biochemical tests and maintained on Nutrient Agar (bacterial) and Sabouraud Dextrose Agar (fungal) at 4℃.

Antibiotic sensitivity test: All the isolates were tested for their antibiotic sensitivity to confirm their multidrug resistant profile. Antibiotics used for sensitivity test included: amikacin (AK), cefuroxime (CXM), amoxicillin (AML), cefoxitin (FOX), erythromycin (E), nitrofurantoin (F), vancomycin (V), doxycycline (DOX), chloramphenicol (C), ceftriaxone (CRO), ceftazidime (Caz), sparfloxacin (SPX), enoxacin (ENX), ofloxacin (OFL), tetracycline (TE), ciprofloxacin (CIP), trimethoprim/ sulfamethoxazole (SXT), nalidixic acid (NA), amoxicillin/calvulanic acid (AMC), norfloxacin (NOR), cefotaxime (CTX), tobramycin (NN), gentamicin (CN), aztreonam (ATZ), meropenem (MEM), imipenem (IPM), piperacillin/ tazobactam (TZP), fluconazole (FCA), polymyxin (PB) and nystatin (NS). Isolates were considered MDR that were found resistant to at least one antibiotic in 3 or more categories of antimicrobials (Magiorakos *et al*., 2012).

Plant collection and extraction: The dried plant (s) of thyme *(Thymus vulgaris*) were obtained from local market of Saudi Arabia. The whole plant was used to prepare thyme water extract and the essential oil.

Thyme water extracts preparation: For water extract, 40 g of dried powdered thyme was soaked in 200ml of distilled water and boiled for 15 to 30 min. The solution was cooled at room temperature and then stored at 4℃ for 24 hours. The extract was filtered and was evaporated by Rotavap (Mostafa *et al.*, 2018).

Phytochemical tests: The methods described by Gurav *et al.*, (2014) and Hossain *et al.*, (2013) were used to identify the presence of steroids, phenols, flavonoids, saponin, and alkaloids in thyme water extract.

Thyme oil extraction: Thyme oil extraction was carried out in a Clevenger apparatus for 3 hrs, yielding 1% oil from water extract. The extracted oil was separated from water and stored in dark brown bottle at 4℃ (Anzlovar *et al.*, 2014).

Agar well diffusion: Bioactivity of thyme water extract and oil was done by agar well diffusion method. Mc Farland 0.5 culture was prepared and seeded on Muller Hinton Agar (MHA). Wells of 9 mm (diameter) were punched on media, and 100 µl of thyme oil and water extract were dispensed into the respective wells. The plates were incubated at 37℃ for 24hr and inhibition zones (mm) were measured. The protocol was repeated in triplicate (Obeidat *et al*., 2012).

Minimum inhibitory concentration (MIC): The concentration of thyme was evaluated by agar well diffusion method (Mostafa *et al.*, 2018). A stock solution of thyme oil (7 mg/ml) and thyme aqueous extract (160 mg/ml) was serially diluted (two fold). Thyme oil was diluted with toluene while thyme water extract was diluted with distilled water. Inoculum of 0.5 Mc Farland was swabbed on MHA and each dilution was loaded into the respective wells followed by incubation for 24hr at 37℃ for ultimate recording of inhibition zones.

Biofilm inhibition assay: Thyme water extract effect on biofilm formation (by clinical *S. aureus)* was studied. Corning tube was poured with 30 ml of BHI broth, 800 µl of thyme water extract, and 500 µl of overnight bacterial culture. Coverslip was placed inside the corning tube and incubated for one week at 37℃. Where after a week the coverslip was removed and stained with 1% crystal violet and observed by scanning electron microscope. (Sample were given for SEM (JSM-6380), which was performed in vaccum and created images of 1000x to 3300x magnifications. SEM images were studied directly). Results were compared with the control (*Staphylococcus* associated biofilm without thyme water extract). A modified method (as per lab condition) of Panda *et al.*, (2016) was used for this purpose.

Phage inhibition assay: The activity of thyme water extract against viruses (lytic coliphage against *E. coli* was isolated as per Dallal *et al.*, (2016)) were estimated by phage inhibition assay. Phages (*E. coli*) were isolated from raw sewage water. Filtered phages 10^{-4} dilution (100 µl) was added to 3.5 ml of molten agar, 500 µl of log-phase *E. coli* culture and different concentration (25 µl, 50 µl, 75 µl and 100 µl) of thyme water extract respectively. The mixture was shaken manually and loaded over the solid media (Nutrient Agar). Each plate was labeled according to concentration of thyme water extract while the control plate only contained the mixture of filtered phages and *E. coli* culture. All the plates were incubated at 37℃ for 24 hrs. Plaque forming unit (pfu) in each plate was counted and compared with the control. The protocol was repeated in triplicate and the graph was plotted accordingly (Chao *et al.*, 2000).

Thyme oil fractionation and identification: Column chromatography (column length = 613cm, diameter = 3cm, resin = glass) was performed to separate thyme oil fractions against the solvent (toluene: ethyl acetate 97:3 ratio) on silica. Fractions were collected in test tubes and identified on TLC (commercially prepared silica plate by MERCK). The separated fractions of oil were visualized by iodine crystals. Rf value of each fraction was calculated (Wagner and Bladt, 1996; Ashnagar *et al*., 2011). Tests were repeated thrice.

Antimicrobial activity of fractions: The antimicrobial activity of each fraction obtained from thyme oil was estimated by agar well diffusion (method). The fraction that showed inhibitory effect was identified on TLC plate with thymol as positive control. Rf value of thyme oil fraction and thymol was calculated (Wagner & Bladt, 1996). Antimicrobial activity assay for thyme oil fractions and thymol was evaluated (Kalemba & Kunicka *et al*., 2003).

Results

Phytochemical profile of thyme: Phytochemical analysis results indicated the presence of phenols, flavonoids, steroids, and saponins, while alkaloids were not detected in aqueous extract of thyme (Table 1) shows the presence of phytochemicals in thyme aqueous extract.

Table 1. Phytochemical profile of thyme water extract.

Secondary metabolite	Reagents	Results
Alkaloids	Dragondroff's reagent	-ve
Phenolic compounds	Ferric chloride	+ve
Flavonoids	NaOH and diluted acid	+ve
Steroids	Chloroform and H_2SO_4	+ve
Saponins	Shaking (manual)	+ve

+ve indicates the presence of biologically active compounds
-ve indicates the absence of biologically active compounds

Antibiogram of clinical isolates: Antibiotic susceptibility test of *E. coli, P. aeruginosa,* and *S. aureus* showed their multidrug resistance (resistance to more than three antibiotics) (Table 2). *P. aeruginosa* was found to be the most resistant among the others.

Effect of thyme oil and aqueous extract against bacterial isolates and Candida: The antimicrobial activity of thyme oil and thyme water extract was monitored against the pathogenic strains (*S. aureus*, *E. coli*, *P. aeruginosa*) and the *C. albicans* (Clinincal samples). Zones of inhibition were recorded (by thyme oil) against *S. aureus* and *C. albicans* as 49 mm, *E. coli* 35 mm and *P. aeruginosa* 31 mm, while thyme water extract produced inhibition zone of 35 mm against *S. aureus*, 20 mm against *P. aeruginosa*, 24 mm against *E. coli* and 30 mm against *C. albicans*. (Figs. 1 & 2) showed the bioactivity of thyme oil and water extract against clinical isolates.

Minimum inhibitory concentration: All the pathogenic strains were sensitive to thyme oil with MICs ranged from 0.2 mg/ml to 3.5 mg/ml. The maximum activity of thyme oil was recorded against *S. aureus* and *C. albicans* with a MIC of 0.2 mg/ml followed by *P. aeruginosa* (MIC of 0.8 mg/ml) and *E. coli* (MIC 3.5 mg/ml). Similarly, the minimum inhibitory concentration of thyme water extract against *S. aureus* was recorded to be 40 mg/ml, for *C. albicans* and *E. coli* to be 80 mg/ml and 160 mg/ml for *P. aeruginosa.*

Thyme (water extract) effect on bacterial cells morphology and biofilm production: The activity of thyme (*Thymus vulgaris*) against the isolated clinical bacterial cells and biofilm formation was studied with the help of scanning electron microscopy. Differences in morphology of the tested bacterial cells were observed (compared with the control). Fig. 3A showed normal cells of *Staphylococcus* while (Fig. 3B) showed cracks, holes and deformation in cells after treatment with thyme extract. Biofilm formation (by *Staphylococcus)* was observed by scanning electron microscopy which showed adherence of *Staphylococcus* cells on the surface to form a smooth layer (Fig. 4A). On the other hand thyme (water extract) treated biofilm producing *Staphylococcus* cells showed a decrease in number of adhering cells and inhibition of biofilm formation (Fig. 4B).

Antiphage activity of thyme water extract: Plaque forming units (pfu) were scored in the control plate and compared with the plates containing different concentrations of the extract. Thyme water extract showed significant inhibition of phages. Phage particles (isolated lytic coliphage against *E. coli*) were inactivated in terms of reduction in plague forming units (pfu). Accordingly, plaque forming units (pfu) percentage was decreased down to 64% at 100 µl (in water) of the drug (*Thymus vulgaris*). (Fig. 5) showed the reduction in plague farming units after treatment of coliphage with thyme water extract.

Table 2. Antibiogram of Escherichia coli, Pseudomonas and Staphylococcus strains.

Antibiotics	*Escherichia coli*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
Amikacin (AK)	S	R	S
Amoxicillin (AML)	R	R	R
Amoxicillin/ clavulanic acid (AMC)	R	R	S
Aztreonam (ATM)	-	R	-
Cefotaxime (CTX)	R	R	S
Ceftazidime (CAZ)	R	R	-
Cefuroxime (CXM)	R	R	S
Ceftriaxone (CRO)	R	-	-
Chloramphenicol (C)	-	R	-
Ciprofloxacin (CIP)	R	S	R
Doxycycline (DOX)	R	-	R
Enoxacin (ENX)	R	R	R
Erythromycin (E)	-	-	R
Gentamicin (CN)	S	S	
Imipenem (IMP)	S	R	S
Meropenem (MEM)	-	R	-
Nalidixic acid (NA)	R	R	R
Nitrofurantoin (F)	S	-	-
Ofloxacin (OFL)	R	-	R
Piperacillin/tazobactam (TZP)	S	R	S
Sparfloxacin (SPX)	R	R	R
Tetracycline (TE)	R	-	-
Tobramycin (NN)	S	-	S
Trimethoprim/ sulfamethoxazole (SXT)	R	R	R
Vancomycin (VA)	R	R	S

Key: R= Resistant, S= Sensitive, - = Indicates antibiotic NT
Susceptible= > 20mm diameter, Intermediate = 15-19mm diameter, Resistant = <14mm

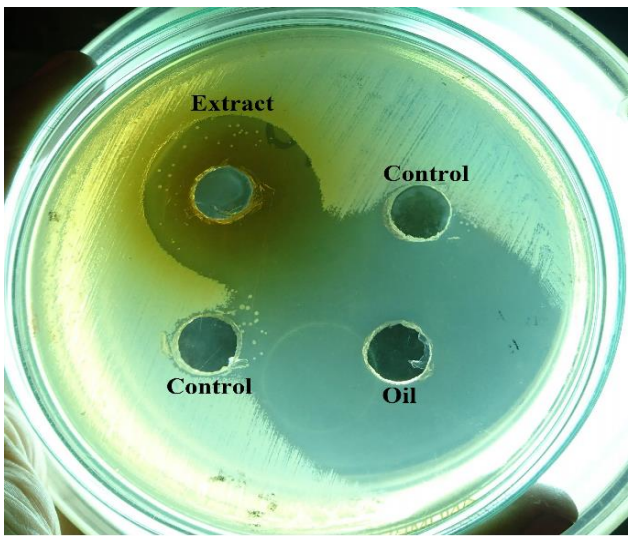


Fig. 1. Antimicrobial activity of thyme oil and water extract targeted against *C. albicans*.

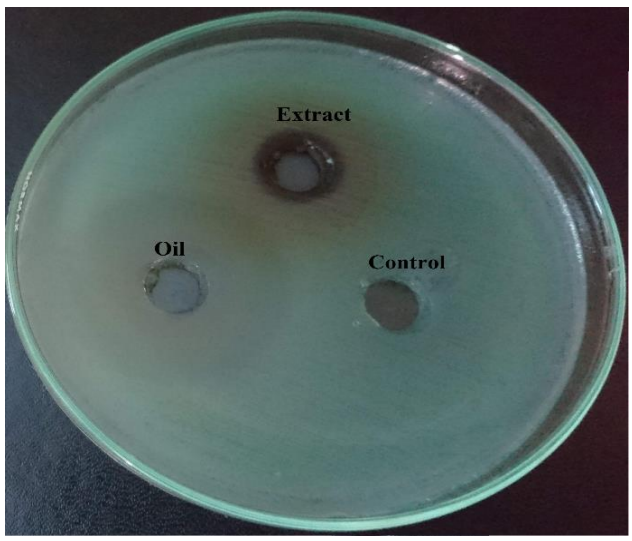
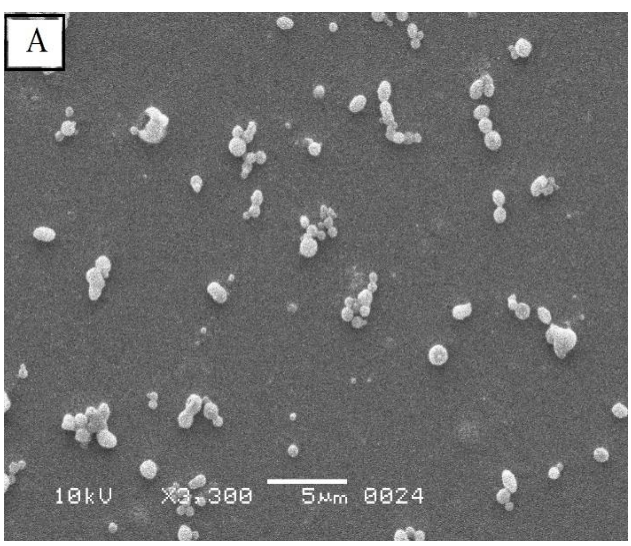


Fig. 2. Antimicrobial activity of thyme oil and water extract directed against *P. aeruginosa*.



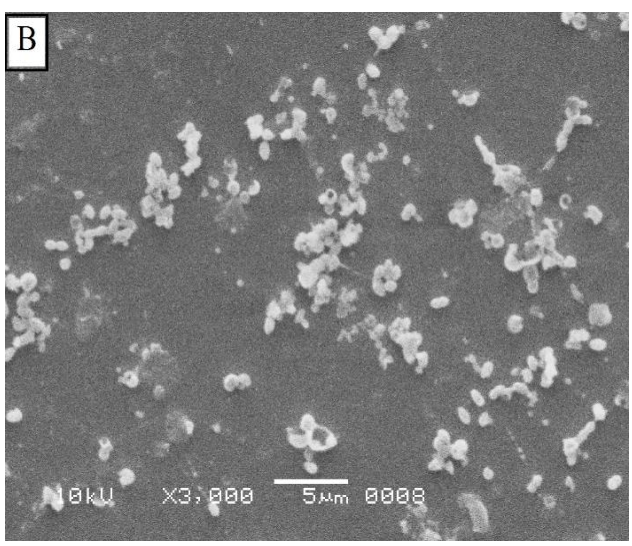
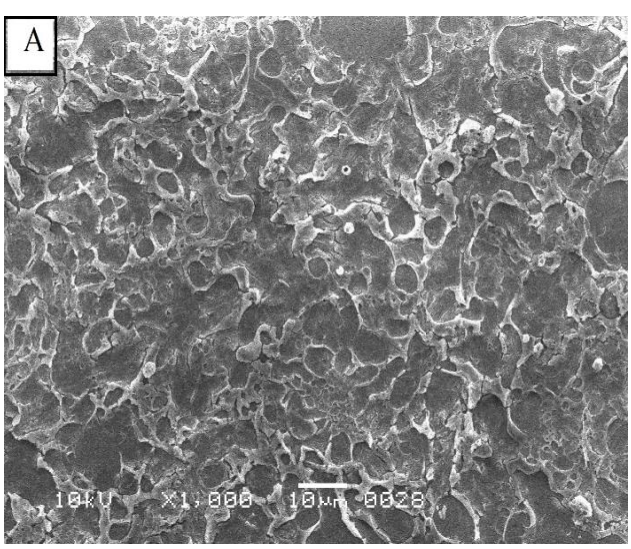


Fig.3. (A) Normal (untreated) cells of S. aureus after 24hr incubation, (B) Deformed S. aureus cells after treatment with thyme water extract after (24hr incubation).



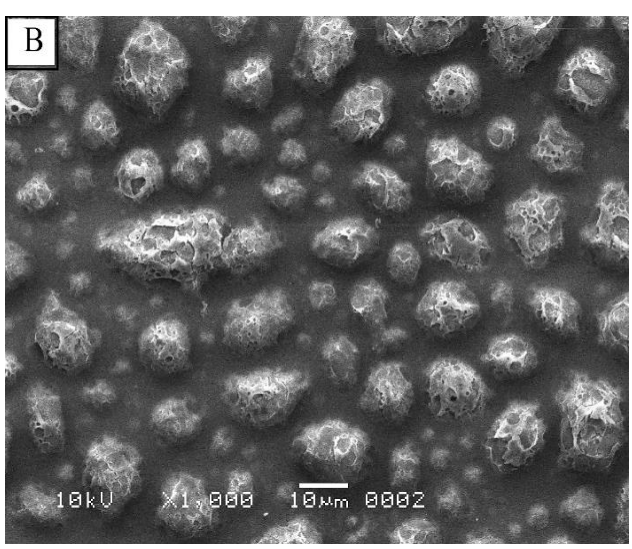


Fig. 4. Scanning electron microscopy (A) Smooth normal biofilm formation by *S. aureus* cells (1week incubation), (B) No biofilm formation in the presence of thyme water extract (1 week incubation).

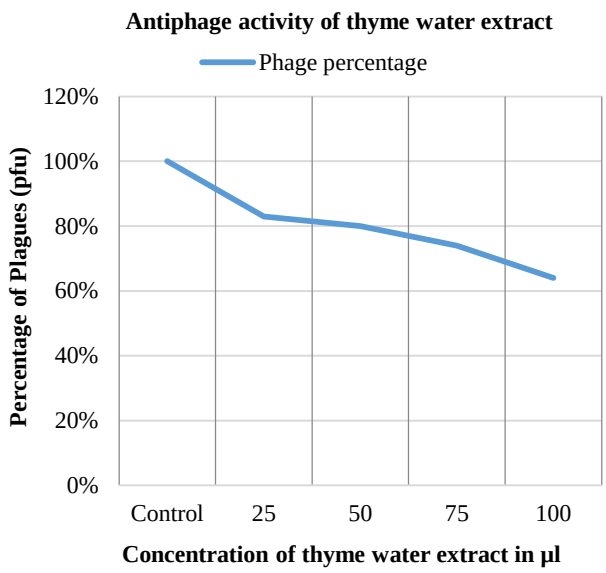


Fig. 5. Graphical presentation of effect of thyme water extract on plaque formation units.

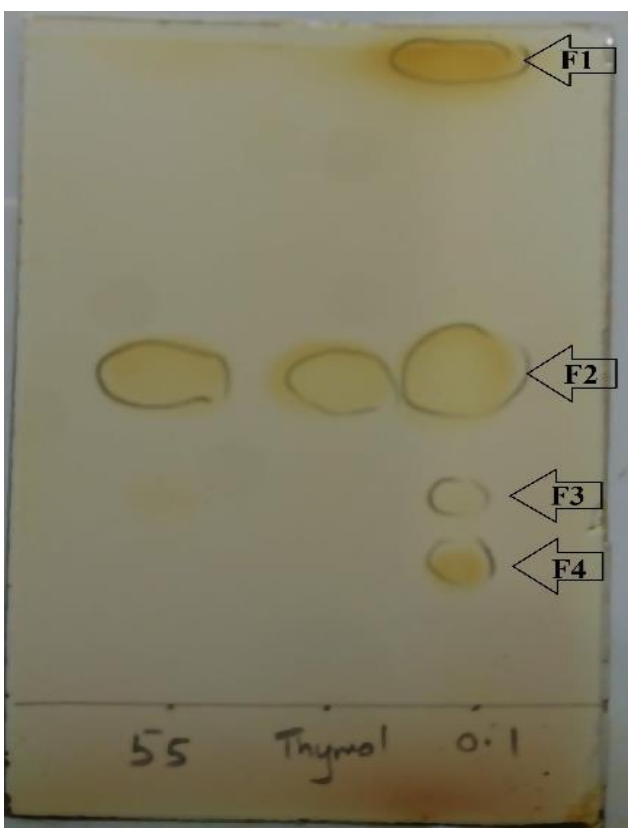


Fig. 6. Thymol compared with the separated fractions of thyme oil on TLC.

Column chromatography and thin layer chromatography: Four bands were observed in the column; these fractions were collected and identified by TLC (each fraction with a different Rf value). (Fig. 6) shows thyme oil fractions and thymol on TLC plate. Rf values of thyme oil fractions (obtained) were compared with the Rf value of thymol. The Rf value of thymol was similar to fraction (2). Table 3 depicts Rf values of all the fractions and Thymol (control).

Antimicrobial activity of thyme oil fractions: The antimicrobial activity of four fractions was assessed by agar well diffusion. Accordingly only fraction F2 exhibited zone of inhibition (35 mm) against *S. aureus*. (Fig. 7) showed the antimicrobial activity of oil fractions. The antimicrobial activity of thymol, fraction F2 and thyme oil was also monitored, whereby, all the three (thymol, thyme oil, and fraction F2) inhibited growth of *S. aureus* (Fig. 8). It is suggested that main bioactive component of thyme oil is thymol.

Table 3. Rf values of thyme oil fractions and thymol.

Sample	Retention factor (Rf) values
Thyme oil Fraction 1	0.91
Thyme oil Fraction 2	0.56
Thyme oil Fraction 3	0.2
Thyme oil Fraction 4	0.18
Thymol (control)	0.55

Discussion

Phytochemicals in thyme water extract: The phytochemical profile of thyme water extract was estimated by different chemical reagents, which showed the presence of phenolic compounds, flavonoids, saponins, and steroids (alkaloids could not be traced in aqueous extract). These results are in agreement with the observations of Alsaidy *et al.,* (2014) who observed the presence of tannins, saponins, flavonoids and carbohydrates in thyme water extract. All these secondary metabolites are associated with different activities resembling antibiotics, antioxidants and anticancer agents (Hussein & El-Anssary, 2018).

Antimicrobial activity of thyme oil and water extract against bacterial and fungal isolates: Thyme oil showed better inhibitory action against the pathogens than the thyme water extract. It could be due to the fact that oil contains high phenolic content while thyme water extract may lose most of essential active compounds during grinding and boiling. Further, both the forms of thyme showed better bioactivity against Gram positive (*S. aureus*) Gram negative (*E. coli* and *P. aeruginosa*) and *C. albicans*, but thyme water extract was less effective against Gram negative isolates (*E. coli* and *P. aeruginosa*). *Pseudomonas* was found the most resistant among all the isolates, but thyme oil was found active against this pathogen even at very low concentration (0.8 mg/ml). These results were in agreement with studies by Burt (2004), Nzeako (2006) and Rota (2008). Nzeako (2006) evaluated the bioactivity of thyme against *S. aureus, P. aeruginosa, E. coli*, *S. pyogenes*, *Corynebacterium* sp., *Salmonella* sp., *B. fragilis* and *C. albicans* and observed thyme oil was active against all the isolates while the aqueous extract was found effective only against *S. aureus*. Burt (2004) suggested that plant essential oils were more effective against Gram positive (*L. monocytogenes, B. cereus* and *S. aureus)* while less effective against Gram negative strains (*S. typhimurium, E. coli O157:H7, S. dysenteriae)*. Rota (2008) suggested thyme as a potential antimicrobial agent with relevance to food industry.

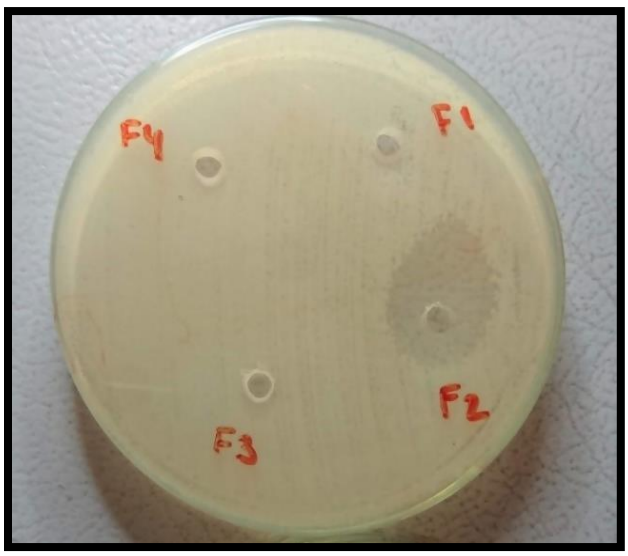


Fig. 7. Antimicrobial activity of thyme oil fractions (F1, F2, F3 and F4).

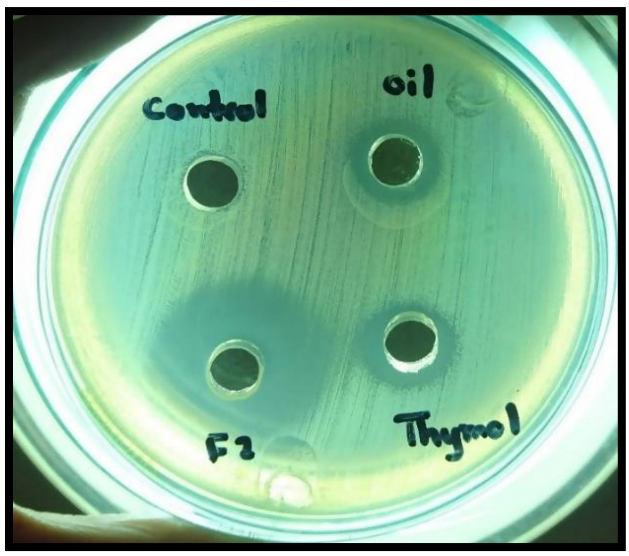


Fig. 8. Antimicrobial activity of thymol, thyme oil and fraction F2 against *Staphylococcus* cells.

Minimum inhibitory concentration: The minimum inhibitory concentration of thyme oil ranged from 0.2 mg/ml to 3.5 mg/ml against multidrug resistant organisms. These observations are supported by the findings of Farag *et al.*, (1989) who found 0.25 mg/ml to 12 mg/ml oil concentrations to inhibit the growth of microbes (*P. fluorescens, E. coli, S. marcescens S. aureus, Micrococcus* sp.*, Sarcina* sp. *and B. subtilis*). However, MIC of thyme water extract was recorded between 40 mg/ml to 160 mg/ml. *P. aeruginosa* was slightly sensitive to thyme extract. These results were in agreement with the work of Man *et al.*, (2019) who observed that essential oils of frankincense, myrtle, thyme, lemon, oregano and lavender showed lower MICs against the bacterial pathogens (used) and therefore exerted better activity than the water extract. These researchers also suggested that essential oil showed better activity than water extract because of the differences in cell wall morphology and composition between Gram positive and Gram negative bacteria. The Gram positive cell wall is more permeable to hydrophobic molecules (like oils) whereas, the Gram negative cell wall are less permeable to hydrophobic molecules due to lipopolysaccharide layer in Gram negative bacteria.

Thyme effect on bacterial cells and biofilm production: The effect of thyme extract on bacterial cells and biofilm formation (by MDR bacterial strains) was studied by scanning electron microscopy (SEM). Accordingly, the thyme treated cells were observed with irregular shaped and with holes and cracks. It is suggested that thyme treatment induces cell membrane damage leading to lysis of bacterial cells. The present findings are in consonance with Helander *et al.*, (1998), who demonstrated the mechanisms of action of thymol (the active component of thyme essential oil) attacking the outer membrane resulting in the disruption of cell wall along with release of cellular components, depletion of intracellular ATP and disruption of the cell membrane. Likewise, thyme effect on biofilm formation was studied by scanning electron microscopy. Normal (untreated) cells formed smooth biofilm along the surface of the slide but thyme treated cells failed to form biofilm (cells were present but did lose the ability to form biofilm). Similar effects (thyme) were observed by Sharifi *et al.*, (2018) at conc. MIC 2 (0.0312 µl.ml^{-}1), MIC 4 (0.0156 µl.ml^{-}1), MIC 8 (0.0078 µl.ml^{-}1) and MIC 16 (0.0039 µl.ml^{-}1) potentially inhibiting biofilm production by *S. aureus*. They concluded that oil of *Thymus sp* has capability to inhibit biofilm formation by *S. aureus* and also revealed that carvacol, terpinene and thymol were the major components of essential oil *T. daenensis* and *S. hortensis.* Mohsenipour and Hassanshahian (2015) studied the inhibitory effect of thyme extract on biofilm formation by some pathogenic bacteria (*S. aureus*, *B. cereus*, *S. pneumoniae*, *P. aeruginosa, E. coli* and *K. pneumoniae*). It was found that the inhibitory effect of thyme extract on biofilm formation was directly related to the concentration of the extract. Antibiofilm properties of *T. vulgaris* indicate the potential of this (wonder) herb may present itself as an alternative to commercially available antibiotics.

Antiphage activity of thyme water extract: Results of the present study have shown the antiphage activity of thyme extract by comparing the plaques forming units (pfu) in control and the thyme treated coliphage particles (in term of p.f.u). The reduction in plaque formation units indicates the antiviral potential of thyme extract. Our results are supported by studies of Nolkemper *et al.*, (2006), Behravan *et al*., (2011) & Kaewprom *et al*., (2017). According to these studies, thyme carries antiviral properties and may be a potential therapy for (treatment) viral infection(s).

Column chromatography and thin layer chromatography: Separation of thyme oil fractions was done by column chromatography followed by identification by thin layer chromatography (TLC). A total of four fractions were obtained with solvent (Toluene: Ethyl acetate in 93:7 ratio) of different Rf values. However,

Ashnagar *et al.*, (2011) could identify two fractions with the solvent containing benzene: chloroform in 3:1 ratio from thyme oil using column chromatography and TLC. This variation in the results may be due to different solvents (used in each study) as mobile phase against the stationary phase (silica gel). Fractions obtained were identified and evaluated for antimicrobial activity. Only one fraction (F2) with 0.55 Rf value showed bioactivity against *Staphylococcus* cells. Antimicrobial activity of Fraction F2, thymol and thyme oil was estimated with toluene as control. All the compounds did cause inhibition of *S. aureus.* Rf value of thymol and the fraction F2 was found to be the same. On the basis of these results, it may be concluded, the fraction F2 (of thyme oil) obtained by column chromatography showed antimicrobial activity. It is suggested that the effectiveness of *T. vulgaris* may be related to its phenolic contents (carvacol and thymol) which are active ingredients of thyme oil with wide spectrum bioactivity (Daferera *et al.*, 2002; Memar *et al.*, 2017).

Conclusions

Antimicrobial, antiphage, and antibiofilm properties of *T. vulgaris* were studied. Accordingly, thyme oil was appreciably more effective than thyme water extract. Thyme oil was effective against all the multidrug resistant isolates. Thyme has stronger bioactivity against Gram positive as compared to the Gram negatives. Thyme water extract inhibited biofilm formation by defying the adherence by bacterial cells. Thyme may attract itself as a possible substitute for antibiotics to counter multidrug resistant microbes as well as against clinical biofilm formers.

References

Alsaidy, H.A.M., I. Qasim and N. Abdala. 2014. The effect of thyme and peppermint extracts on some species of candida yeast. *J. Biol. Agri. Healthcare,* 4(11): 38-48.

Alkowni, R., E.S.R.A. Solyman and H.A. Qauod. 2017. Introducing some of threatened thymus species to *In vitro* tissue culturing as an approach for their conservation. *Pak. J. Bot.,* 49(1): 259-264.

Anzlovar, S., D. Baricevic, J.A. Avgustin and J.D. Koce. 2014. Essential oil of common thyme as a natural antimicrobial food additive. *Food Technol. Biotech*., 52(2): 263-268.

Ashnagar, A., N.N. Gharib and M. Ramazani. 2011. Characterization of the major chemical compounds found in *Thymus vulgaris* plant grown wildly in Chahar Mahal and Bakhtaran province of Iran. *Int. J. Pharm. Tech. Res*., 3(1): 1-4.

Behravan, J., M. Ramezani, E.F.N. Melika and E. Gharaee. 2011. Antiviral and antimicrobial activity of *Thymus trascaspicus* essential oil. *Pharmacologyonline,* 1: 1190-1199.

Burt, S. 2004. Essential oils: Their antibacterial properties and potential applications in foods-a review. *Int. J. Food Microbiol*., 94(3): 223-253.

Chao, S.C., D.G. Young and C.J. Oberg. 2000. Screening for inhibitory activity of essential oils on selected bacteria, fungi and viruses. *J. Essent Oil Res.*, 12(5): 639-649.

Daferera, D.J., P.A. Tarantilis and M.G. Polissiou. 2002. Characterization of essential oils from Lamiaceae species by Fourier transform Raman spectroscopy. *J. Agr. Food Chem*., 50(20): 5503-5507.

Dallal, M.M.S., Z. Rajabi, S.M. Imeni, S.P. Salas and F. Nikkhahi. 2016. Isolation of *E. coli* bacteriophage from raw sewage and comparing its antibacterial effect with ceftriaxone antibiotic. *Int. J. Adv. Biotechnol. Res.*, 7(3): 385-391.

Farag, R.S., Z.Y. Daw, F.M. Hewedi and G.S.A. El-Baroty. 1989. Antimicrobial activity of some Egyptian spice essential oils. *J. Food Protect.*, 52(9): 665-667.

Fayad, N.K., O.H.S. AL-Obaidi, T.H. Al-Noor and M.O. Ezzat. 2013. Water and alcohol extraction of Thyme plant (*Thymus vulgaris*) and activity study against bacteria, tumors and used as anti-oxidant in margarine manufacture. *Innov. Syst. Desi. & Eng*., 4(1): 41-51.

Gurav, A., D.B. Mondal and H. Vijayakumar. 2014. *In vitro* qualitative and quantitative phytochemical analysis of ethanolic and 50% ethanolic extracts of *Tinospora cordifolia, Momordica charantia, Cucurbita maxima* and *Raphanus sativus. Int. J. Pharm. Sci. Res.*, 5(5): 1937.

Helander, I.M., H.L. Alakomi, K. Latva-Kala, T. Mattila-Sandholm, I. Pol, E.J. Smid and A. Wright. 1998. Characterization of the action of selected essential oil components on Gram-negative bacteria. *J. Agr Food Chem*., 46(9): 3590-3595.

Hossain, M.A., K.A.S. AL-Raqmi, Z.H. AL-Mijizy, A.M. Weli and Q. Al-Riyami. 2013. Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown *Thymus vulgaris*. *Asian Pac. J. Trop. Biomed.*, 3(9): 705-710.

Hussein, R.A. and A.A. El-Anssary. 2018. Plants secondary metabolites: The key drivers of the pharmacological actions of medicinal plants. In Herbal Medicine. *Intech. Open,* 11-30.

Kaewprom, K., Y.H. Chen, C.F. Lin, M.T. Chiou and C.N. Lin. 2017. Antiviral activity of *Thymus vulgaris* and *Nepeta cataria* hydrosols against porcine reproductive and respiratory syndrome virus. *Thai J. Vet. Med.*, 47(1): 25.

Kalemba, D.A.A.K. and A. Kunicka. 2003. Antibacterial and antifungal properties of essential oils. *Curr. Med. Chem.*, 10(10): 813-829.

Khalili, S.T., A. Mohsenifar, M. Beyki, S. Zhaveh, T. Rahmani-Cherati, A. Abdollahi, M. Bayat and M. Tabatabaei. 2015. Encapsulation of Thyme essential oils in chitosan-benzoic acid nanogel with enhanced antimicrobial activity against *Aspergillus flavus*. *LWT-Food Sci. Technol.,* 60(1): 502-508.

Lewis, K. 2001. Riddle of biofilm resistance. *Antimicrob. Agents Chemother*., 45(4): 999-1007.

Magiorakos, A.P., A. Srinivasan, R.B. Carey, Y. Carmeli, M.E. Falagas, C.G. Giske and D.L. Paterson. 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin. Microbiol. Infect*., 18(3): 268-281.

Majeed, H., H. N. Bhatti and I. A. Bhatti. 2019. Replacement of sodium alginate polymer, urea and sodium bicarbonate in the conventional reactive printing of cellulosic cotton. *J. Polym Eng*., 39(7): 661-670. DOI: 10.1515/polyeng-2019-0076.

Man, A., L. Santacroce, R. Iacob, A. Mare and L. Man. 2019. Antimicrobial activity of six essential oils against a group of human pathogens: A comparative study. *Pathogens,* 8(1): 15.

Memar, M.Y., P. Raei, N. Alizadeh, M.A. Aghdam and H.S. Kafil. 2017. Carvacrol and thymol: strong antimicrobial agents against resistant isolates. *Rev. Med. Microbiol*., 28(2): 63-68.

Mohsenipour, Z. and M. Hassanshahian. 2015. The inhibitory effect of *Thymus vulgaris* extracts on the planktonic form and biofilm structures of six human pathogenic bacteria. *Avicen. J. Phytomed.*, 5(4): 309.

Mostafa, A.A., A.A. Al-Askar, K.S. Almaary, T.M. Dawoud, E.N. Sholkamy and M.M. Bakri. 2018. Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. *Saudi J. Biol Sci*., 25(2): 361-366.

Nakano, M.M. 2018. Role of Streptococcus mutans surface proteins for biofilm formation. *Japn Dent. Sci. Rev*., 54: 22-29.

Nolkemper, S., J. Reichling, F.C. Stintzing, R. Carle and P. Schnitzler. 2006. Antiviral effect of aqueous extracts from species of the Lamiaceae family against Herpes simplex virus type 1 and type 2 *In vitro*. *Planta Medica.,* 72(15): 1378-1382.

Nzeako, B.C., Z.S. Al-Kharousi and Z. Al-Mahrooqui. 2006. Antimicrobial activities of clove and thyme extracts. *Sultan Qaboos Univ. Med. J*., 6(1): 33-39.

Obeidat, M., M. Shatnawi, M. Al-alawi, E. Al-Zubi, H. Al-Dmoor, M. Al-Qudah, J. El-Qudah and I. Otri 2012. Antimicrobial activity of crude extracts of some plant leaves. *Res. J. Microbiol*., 7(1): 59-67.

Panda, P.S., U. Chaudhary and S.K. Dube. 2016. Comparison of four different methods for detection of biofilm formation by uropathogens. *Ind. J. Pathol. Microbiol.,* 59(2): 177.

Panezai, G.M., M. Javaid, S.A.D.A.F. Shahid, W. Noor, Z.O.H.R.A. Bibi and A. Ejaz. 2019. Effect of four plant extracts against *Trogoderma granarium* and *Tribolium castaneum*. *Pak. J. Bot.*, 51(3): 1149-1153

Rota, M.C., A. Herrera, R.M. Martínez, J.A. Sotomayor and M.J. Jordán. 2008. Antimicrobial activity and chemical composition of *Thymus vulgaris*, *Thymus zygis* and *Thymus hyemalis* essential oils. *Food Control*., 19(7): 681-687.

Salimena, A.P.S.A., A.C.S. Junior, C. M. das Graças, E. Alves and R.H. Piccoli. 2014. Scanning electron microscopy of biofilm formation by *Staphylococcus aureus* on stainless steel and polypropylene surfaces. *Afr. J. Microbiol. Res*., 8(34): 3136-3143.

Šegvić, K.M., I. Kosalec, J. Mastelić, E. Pieckova and S. Pepeljnak. 2007. Antifungal activity of thyme (*Thymus vulgaris* L.) essential oil and thymol against moulds from damp dwellings. *Lett. Appl. Microbiol*., 44(1): 36-42.

Sharifi, A., A.M. zadeh, T. Z. Salehi and P. Mahmoodi. 2018. Antibacterial, antibiofilm and antiquorum sensing effects of *Thymus daenensis* and *Satureja hortensis* essential oils against *Staphylococcus aureus* isolates. *J. Appl Microbiol*., 124(2): 379-388.

Stephens, C. 2002. Microbiology: breaking down biofilms. *Curr. Biol*., 12(4): 132-134.

Wagner, H. and S. Bladt. 1996. Plant drug analysis: a thin layer chromatography atlas. (ISBN 3-540-58676-8.; Springer-Verlag Berlin Heidelberg): 385.

Zaborowska, Z., K. Przygoński and A. Bilska. 2012. Antioxidative effect of thyme (*Thymus vulgaris*) in sunflower oil. *Acta Sci. Pol. Technol. Aliment*., 11(3): 283-291.

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