Journal of Animal & Plant Sciences, 31(5): 2021, Page: 1270-1276
ISSN (print): 1018-7081; ISSN (online): 2309-8694
https://doi.org/10.36899/JAPS.2021.5.0327

ANTIMICROBIAL POTENTIAL OF TEA TREE, CLOVE, BASIL AND THYME ESSENTIAL OILS AGAINST BACTERIAL ISOLATES OF BOVINE WOUNDS

F. Leghari[1], A. A. Kamboh[1*], R. A. Leghari[2], N. A. Korejo[2] and K. K. Malhi[1]

[1]Department of Veterinary Microbiology, [2] Department of Veterinary Medicine, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam, 70060, Pakistan
[*] Corresponding author's E-mail: drasgharkamboh@yahoo.com

ABSTRACT

The present research aimed to investigate the individual and combined antimicrobial potential of plant essential oils (EOs) viz., tea tree (*Melaleuca alternifolia*), clove (*Syzygium aromaticum*), basil (*Ocimum basilicum*), and thyme (*Thymus vulgaris*) against bovine wound isolates. *Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes, Escherichia coli* and *Klebsiella spp.* were isolated from bovine wound samples. Inhibition of microbial growth via disk diffusion method and minimum inhibitory concentration (MIC) was analyzed for EOs and standard antibiotics both individually and in combination. Tea tree and clove produced significantly higher ($p \leq 0.05$) inhibitory effects against all the bacteria compared to one or other EOs and standard antibiotics. Basil+ neomycin, thyme+ ampicillin and tea tree+ ampicillin exhibited synergisms against all the wound isolates except *Klebsiella spp.* The MIC result indicated that clove followed by basil and tea tree exhibited significantly lower ($p \leq 0.05$) MIC values against tested isolates compared to one or other EOs and antibiotics. The combination of thyme neomycin and clove norfloxacin exhibited a significantly lower ($p \leq 0.05$) MIC against *S.pyogenes* and *E.coli* compared to other combination of EOs and antibiotics. Likewise, the combination of basil oxytetracycline and clove norfloxacin exhibited a significantly lower ($p \leq 0.05$) MIC against *S.pneumoniae* compared to one or other combinations of EOs and antibiotics. These results demonstrated the significant antimicrobial potential of EOs against wound isolates; individually tea tree and clove showed superior effects, whereas combinations of basil+ neomycin, thyme+ ampicillin and tea tree+ ampicillin showed synergisms compared to one or other EOs and antibiotics.

Key words: antibacterial, bovine wound, plant essential oil, synergistic effect

Published first online January 21, 2021. Published Final August 07, 2021.

INTRODUCTION

The skin is the outer most organ of the body that plays a critical role in the primary defense system besides external environment. It provides constant security against infection from micro-organism (Lee *et al.*, 2006). Skin is a major multi-functional organ of the body that play a key role in sensation, thermoregulation, biochemical, metabolic and immune functions as well (Wysocki, 1999)

Wound repair is a sequence of body regulatory mechanisms and the innate skin repair response. It is a multi-stage procedure that is influenced by various intrinsic and extrinsic factors. Microorganisms are present in wounds; they are attained from the indigenous flora of the host or the external environment (Schultz *et al.*, 2003). The progress of wound infection negatively affects this process and delays healing (Siddiqui and Bernstein, 2010). Polymicrobial wounds have numerous potential pathogens, of which some can cause infection. To improve wound management practices and prevent complicated wound infections, various efforts were carried out to diminish the emergence of antibiotic-resistant bacteria (Wolcott *et al.*, 2010). The development of microbial resistance to currently available antimicrobials addressed the need to explore novel antimicrobial agents against drug-resistant bacteria like *Staphylococcus aureus* that is a most common cause of food poisoning, post-surgical wound infection, toxic shock syndrome, osteomyelitis and endocarditis (Taylor and Unakal, 2019). It has been estimated that undiscriminated use of synthetic antibiotics as a curative or prophylactic agent in farm animals is a-probable cause of the emergence of drug-resistant pathogenic microorganism (Ariffin *et al.*, 2019). Moreover, the production cost of synthetic drugs is very high, and they also produce several undesirable effects compared to plant-derived drugs (Benhassaini *et al.*, 2003). This warrants to explore new antimicrobial constituents from numerous sources as novel antimicrobial chemotherapeutic agents (Abiramasundari *et al.*, 2011).

Medicinal plants are considered a chief source of novel chemical substances having significant therapeutic functions (Blumenthal *et al.*, 2000). They contain a wide variety of compounds that can be used to treat infectious and noninfectious chronic disorders. Essential oils (EOs) are a very exciting group of secondary metabolites that are potentially important source of antimicrobial agents (Toroglu, 2007). Many pieces of researches have been published on the antimicrobial activity of EOs like tea tree, lavender, oregano, clove, peppermint etc., (Mihajilov-Krstev *et al*., 2010 and Sepahvand *et al.,* 2014). The essential oils unlike, antibiotics, are composed of numerous molecules so that bacteria cannot resist in the mutant. The combination of essential oils with antibiotics is a new therapeutic method that may lead to new ways to overcome resistant infections (Enrico *et al.*, 2004). Many investigators have carried out trials for the synergistic effects by combining the different plant extracts with antimicrobials against bacterial isolates of the human wound (Adwan & Mhanna, 2009 and Olajuyigbe & Afolayan, 2012). However, to the best of our knowledge, no work has been done on the animal wound isolates. Therefore, the current study aimed to evaluates the antimicrobial potential of some EOs (clove, thyme, basil, and tea tree) individually or in combinations with antibiotic drugs (ampicillin, neomycin, norfloxacin and oxytetracycline) against bacterial isolates of animal wounds. This study result illuminates the potential of EOs to control wound microbiota and also its synergistic potential for therapeutic uses with antibiotics against bacterial organisms of animal wounds.

MATERIALS AND METHODS

Sample collection: The whole study design regarding ethical procedures was approved by the institutional ethical committee and endorsed by the Directorate of Advanced Studies, Sindh Agriculture University (SAU), Tandojam. The study was carried out from February to August 2018 at Department of Veterinary Microbiology, SAU Tandojam and Central Veterinary Diagnostic Laboratory Tandojam. Samples (exudate or pus) were collected from wounds (n=30) of cattle and buffalos. These were collected aseptically using sterile swabs that were kept in sterile bottles. These were transported to the laboratory within a few hours of collection under refrigerated conditions to prevent any deterioration. Un-treated open wounds were used for sample collection and during collection sterile cotton swabs were introduced deeply in the wound (after cleaning the exposed area with sterile cotton and normal saline) to collect the wound material. In the laboratory, samples were stored at 4℃ and processed within 24h for isolation of bacterial organisms.

Isolation and identification of bacteria from bovine wounds: Isolation of wound harbored bacterial organisms was done by using conventional culture procedure as defined previously (Pirzada *et al.*, 2016). In brief, wound samples were cultured on different culture media using sterilize wire loop by dipping it in sample solution and then streaked agar plates were incubated aerobically overnight at 37 degree Celsius. Sample solution was prepared by dipping vigorously each swab sample separately in 0.5 ml of normal saline. Single/Individual colonies were chosen up from these Petri plates with a sterile inoculating loop and transported to distinct agar plates or culture tube to form a pure culture. *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumoniae, Escherichia coli* and *Klebsiella* bacteria were isolated and identified using standard identification technique (Smibert *et al.*, 1974). The pure bacterial cultures were stored and analysed for antimicrobial sensitivity.

Antibacterial activity of essential oils (EOs): Antibacterial activity of essential oils was determined by disk diffusion method following the guideline of Clinical and Laboratory Standard Institute (CLSI, 2012). Briefly, Muller Hinton agar plates were prepared, and their sterility was confirmed. Microorganism culture having optical density (OD) equivalent to 0.5 McFarland units was spread over media surface. The sterile paper discs of 6 mm diameter were impregnated with 2µl of each pure EOs (tea tree (*Melaleuca alternifolia*), basil (*Ocimum basilicum*), thyme (*Thymus vulgaris*), and clove (*Syzygium aromaticum*) that were purchased from commercial source with 100% purity (Xpel Marketing Ltd. UK) and were placed on the inoculated agar surface. Standard antibiotic discs (viz., norfloxacin, oxytetracycline, ampicillin and neomycin; Oxoid, UK) were used to compare the zone of inhibition with EOs. Plates were incubated at 37°C for 24hrs then activity was determined by measuring the diameter of zone of inhibitions. All tests were performed in triplicate (Sharma *et al.*, 2017). To determine the combined effect of EOs with antibiotics 2µl of each EOs was saturated with the antibiotic disc to determine the zone of inhibition (Toroglu, 2007). The obtained results were compared with those of the antibiotics and EOs tested on the same bacteria alone. The synergistic (S) or antagonistic (A) effects were calculated by comparing the combined antimicrobial activity with the sum of individual antimicrobial activity of corresponding essential oil and antibiotic. Water and DMSO (dimethyl sulfoxide, 10%) were used as negative controls.

Minimum inhibitory concentration: The separate and combined minimum inhibitory concentration (MIC) of EOs with antibiotics was determined using micro broth dilution method (Lalitha, 2004). Serial tow folds dilutions in Muller Hinton broth (containing 0.5% Tween 80) were prepared using essential oils in a 96 well micro titration plate containing test organisms at a concentration of 2×10^6 CFU/ml. well # 12 of micro plate was used to negative control having only media and well # 11 was taken as a positive control by adding media and bacterial suspension. The well OD values were established by ELISA reader at 524nm. Post 24 hours incubation at

37°C, the OD values again recorded. The minor concentration showing inhibition of growth by a decrease in OD values was considered the MIC of EOs (with or without antibiotic) against test organisms. Water and DMSO (10%) were used as negative controls. All tests were repeated and two replicates done for each assay.

Statistical analysis: Mean MIC values and inhibitory zones of both EOs and standard antimicrobials were compared statistically by one-way ANOVA followed by Duncan's multiple range post hoc test using JMP 5.0.1a statistical software (SAS Institute Inc., Cary, NC). The limit of significance was considered at 5% level.

RESULTS

Individual antibacterial activity of essential oils and antibiotics against animal wound isolates: The results presented in table 1 showing the individual inhibitory activity of essential oils and antibiotics against the bacteria *Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes, E. coli* and *Klebsiella spp*. The data showed that tea tree and clove produced significantly higher ($p \leq 0.05$) inhibitory effects against *Staphylococcus aureus* as compared to basal and thyme essential oils. Tea tree produced significantly higher ($p \leq 0.05$) inhibitory effect against *Streptococcus pyogenes* as compared to basil, thyme, antibiotic oxytetracycline and antibiotic ampicillin whereas clove also produce significantly higher ($p \leq 0.05$) inhibitory effects against *Streptococcus pyogenes* as compared to basil, thyme and antibiotic oxytetracycline, Tea tree and clove produced significantly higher ($p \leq 0.05$) inhibitory effects against *Streptococcus pneumoniae* as compared to basil, thyme, antibiotic oxytetracycline and antibiotic ampicillin, whereas thyme also produced significantly higher ($p \leq 0.05$) inhibitory effects against *Streptococcus pneumoniae* as compare to basil and antibiotic oxytetracycline. However, tea tree and clove produced significantly higher ($p \leq 0.05$) inhibitory effects against *Escherichia coli* and *Klebsiella spp.* as compare to basil and thyme.

Combined antimicrobial activity of essential oils and antibiotics against animal wound isolates: Table 2 showed that the clove+ neomycin, clove+ ampicillin, basil+ neomycin, thyme+ neomycin, thyme+ampicillin, thyme+ oxytetracycline, tea tree+ ampicillin, tea tree+ norfloxacin, and tea tree+ oxytetracycline produced antimicrobial synergism against *Staphylococcus aureus*. Clove+ ampicillin and clove+ oxytetracycline, basil+ ampicillin, basil+ neomycin, basil+ norfloxacin, basil+ oxytetracycline, thyme+ ampicillin, thyme+ oxytetracycline, tea tree+ ampicillin and tea tree+oxytetracycline produced antimicrobial synergism against *Streptococcus pyogenes*. clove+ampicillin, clove+oxytetracycline, basil+neomycin, basil+norfloxacin, basil+ampicillin, basil+oxytetracycle, thyme+norfloxacin, thyme+ampicillin, thyme+oxytetracyline, tea tree+ampicillin and tea tree+oxytetracycline exhibited synergism effect against *Streptococcus pneumoniae* whereas all other combinations of EOs produce antagonistic effects. Clove+ neomycin, basil+ neomycin, thyme+ norfloxacin, thyme+neomycin, thyme+ ampicillin, tea tree+ neomycin, tea tree+ ampicillin and tea tree+ norfloxacin produced synergistic antimicrobial activity against *Escherichia coli*. Whereas all other combination of EOs produced antagonistic effects. All combinations of EOs and antibiotics exhibited the antagonistic effect against *Klebsiella spp*.

Table 1. Individual antibacterial activity of essential oils and antibiotics against animal wound isolates.

Name of agent*	Inhibition zone diameter (mm)				
	Staphylococcus aureus	***Streptococcus pyogenes***	***Streptococcus pneumoniae***	***Escherichia coli***	***Klebsiella spp.***
Basil	3.36	3.66	4.00	1.66	4.30
Tea tree	9.45#	12.66#^~	10.00#^~	6.33#	9.30#
Thyme	4.45	5.00	7.00#^	2.33	2.00
Clove	7.81#	6.60#^	11.00#^~	11.30#	10.00#
N	14.36	17.33	13.66	16.60	17.60
NOR	18.36	15.00	16.00	16.00	20.60
AMP	16.09	8.33	6.60	22.60	18.60
OT	20.45	5.00	5.00	28.00	15.30

* N: neomycin, NOR: norfloxacin, AMP: ampicillin, OT: oxytetracycline
# Significantly higher than other essential oils at $p \leq 0.05$
^ Significantly higher than reference antibiotic oxytetracycline at $p \leq 0.05$
~ Significantly higher than reference antibiotic ampicillin at $p \leq 0.05$

Individual MIC of essential oils and antibiotics against animal wound isolates: The result of table 3 showed that basil and clove exhibited significantly lower ($p \leq 0.05$) MIC against *Staphylococcus aureus* (2.650 and 2.278 µl/ml respectively) and *Escherichia coli* (3.151 and 3.062 µl/ml) as compared to other essential oils and antibiotics ampicillin and oxytetracycline. Clove exhibited significantly lower ($p \leq 0.05$) MIC against *Streptococcus pyogenes* and *Streptococcus pneumoniae* (1.699 and 2.130 ul/ml respectively) as compared to other essential oils and reference antibiotics ampicillin and oxytetracycline. Moreover, clove and Tea tree exhibited significantly lower ($p \leq 0.05$) MIC against *Klebsiella spp*. (2.687 and 2.750ul/ml respectively) as compare to other EOs (thyme and basil) and antibiotic oxytetracycline.

Combined MIC of essential oils and antibiotics against animal wound isolates: The result regarding the combined minimum inhibitory concentration of essential oils and antibiotics against *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumoniae, E.coli* and *Klebsiella* has been presented in table 4. The combination of thyme+neomycin and clove+norfloxacin exhibited a significantly lower ($p \leq 0.05$) MIC against *Streptococcus pyogenes* and *Escherichia coli* as compared to other combination of EOs and antibiotics. Likewise, against *Streptococcus pneumoniae* the combination of basil+oxytetracycline and clove+norfloxacin exhibited a significantly lower ($p \leq 0.05$) MIC as compared to other combination of EOs and antibiotics.

Table 2. Combined antimicrobial activity of essential oils and antibiotics against animal wound isolates.

Name of agent*	Inhibition zone diameter (mm)				
	Staphylococcus aureus	***Streptococcus pyogenes***	***Streptococcus pneumoniae***	***Escherichia coli***	***Klebsiella spp.***
Clove + N	22.5(S)	16.0(A)	20.0(A)	35.3(S)	10.0(A)
Clove + NOR	20.0(A)	14.5(A)	12.4(A)	20.5(A)	12.0(A)
Clove + AMP	25.0(S)	20.5(S)	18.5(S)	22.4(A)	15.0(A)
Clove + OT	20.6(A)	25.4(S)	20.2(S)	18.8(A)	8.5(A)
Basil + N	18.9(S)	27.6(S)	24.4(S)	18.5(S)	16.7(A)
Basil + NOR	20.6(A)	25.3(S)	26.3(S)	12.2(A)	8.7(A)
Basil + AMP	15.6(A)	29.4(S)	18.4(S)	9.5(A)	10.5(A)
Basil + OT	14.5(A)	23.2(S)	16.0(S)	15.4(A)	14.7(A)
Thyme + N	20.6(S)	20.0(A)	14.5(A)	19.3(S)	15.0(A)
Thyme + NOR	19.2(A)	18.5(A)	25.5(S)	38.4(S)	12.5(A)
Thyme +AMP	42.4(S)	22.4(S)	18.2(S)	25.8(S)	5.0(A)
Thyme + OT	32.0(S)	26.5(S)	18.0(S)	30.0(A)	9.0(A)
Tea tree + N	20.7(A)	20.8(A)	19.2(A)	37.0(S)	19.7(A)
Tea tree + NOR	31.5(S)	18.3(A)	22.5(A)	35.5(S)	23.5(A)
Tea tree + AMP	29.0(S)	22.5(S)	18.2(S)	30.8(S)	17.5(A)
Tea tree + OT	32.5(S)	25.3(S)	16.5(S)	28.2(A)	22.2(A)

* N: neomycin, NOR: norfloxacin, AMP: ampicillin, OT: oxytetracycline

Note: values in parentheses showing combined effect in terms of Synergism (S) or Antagonism (A); that was calculated by comparing the combined antimicrobial activity with sum of individual antimicrobial activity of corresponding essential oil and antibiotic.

Table 3. Individual minimum inhibitory concentration of essential oils (µl/ml) and antibiotics (µg/ml) against animal wound isolates.

Name of agent*	Minimum inhibitory concentration				
	Staphylococcus aureus	***Streptococcus pyogenes***	***Streptococcus pneumoniae***	***Escherichia coli***	***Klebsiella spp.***
Basil	2.650#†	2.95	3.11	3.15#†	3.50
Tea tree	4.49	4.28	5.58	5.41	2.75#†
Thyme	3.59	3.17	3.12	5.35	3.21
Clove	2.28#†	1.69#†	2.13#†	3.07#†	2.69#†
N	1.09	2.68	2.04	1.43	2.07
NOR	1.84	2.82	1.11	1.43	1.80
AMP	2.91	3.69	3.52	4.44	2.96
OT	3.03	3.73	3.02	4.54	3.05

* N: neomycin, NOR: norfloxacin, AMP: ampicillin, OT: oxytetracycline

Significantly different from MIC of other essential oils at $p \leq 0.05$

† Significantly different from MIC of reference antibiotic ampicillin and/or oxytetracycline at $p \leq 0.05$

Table 4. Combined MIC of essential oils (µl/ ml) and antibiotics (µg/ mg) against animal wound isolates.

Name of agent*	Minimum inhibitory concentration				
	Staphylococcus aureus	***Streptococcus pyogenes***	***Streptococcus pneumoniae***	***Escherichia coli***	***Klebsiella spp.***
Basil + OT	2.48	2.79	2.91#	3.49	2.73
Tea tree + AMP	2.45	2.79	3.26	3.56	2.62
Thyme + N	2.46	1.71#	3.81	2.06#	2.95
Clove + NOR	2.46	1.74#	2.80#	1.77#	2.87

* NOR: norfloxacin, AMP: ampicillin, N: neomycin, OT: oxytetracycline
# Significantly different from MIC of others at p≤0.05

DISCUSSION

Extensive antimicrobial resistance among microorganisms results an unnecessary burden of antibiotic therapy in routine practice (Rice, 2009). Moreover, an abuse or consumption of untargeted antimicrobial agents might lead to severe concerns for the health of the community. In fact, the current approaches of World Health Organization ensured to limit the usage of antibiotic in livestock, mostly in food animals (Normand *et al.*, 2000). Therefore, it is vital to build up harmless and ordinary alternative methods for controlling the infection. Aromatic and therapeutic plants are generally known to have an antibacterial effect against various disease-causing agents (Baskaran *et al.*, 2009). The essential oils, unlike antibiotics are composed of several bioactive molecules; hence the bacteria cannot resist against them. Curatively and preventively, they are generally well-known for their potent antiviral, antimicrobial, antifungal, antiparasitic, expectorant, mucolytic, antipyretic and anti-inflammatory effects. Besides, the combination of antibiotics with essential oils may lead on the way to cure infectious disease with an extended degree of efficacy (Enrico *et al.*, 2004).

In the current study, tea tree essential oil exhibited high antimicrobial effects against *Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes* and *Klebsiella spp*. Tea tree (*Melaleuca alternifolia)* oil (TTO) or melaleuca oil has long been recognized for numerous medicinal uses (Carson *et al.*, 2006). It is famous for its antibacterial benefits (Carson & Riley, 1993). It is toxic on oral intake and for injection but can be used for topical applications (Hammer *et al.*, 2006) nevertheless; researches discovered that TTO is not genotoxic in vitro in mammalian cells (Pereira & Bartolo, 2016). It has been adopted as the active constituent in many current preparations used against the skin infections and controlling the acne, herpes, lice, dandruff and other cutaneous infections (Pazyar *et al.*, 2013). Recent work on ATCC reference clinical strains of various pathogens including *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella enteritidis, S. typhimurium,* and *E. faecalis* showed the significant antimicrobial potential of tea tree against all strains except *P. aeruginosa* (Andrade *et al.*, 2016). It was reported that chemical constituents of tea tree might increase the porousness of liposomal systems thus producing lysis and the loss of membrane integrity that exhibited by the leak of ions and also the inhibition of respiration that finally destroy the bacterium (Carson *et al.*, 2002).

In current study clove oil has been demonstrated as second topmost effective antibacterial agent against *Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes* and *Klebsiella spp*. Its' antibacterial potential has been reported in the literature against many Gram-negative and Gram-positive organisms as well as some fungi (Gijzen *et al.*, 1993). The antibacterial action of clove is attributable to oleic acids, eugenol, and lipids which are the significant part of its essential oil (Hammer *et al.*, 1999).

As described previously that combined activities of antimicrobial drugs may be due to complex formulation achieved after combination than individual drugs; that results in comparatively high degree of inhibition of microbial activity. This phenomenon has also been observed in the current study that showed synergistic effects of EOs and antibiotics against wound isolates. The combined antimicrobial act of antibiotics and essential oils are one of the current approaches to overcome multidrug-resistant bacteria. Such synergistic treatment potency leads to decrease in the dose of antibacterial agents required for therapy and also have minimum side effects (Mahboubi & Bidgoli, 2010 and Tohidpour & Sattari, 2010). Our current investigation suggested that basil+ neomycin, thyme+ ampicillin and tea tree+ ampicillin are superior combinations as they had shown synergistic effects against 80% of tested bacteria. A previous study has also reported the synergistic potential of some EOs including peppermint, cinnamon bark and lavender with antibiotic piperacillin against multidrug-resistant *E.coli* (Yap *et al.*, 2013), while another study demonstrated the synergistic antibacterial effect of tea tree oil and tobramycin against *Staphylococcus aureus* (D'Arrigo *et al.*, 2010).

Conclusion: In conclusion, essential oils of tea tree, clove, basil and thyme showed antibacterial activity against bacterial isolates of animal wound i.e., *Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes*, *Streptococcus pneumoniae* and *Klebsiella spp*. Individually tea tree and clove exhibited superior antimicrobial effect compared to thyme and basil essential oils. In combinations, basil+ neomycin, thyme+ ampicillin and tea tree+ ampicillin showed superior antimicrobial effects and synergism against most of the isolates of animal wound samples compared to other combination of EOs and antibiotics.

Acknowledgements: This work is supported by the Central Veterinary Diagnostic Laboratory (CVDL), Tandojam. The authors highly acknowledged the support and help of all staff of CVDL during this project.

REFERENCES

Abiramasundari, P., V. Priya, G. Jeyanthi, and D. Gayathri (2011). Evaluation of the Antibacterial activity of *Cocculus hirsutus*. J Drugs Med. 3: 26-31.

Adwan, G., and M. Mhanna (2009). Synergistic effects of plant extracts and antibiotics on *Staphylococcus aureus* strains isolated from clinical specimens. Asian Pacific J. Trop. Med. 2: 46-51.

Ariffin, S., N. Hasmadi, N. Syawari, M. Sukiman, T. Faiq, M. Chai, and M. Ghazali (2019). Prevalence and antibiotic susceptibility pattern of *Staphylococcus aureus*, *Streptococcus agalactiae* and *Escherichia coli* in dairy goats with clinical and subclinical mastitis. J. Anim. Health Prod. 7: 32-37.

Andrade, M.T., B. Farnanda, L. Nunes Barbosa, F.C. Bérgamo Alves, M. Albano, V.L Mores Rall, J.M. Sforcin, A.A.H. Fernandes, and A. Fernandes Júnior (2016). The antibacterial effects of *Melaleuca alternifolia, Pelargonium graveolens* and *Cymbopogon martinii* essential oils and major compounds on liquid and vapor phase. J. Essential Oil Res. 28: 227-233.

Baskaran, S. A., G. Kazmer, L. Hinckley, S. Andrew, and K. Venkitanarayanan (2009). Antibacterial effect of plant-derived antimicrobials on major bacterial mastitis pathogens in vitro. J Dairy Sci. 92: 1423-1429.

Benhassaini, H., K. Enabderrahmane, and K. Chi (2003). Contribution to the assessment of the antiseptic activity of essential oils and oleoresin of Pistaciatial Atlas on some microbial sources: *Candida albicans* (ATCC 20027), *Candida albicans* (ATCC 20032) and *Saccharomyces cerevisiae*. *Ethnopharmacology* 30: 38-46.

Blumenthal, M., A. Goldberg, and J. Brinckmann (2000). *Herbal Medicine. Expanded Commission E monographs* (1 ed.): Integrative Medicine Communications.

Carson, C., K. Hammer, and Riley (2006). *Melaleuca alternifolia* (tea tree) oil: a review of antimicrobial and other medicinal properties. Clin. Microbiol. Rev. 19: 50-62.

Carson, C., and T. Riley (1993). Antimicrobial activity of the essential oil of *Melaleuca alternifolia*. Lett. Appl. Microbiol. 16(2): 49-55.

Carson, C. F., B.J. Mee, and T.V. Riley (2002). Mechanism of action of *Melaleuca alternifolia* (tea tree) oil on *Staphylococcus aureus* determined by time-kill, lysis, leakage, and salt tolerance assays and electron microscopy. Antimicrob. Agents Chemo. 46: 1914-1920.

CLSI, (2012). Performance standards for antimicrobial susceptibility testing. Clinical and Laboratory Standards Institute (M100eS22) (s22nd Informational Supplement).

D'Arrigo, M., G. Ginestra, G. Mandalari, P. Furneri, and G. Bisignano (2010). Synergism and postantibiotic effect of tobramycin and *Melaleuca alternifolia* (tea tree) oil against *Staphylococcus aureus* and *Escherichia coli*. Phytomedicine 17: 317-322.

Enrico, V., P. Andrea, B. Francesca, and Z. Min (2004). Experimental study of reinforcement of immunity by syrup of father Michel (POE 20). Italy, Institute of Naturopathic Sciences (ISN).

Gijzen, M., E. Lewinsohn, T.J. Savage, and R.B. Croteau (1993). Conifer monoterpenes: biochemistry and bark beetle chemical ecology Ch. 2 ed. Washington DC: American Chemistry Society: ACS Publications.

Hammer, K. A., C.F. Carson, and T.V. Riley (1999). Antimicrobial activity of essential oils and other plant extracts. J. Appl. Microbiol. 86: 985-990.

Hammer, K. A., C.F. Carson, T.V. Riley, and J.B. Nielsen (2006). A review of the toxicity of *Melaleuca alternifolia* (tea tree) oil. Food Chem. Toxicol. 44: 616-625.

Lalitha, M (2004). Manual on antimicrobial susceptibility testing. Performance standards for antimicrobial testing: Twelfth Informational Supplement 56238: 454-456.

Lee, S. H., S.K. Jeong, and S.K. Ahn (2006). An update of the defensive barrier function of skin. Yonsei Med. Jo. 47: 293-306.

Mahboubi, M., and F.G. Bidgoli (2010). Antistaphylococcal activity of *Zataria multiflora* essential oil and its synergy with vancomycin. Phytomedicine 17: 548-550.

Mihajilov-Krstev, T., D. Radnovic, D. Kitic, Z. Stojanovic-Radic, and B. Zlatkovic (2010). Antimicrobial activity of *Satureja hortensis* L.

essential oil against pathogenic microbial strains. Arch. Biol. Sci. 62: 159-166.

Normand, E., N. Gibson, S. Reid, S. Carmichael, and D. Taylor (2000). Antimicrobial-resistance trends in bacterial isolates from companion-animal community practice in the UK. Prev. Vet. Med. 46: 267-278.

Olajuyigbe, O. O., and A.J. Afolayan (2012). Synergistic interactions of methanolic extract of *Acacia mearnsii* De Wild. with antibiotics against bacteria of clinical relevance. Int. J. Mol. Sci. 13: 8915-8932.

Pazyar, N., R. Yaghoobi, N. Bagherani, and A. Kazerouni (2013). A review of applications of tea tree oil in dermatology. Int. J. Dermatol. 52: 784-790.

Pereira, R. F., and P.J. Bartolo (2016). Traditional therapies for skin wound healing. Adv. Wound Care 5: 208-229.

Pirzada, M., K. Malhi, M. Memaon, R. Leghari, A. Leghari, F. Habib, H. Baloch, R. Rind, and S. Alam (2016). In vitro antimicrobial susceptibility profile of sub-clinical mastitis isolates from dairy goats. J. Anim. Health Prod. 4: 72-77.

Rice, L. B. (2009). The clinical consequences of antimicrobial resistance. Curr. Opinion Microbiol. 12: 476-481.

Schultz, G. S., R. G. Sibbald, V. Falanga, E. A. Ayello, C. Dowsett, K. Harding, M. Romanelli, M. C. Stacey, L. Teot, and W. Vanscheidt (2003). Wound bed preparation: a systematic approach to wound management. Wound Repair Regen. 11: S1-S28.

Sepahvand, R., B. Delfan, S. Ghanbarzadeh, M. Rashidipour, G. H. Veiskarami, and J. Ghasemian-Yadegari (2014). Chemical composition, antioxidant activity and antibacterial effect of essential oil of the aerial parts of *Salvia sclareoides*. Asian Pac. J. Trop. Med. 7: S491-S496.

Sharma, S., V. Galav, M. Agrawal, F. Faridi, and B. Kumar (2017). Multi-drug resistance pattern of bacterial flora obtained from necropsy samples of poultry. J. Anim. Health Prod. 5: 165-171.

Siddiqui, A. R., and J. M. Bernstein (2010). Chronic wound infection: facts and controversies. Clinics Dermatol. 28: 519-526.

Smibert, R., R. Buchanan, and N. Gibbons (1974). Bergey's manual of determinative bacteriology. The Williams and Wilkins Co., Baltimore, 208.

Tohidpour, A., M. Sattari, R. Omidbaigi, A. Yadegar, and J. Nazemi (2010). Antibacterial effect of essential oils from two medicinal plants against Methicillin-resistant *Staphylococcus aureus* (MRSA). Phytomedicine 17: 142-145.

Toroglu, S (2007). In vitro antimicrobial activity and antagonistic effect of essential oils from plant species. J. Environ. Biol. 28: 551-559.

Taylor, T.A., and C.G. Unakal (2019). *Staphylococcus aureus*. In StatPearls [Internet]. StatPearls Publishing.

Wolcott, R. D., K. F. Cutting, S. E. Dowd, and S. L. Percival (2010). Types of wounds and infections. Microbiology of Wounds. CRC Press, pp. 232-245.

Wysocki, A. B (1999). Skin anatomy, physiology, and pathophysiology. Nursing Clin. North Amer. 34: 777-797.

Yap, P. S. X., S. H. E. Lim, C. P. Hu, and B. C. Yiap (2013). Combination of essential oils and antibiotics reduce antibiotic resistance in plasmid-conferred multidrug resistant bacteria. Phytomedicine 20: 710-713.