Malaysian Journal of Microbiology, Vol 17(4) 2021, pp. 380-389
DOI: http://dx.doi.org/10.21161/mjm.200895

Malaysian Journal of Microbiology
Published by Malaysian Society for Microbiology
(In SCOPUS since 2011)

Assessment of antibacterial activity of *Syzygium aromaticum* extracts, antibiotics and silver sulphadiazine ointment against pathogenic bacteria isolated from the burned and unburned skin

Iffat Naz[1]*, Afsheen Fatima[2], Saleh S. Alhewairini[3] and Abdul Rehman[2,4]*

[1]Department of Biology, Scientific Unit, Deanship of Educational Services, Qassim University, Buraidah, 51452, Qassim, Kingdom of Saudi Arabia (KSA).
[2]Department of Microbiology and Biotechnology, Abasyn University Peshawar, Khyber Pakhtunkhwa, 25000, Pakistan.
[3]Department of Plant Production and Protection, College of Agriculture and Veterinary Medicine, Qassim University, Buraidah 51452, Qassim, Kingdom of Saudi Arabia (KSA).
[4]Department of Microbiology, Kohat University of Science and Technology (KUST), Kohat, Khyber Pakhtunkhwa, Pakistan.
Email: I.Majid@qu.edu.sa; abdulrehman@kust.edu.pk

Received 12 June 2020; Received in revised form 2 August 2020; Accepted 2 June 2021

ABSTRACT

Aims: Skin burns remain a noteworthy general medical issue throughout the world, as it boosts a condition of immuno-suppression. The present research aimed to evaluate the efficacy of *Syzygium aromaticum* extracts, silver sulphadiazine ointment, and different commercially available topical antibiotics against pathogenic bacteria, isolated from the skin of burn patients.
Methodology and results: A total of 124 clinical pus samples were collected from the skin of burn patients, admitted to two different tertiary care burn units at Peshawar, Pakistan. From these pus samples, 6 bacterial isolates from burned skin (*Staphylococcus epidermidis*, *Streptococcus*, *Klebsiella*, *Enterobacter*, *Bacillus* and *Pseudomonas* spp.) were isolated, while 4 different bacterial isolates (*Staphylococcus epidermidis*, *Staphylococcus aureus*, *Bacillus* and *Streptococcus* spp.) were isolated from unburned skin via conventional culturing techniques. Further, antibacterial assays were performed to compare the efficacy of *S. aromaticum* extracts (methanolic and aqueous extract), silver sulphadiazine ointment, and different commercially available antibiotics against tested bacteria. It was observed that both methanolic and aqueous extracts of *S. aromaticum* were effective at all concentrations against all the tested bacteria. In addition, all the tested antibiotics expressed substantial activity against most of the bacterial isolates. While silver sulphadiazine ointment was observed to be less potent against isolated bacteria as compared to *S. aromaticum* extracts.
Conclusion, significance and impact of study: It was concluded that both aqueous and methanolic extracts of *S. aromaticum* were effective antimicrobial agents and could be used as an alternative to control bacterial infections of burn patients. This study would help to distinguish the risk factors of bacterial pathogenicity in burn patients and would also provide a guideline to utilize medicinal plants and their extracts to minimize the chances of antibiotic resistance phenomenon in burn patients.

Keywords: Infected burned skin, pathogenic bacteria, *Syzygium aromaticum* extracts, silver sulphadiazine ointment, antibiotics

INTRODUCTION

Skin is the outer most epithelial tissue, covering the human body and is considered as the most conspicuous organ. It has seven layers of ectodermal tissue and protects the underlying bones, ligaments, muscles, and internal organs (Pappas *et al.*, 2009; Mostafa *et al.*, 2018). Skin plays various important roles such as homeostasis of water, protection against different infections, thermo-regulation (insulation), sensation and the synthesis of vitamin B and D (Proksch *et al.*, 2008; Simard-Bisson *et al.*, 2018). It is also a site for the propagation of varieties of bacterial pathogens and the most dominant surface colonizers are *Staphylococcus epidermidis* and *Staphylococcus aureus* (Grice *et al.*, 2009; Nagoba *et al*., 2010; Cong and Zhang, 2018).

Skin burns or blaze is a sort of skin or mucosa damage, brought on by fire, electricity, chemicals, friction,

*Corresponding author

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 17(4) 2021, pp. 380-389
DOI: http://dx.doi.org/10.21161/mjm.200895

radiations, and so on. There are three types of blazes which are first-degree burn that causes the least harm and only influence the external layer of skin; second-degree burn that causes more serious damage as they affect the top layer and the layer beneath; and third-degree burn is the most severe, because they damage all layers of the skin and tissues (Allegranzi *et al*., 2011; Darlenski and Fluhr, 2017).

Skin burns are remarkable medical issues throughout the world, particularly in underdeveloped countries, as it expedites a condition of immuno-suppression that effects patients to irresistible intricacies. Many researchers reported patients with general burn injuries, were mainly vulnerable to infection with methicillin resistant *Staphylococcus aureus* (MRSA) (Oncul *et al*., 2002; Benchamkha *et al*., 2017). Different topical antimicrobial agents are available in the market to prevent skin infections, but most of them failed to prevent the wounds from other invasive fungi and bacteria. These fungi and bacteria start to penetrate in the internal tissues which depend on their local wound factors, the capacity of invasion, and the level of the patient's immuno-suppression (Oncul *et al*., 2002). Further, *Pseudomonas aureginosa*, which exists widely in the environment, makes it extremely feasible that an individual suffering from severe burns would be susceptible to this bacterial pathogen (Gallo and Nakatsuji, 2011; Espiritu *et al.*, 2016; Moissl-Eichinger *et al*., 2017; Chen *et al*., 2018).

Syzygium aromaticum (clove) is an extensively cultivated plant in Spice Islands, Indonesia, Pemba, and Zanzibar, though it's earlier production was reported in China. It is also used in the seasoning of food like thyme and its antimicrobial potential was established, when its essential oil extracts destroyed various Gram-positive and Gram-negative bacteria as well as some fungi (Cressy *et al*., 2003; Shoaib *et al*., 2014; Atanasova-Pancevska *et al*., 2017). Clove is the dried flower bud of *Eugenia caryophyllus*, *Mytraceae* family. It has wide range of medicinal properties and now is commonly used in Western medicine. Its extract contains eugenol, which has tremendous antimicrobial properties against pathogenic organisms such as *Listeria monocytogenes*, *Campylobacter jejuni*, *Salmonella enteritidis*, *Escherichia coli* and *Staphylococcus aureus* (Cressy *et al*., 2003; Kim *et al.*, 2005). In addition to this, cloves are very rich in manganese and are also being an excellent source of vitamin C dietary fibers, vitamin K, magnesium, calcium, and fatty acids (Kim *et al*., 2005 Shoaib *et al*., 2014). Further, it consists of a sufficient quantity of carbohydrates, calcium, iron, phosphorus, sodium, potassium, proteins, and hydrochloric acid (Im *et al.*, 2016; Atanasova-Pancevska *et al*., 2017).

The present study was designed to evaluate the efficacy of *S. aromaticum* extracts, silver sulphadiazine ointment and different commercially available topical antibiotics against pathogenic bacteria isolated from the skin of burn patients. This study would help us to identify the hospital-acquired infections, as well as the appropriate level of treatment for burn injuries. Besides, it would also give an idea of the consumption of medicinal plants and their extracts as an alternative to commercially available antibiotics. By doing this practice, there might be a reduction in the emergence of antibiotic resistant bacterial species. Moreover, it is expected that it will reduce the burden of purchasing expensive antibiotics for the treatment of burn-wound infections.

MATERIALS AND METHODS

Sample collection

A total of 124 clinical pus samples were collected from the skin of patients admitted in burn units of two different hospitals at Peshawar, Khyber Pakhtunkhwa, Pakistan. Samples from the burned (n=62) and unburned (n=62) skin areas were collected through pre-sterilized cotton swab using aseptic techniques and then transported to the Microbiology Research Laboratory, Abasyn University, Peshawar, Pakistan in an icebox and preserved at 4 °C until further analysis.

Isolation of pathogenic bacteria from clinical samples

About 25 mL of nutrient broth was prepared for each sample in flasks. After inoculation, the nutrient broth was incubated in shaking incubator at 37 °C for 24-48 h. After incubation, 1 mL of freshly grown culture was successively diluted up to 10^{-5} with sterile distilled water and then nutrient agar plates were prepared for each dilution. After this, 0.1 mL of diluted samples were inoculated into nutrient agar plates, and then culture plates were kept in an incubator at 37 °C under aerobic conditions for about 24-72 h. After incubation, bacterial isolates were identified using conventional culturing technique i.e., determining culture characteristics, Gram staining and performing different biochemical tests (catalase, oxidase, urease, indole, citrate utilization, coagulase, and triple sugar iron) according to their standard procedures (Pahlow *et al*., 2015; Yoo *et al*., 2016).

Preparation of *Syzygium aromaticum* extract

Fresh cloves (*Syzygium aromaticum*) were purchased from the local grain market, Peshawar, Khyber Pakhtunkhwa, Pakistan, and then grinded into powder form by blender and kept in airtight bottles. Two kinds of *S. aromaticum* extracts (aqueous and methanolic extracts) were prepared for evaluating their antimicrobial activities towards pathogenic bacterial isolates. For the preparation of aqueous extract of *S. aromaticum*, 25 g of *S. aromaticum* powder was dissolved in 150 mL of distilled water. After dissolution, the mixture was left overnight at room temperature and then it was filtered. At the end of this procedure, a dark color solution (primary solution) having a concentration of 166 mg/mL was obtained. Further, different working solutions i.e., 1, 2, 3, 4, and 5 mg/mL were prepared from the primary solution using sterile distilled water. The sample extracts were

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 17(4) 2021, pp. 380-389
DOI: http://dx.doi.org/10.21161/mjm.200895

kept refrigerated at 4 °C until further analysis (Ahmad and Aqil, 2007).

Whereas, for the preparation of methanolic extract of *S. aromaticum*, 150 mL of methanol was added to 25 g of already finely grounded *S. aromaticum* powder and then the mixture was left overnight at room temperature. Later, the prepared mixture was filtered, and methanol was let to evaporate in a rotary evaporator. Eventually, dark-colored extract having a concentration of 166 mg/mL was obtained at the end of this procedure. With the help of dimethyl sulphoxide (DMSO), different working solutions i.e., 1, 2, 3, 4 and 5 mg/mL respectively were prepared from the stock solution. The sample extracts were kept refrigerated at 4 °C until the accomplishment of further analysis (Ahmad and Aqil, 2007).

Comparative antibacterial activity of *S. aromaticum* extracts and silver sulphadiazine

The antibacterial activity of *S. aromaticum* extracts (aqueous and methanolic extract) and commercially available silver sulphadiazine (Silvadene®, Thermazene®, Flamazine®, SSD®, a 1% water-soluble ointment, is a combination of sulfadiazine plus silver, and used to treat wound infections in patients with second- and third-degree burns) against pathogenic bacterial isolates were performed using Kirby-Bauer agar well diffusion method. Nutrient agar media was prepared in the Petri plates, the turbidity of the inoculum was adjusted with 0.5 McFarland solution and then approximately 50 µL inoculum of every selected bacterium was homogeneously spread on their specified plates using glass spreader. After 5 min, six wells of 6 mm diameter were bored via borer having 6 mm of diameter. One well was used for the positive control (methanol) and two wells were used for negative control (distilled water and DMSO). While, the remaining three wells were used for silver sulphadiazine ointment, aqueous and methanolic extracts of *S. aromaticum* respectively. An equal volume (50 µL) of control as well as extracts and silver sulphadiazine ointment, were added into these wells and then incubated at 37ºC for 24 h. After incubation, zones of inhibition were measured to the nearest millimeter to evaluate the potency of *S. aromaticum* extracts and silver sulphadiazine ointment against isolated bacterial species (Dai *et al.*, 2010; CLSI, 2017).

Antibiotic sensitivity assay

Antibiotic sensitivity assay was performed according to the Kirby-Bauer disc diffusion assay to determine the efficacy of narrow and broad-spectrum antibiotics. Antibiotic selection was based on CLSI (2017) guidelines and 9 different commercially available antibiotics disc i.e., azithromycin, clindamycin, ciprofloxacin, ampicillin, ceftazidime, cefepime, meropenem, gentamicin, and amikacin, were used against test bacterial strains. In antibiotic sensitivity assay, Mueller-Hinton agar plates were prepared by spreading 0.1 mL of diluted inoculum of each test bacterium over the media surface. After spreading, it was allowed to dry for about 5 min and then with the help of sterilized forceps, antibiotic discs were placed gently on the surface of the bacterial lawn at equal distance. The plates were then incubated at 37 °C for 24 h, the antibiotics sensitive bacteria had made clear rings or zones of inhibition around the discs during incubation. Then, these zones were measured in millimeter to evaluate the *in vitro* potency of antibiotics against test bacterial species.

RESULTS

Bacteriological assessment of clinical samples

Isolated bacterial species were characterized according to Bergey's Manual of Determinative Bacteriology (9th edition). Based on microscopic and biochemical tests, 10 different bacterial isolates were characterized, 4 were identified from unburned skin samples and 6 isolates were identified from burned skin samples of patients admitted in the tertiary care hospitals. Out of these 10 different bacterial isolates, 7 were Gram-positive and 3 were Gram-negative. Bacterial isolates encoded B_3, B_4 and B_6 were Gram-negative rods and showed scattered arrangement, while UN_1, UN_2, UN_3, UN_4, B_1, B_2, and B_5 were Gram-positive cocci and displayed cluster as well as chain-like arrangement under a microscope. After microscopic analysis, these bacterial isolates were sub-cultured on nutrient agar media plates to observe their cultural characteristics. Then identification of these bacterial isolates was carried out by performing different biochemical tests. The detailed description of microscopic, cultural, and biochemical characteristics of all identified bacterial isolates is given in Table 1.

Antibacterial activity of *S. aromaticum* extracts

In the current research, the methanolic extract of *S. aromaticum* at concentration of 1 and 2 mg/mL revealed an excellent antibacterial activity against *Streptococcus* sp. from unburned skin. While at a concentration of 3 mg/mL, substantial activity was observed against *Bacillus* sp. and *S. aureus* isolated from the unburned skin samples of patients. However, at concentrations of 4 and 5 mg/mL, maximum antibacterial activity was observed against *Bacillus* sp. and *S. aureus* respectively as shown in Figure 1A. While in the case of indicator bacteria isolated from burned skin samples, the maximum antibacterial activity of methanolic extracts were illustrated against *Bacillus* sp. at 1 and 2 mg/mL and *Pseudomonas* sp. at concentrations of 3 mg/mL. Further, at concentrations of 4 and 5 mg/mL, the substantial activity of methanolic extract was observed against *Bacillus* sp. as shown in Figure 1B.

On the other hand, aqueous extract of *S. aromaticum* had also been used to evaluate their antibacterial activity against the tested bacteria. For unburned skin (Figure 2A), it was noticed that the maximum antibacterial activity of the aqueous extract was observed against *Bacillus* sp. at concentrations of 1 and 2 mg/mL, while at a

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 17(4) 2021, pp. 380-389
DOI: http://dx.doi.org/10.21161/mjm.200895

Table 1: Bacteriological assessment of clinical samples.

	Isolate code	Cultural characteristics on nutrient agar	Gram's reaction	Citrate	Indole	Oxidase	Coagulase	Urease	Catalase	Triple sugar iron	Identified organisms
Unburned skin samples	UN_1	White, circular, smooth, opaque growth	+	-	-	-	-	+	+	K/A	*Staphylococcus epidermidis*
	UN_2	Circular, yellow, small, opaque growth	+	+	-	-	+	+	+	K	*Staphylococcus aureus*
	UN_3	Small, white, smooth, opaque growth	+	+	-	±	-	-	+	KH_2S	*Bacillus* sp.
	UN_4	Small, circular, golden, smooth growth	+	-	-	-	-	-	-	K/A, G	*Streptococcus* sp.
Burned skin samples	B_1	Small, circular, golden, smooth growth	+	-	-	-	-	-	-	K/A, G	*Streptococcus* sp.
	B_2	White, circular, smooth, opaque growth	+	-	-	-	-	+	+	K/A	*Staphylococcus epidermidis*
	B_3	Circular, small, white opaque growth	-	+	-	-	-	+	+	NC	*Klebsiella* sp.
	B_4	Large, off white, irregular, opaque growth	-	+	-	-	-	-	+	NC	*Enterobacter* sp.
	B_5	Small, circular, white, opaque growth	+	+	-	±	-	-	+	K, H_2S	*Bacillus* sp.
	B_6	Small, greenish, smooth, opaque growth	-	+	-	+	-	-	+	NC	*Pseudomonas* sp.

UN = Unburned skin samples; B = Burned skin samples; + = Positive; - = Negative; ± = Variable; A = Acid production; K = alkaline reaction; NC = No change; H_2S = Sulfur reduction; K/A = Red/yellow; K/AG = Red/yellow with gas production.

ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 17(4) 2021, pp. 380-389
DOI: http://dx.doi.org/10.21161/mjm.200895

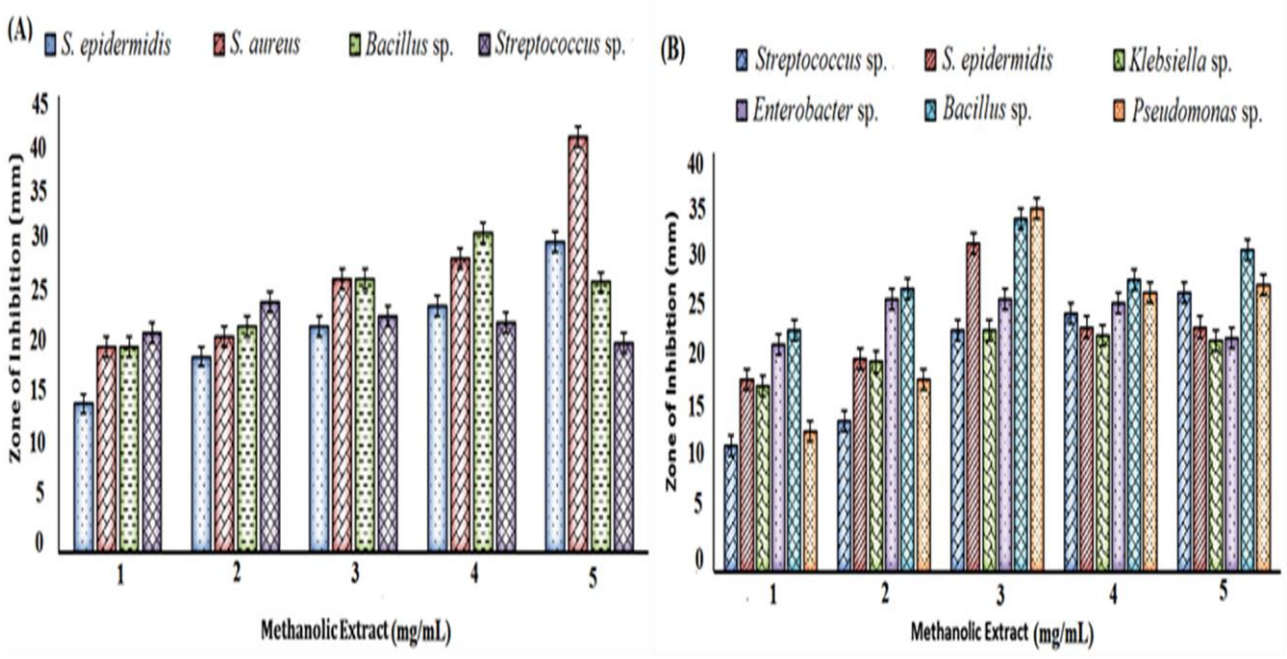

Figure 1: Antibacterial activity of methanolic extract of *S. aromaticum* in different concentrations against test bacterial isolates from unburned skin (A) and burned skin (B).

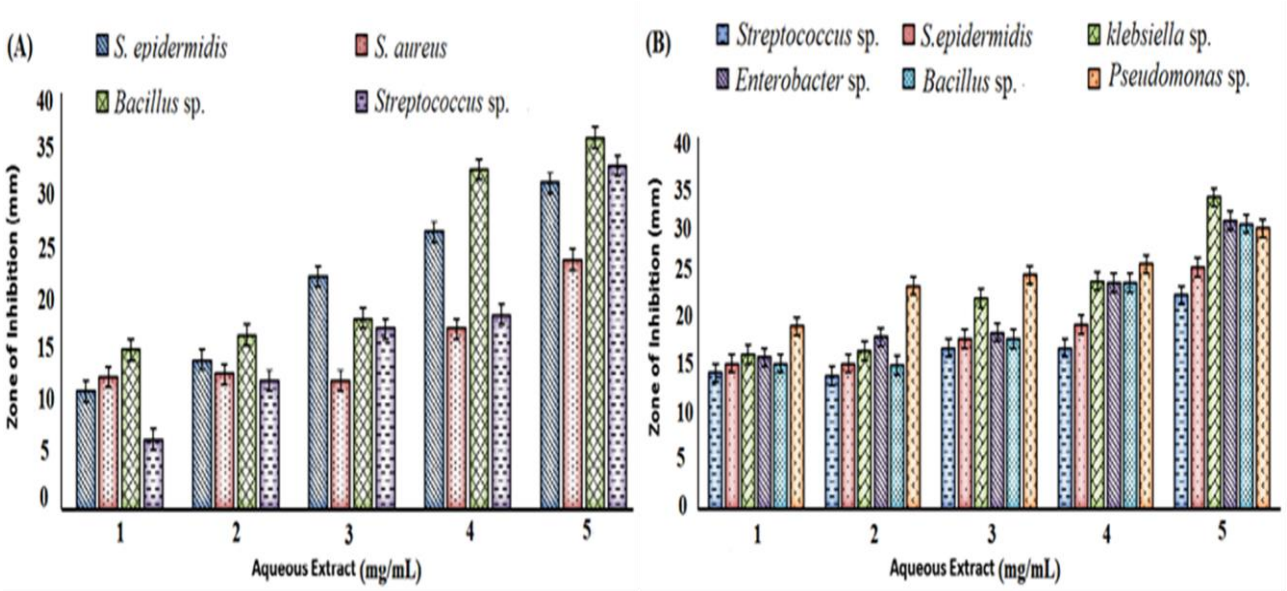

Figure 2: Antibacterial activity of aqueous extract of *S. aromaticum* in different concentrations against test bacterial isolates from unburned skin (A) and burned skin (B).

concentration of 3 mg/mL, strongest activity was observed against *S. epidermidis*. In addition, at concentrations of 4 and 5 mg/mL, the maximum antibacterial activity of the aqueous extract was revealed against *Bacillus* and *Streptococcus* spp. While in the case of bacterial species isolated from infected burned skin, the maximum antibacterial activity of the aqueous extract was found against *Pseudomonas* sp. at concentrations of 1, 2, 3 and 4 mg/mL. Though at a concentration of 5 mg/mL, the substantial antibacterial activity of the aqueous extract was perceived against *Klebsiella* sp. as shown in Figure 2B.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 17(4) 2021, pp. 380-389
DOI: http://dx.doi.org/10.21161/mjm.200895

Table 2: Antibacterial activity of tested antibiotics against bacteria isolated from unburned skin samples.

No.	Antibiotics	CLSI (2017) standard sensitivity limits (mm)	Zone of inhibition against bacteria isolated from unburned skin samples (mm)			
			S. epidermidis	*S. aureus*	*Bacillus* sp.	*Streptococcus* sp.
1	Cef	14	15.3±2.5	21.6±2.0	28.0±2.0	18.3±0.5
2	Mer	16	31.6±1.5	27.3±2.0	27.6±1.5	36.6±1.5
3	Clind	15	31.3±1.5	16.0±2.0	7.3±1.5	6.6±1.5
4	Gen	15.6	27.0±2.0	24.0±1.0	16.6±2.5	18.0±2.6
5	Cip	16.5	30.6±2.0	29.3±1.5	27.6±2.0	33.3±1.5
6	Azith	14.5	20.3±2.0	22.3±2.5	16.6±1.5	20.3±0.5
7	Ceftz	13.6	8.6±2.5	11.0±2.6	20.0±1.0	7.3±2.0
8	Amp	14.5	17.3±1.5	19.6±1.5	15.0±2.0	6.6±1.1
9	Amik	15	22.3±2.5	22.6±1.5	20.0±2.0	24.6±1.5

Cef = cefepime; Mer = meropenem; Clind = clindamycin; Gen = gentamycin; Cip = ciprofloxacin; Azith = azithromycin; Ceftz = ceftazidime; Amp = ampicillin; Amik = amikacin

Antibacterial activity of commercially available antibiotics against bacterial isolates

Antibacterial activity of test antibiotics against bacterial species isolated from unburned skin samples are shown in Table 2. It was observed that all the test antibiotics against ceftazidime, clindamycin, and ampicillin have illustrated a noteworthy activity against *Streptococcus* sp. and the highest zone of inhibition was produced by meropenem i.e., 36.6 mm. Further, it was observed that *Bacillus* sp. showed sensitivity to all the test antibiotics except clindamycin while highest sensitivity was observed against cefepime i.e., 28 mm according to CLSI (2017) guidelines. Similarly, *S. aureus* displayed sensitivity to all the test antibiotics except ceftazidime, and largest zone of inhibition was observed against ciprofloxacin i.e., 29.3 mm. Moreover, *S. epidermitdis* had demonstrated resistance to only ceftazidime (8.6 mm) according to CLSI (2017) guidelines while the rest of test antibiotics presented substantial activity against *S. epidermidis*.

Similarly, the same antibiotics were used to evaluate their antibacterial activity against test bacterial species isolated from the burned skin samples (Table 3). According to the observations, it was found that *S. epidermidis* demonstrated maximum sensitivity against clindamycin (32.6 mm), *Pseudomonas* sp. was more sensitive to ciprofloxacin (33.6 mm) while, *Streptococcus* sp., *Klebsiella* sp., *Enterobacter* sp., and *Bacillus* sp., presented highest sensitivity to meropenem i.e., 31 mm, 31 mm, 32 mm, and 32.3 mm respectively.

Antibacterial activity of silver sulphadiazine ointment against bacterial isolates

Silver sulphadiazine ointment was also assessed in the current study to evaluate their antibacterial activity against bacterial isolates (Figures 3 and 4). The maximum antibacterial activity of silver sulphadiazine ointment was observed against *Streptococcus* sp. (16.0 ± 1.8 mm), followed by *S. aureus* (15.0 ± 1.5 mm), *Bacillus* sp. (14.6 ± 1.4 mm) and *S. epidermidis* (14.6 ± 1.3 mm) isolated from unburned skin samples as shown in Figure 3. While, in the case of bacterial isolates from burned skin samples (Figure 4), maximum activity was observed against *Klebsiella* sp. (15.0 ± 1.7 mm), *Bacillus* sp. (14.6 ± 1.4 mm), *Streptococcus* sp. (14.3 ± 0.8 mm) and *Pseudomonas* sp. (14.3 ± 1.0 mm). Whereas, against *Enterobacter* sp. and *S. epidermidis,* the zones of inhibition produced by silver sulphadiazine were 12.6 ± 1.3 mm and 11.6 ± 1.1 mm respectively. These results were in accordance with the investigations of Oaks and Cindass (2020).

DISCUSSION

The current research is focused on the evaluation of the efficacy of *S. aromaticum* extracts, silver sulphadiazine ointment and different commercially available topical antibiotics against 10 different pathogenic bacteria, isolated from the skin of burn patients. Among them, 4 bacterial isolates (*S. epidermidis*, *S. aureus*, *Bacillus* and *Streptococcus* spp.) were identified from unburned skin samples and 6 isolates (*S. epidermidis*, *Streptococcus Klebsiella*, *Enterobacter*, *Bacillus and Pseudomonas* spp.) from burned skin samples of the admitted patients as shown in Table 1. All these isolated bacteria were highly pathogenic and capable to produce severe infection especially in burn patients. Microbial infection in burn wards might be due to nosocomial pathogens and due to sub-standard hygienic conditions (Ahmad and Al-Kafri,

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 17(4) 2021, pp. 380-389
DOI: http://dx.doi.org/10.21161/mjm.200895

Table 3: Antibacterial activity of tested antibiotics against bacteria isolated from burned skin samples.

No.	Antibiotics	CLSI (2017) standard sensitivity limits (mm)	Zone of inhibition against bacteria isolated from burned skin samples (mm)					
			Streptococcus sp.	*S. epidermidis*	*Klebsiella* sp.	*Enterobacter* sp.	*Bacillus* sp.	*Pseudomonas* sp.
1	Cef	14.0	14.3±1.1	14.0±1.0	14.0±1.0	19.6±1.5	14.0±1.0	26.0±1.4
2	Mer	16.0	31.0±1.0	30.3±1.5	31.0±2.0	32.0±1.0	32.3±1.1	31.3±1.5
3	Clind	15.0	21.3±1.5	32.6±1.5	5.6±0.5	8.3±2.0	7.3±1.5	9.8±1.6
4	Gen	15.6	16.6±1.1	26.6±1.5	23.6±1.5	21.3±2.0	21.6±2.0	15.3±2.0
5	Cip	16.5	30.6±2.0	30.6±1.5	26.3±1.5	30.0±1.0	32.0±1.7	33.6±1.5
6	Azith	14.5	21.3±1.1	22.0±2.0	14.0±2.0	18.0±1.7	14.6±1.5	22.0±1.7
7	Ceftz	13.6	7.3±1.5	5.6±0.5	7.0±2.0	20.0±2.0	24.3±1.5	19.0±2.6
8	Amp	14.5	24.3±0.5	23.6±2.0	15.3±1.5	18.6±2.5	16.3±1.5	6.6±2.0
9	Amik	15.0	20.6±2.0	27.0±2.0	15.6±1.5	23.0±1.0	22.3±2.5	25.1±1.7

Cef = cefepime; Mer = meropenem; Clind = clindamycin; Gen = gentamycin; Cip = ciprofloxacin; Azith = azithromycin; Ceftz = ceftazidime; Amp = ampicillin; Amik = amikacin

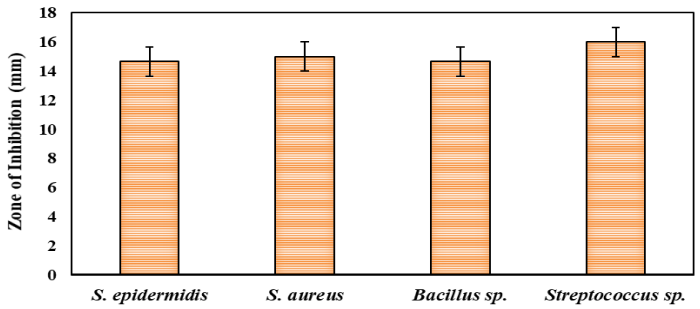

Figure 3: Antibacterial activity of silver sulphadiazine ointment against tested bacterial isolates from unburned skin samples.

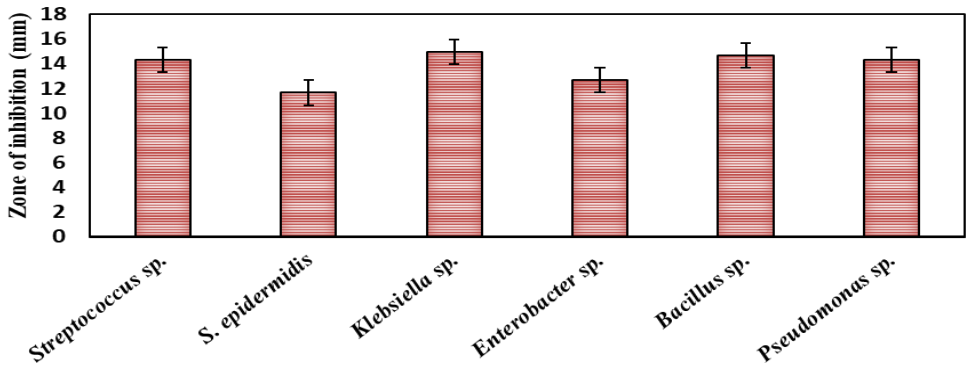

Figure 4: Antibacterial activity of silver sulphadiazine ointment against tested bacterial isolates isolated from burned skin samples.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

2016). Rajbahak *et al.* (2014) isolated a total of 215 bacterial species from burn wounds, in which *P. aeruginosa* accounted for 45.6%, followed by *S. aureus* (19.1%), *Acinetobacter* sp. (17.7%) and coagulase-negative *Staphylococci* (CONS) (5.6%). Further, they concluded that Gram-negative bacteria were the dominating bacteria in burn patient's skin samples due to nosocomial infection. Moreover, different pathogenic bacteria (*P. aeruginosa*, *S. epidermidis*, *Bacillus subtilis* and *Enterobacter* spp.) were isolated from the skin of burned patients via conventional culturing technique. While, among them, *P. aeruginosa* was the most widely recognized and dominating in burned skin samples (Alwan, 2011; Azimi *et al.*, 2011).

Presently, *S. aromaticum* was used as a natural remedy for the treatment of skin burns. Two different extracts such as methanolic extract and aqueous extracts were prepared in five different concentrations i.e. 1, 2, 3, 4, and 5 mg/mL and it was observed that both methanolic and aqueous extract showed a noteworthy activity against tested bacterial pathogens as shown in Figures 1 and 2. A possible reason for this might be that both methanol and water are polar solvents and chemical constituents in *S. aromaticum* had more affinity towards polar solvents that's why methanolic and aqueous extracts of *S. aromaticum* displayed excellent activity against tested bacterial isolates (Pandey and Kim, 2011). Further, Ali *et al.* (2011) also conducted a study to assess the antibacterial activity of aqueous and alcoholic extract of *S. aromaticum* against pathogenic bacteria (*S. aureus*, *S. epidermidis* and *Pseudomonas* sp.) causing hospital-acquired infections. They reported that alcoholic extract of *S. aromaticum* displayed maximum MIC values (780 µg/mL) against all the tested bacterial pathogens as compared to aqueous extract, showing MIC values in the range of 62-250 µg/mL. They further concluded that aqueous extract of *S. aromaticum* was more effective as compared to alcoholic extract of *S. aromaticum* and a possible reason might be that chemical constituents in *S. aromaticum* had more affinity towards highly polar solvents as compared to less polar or non-polar solvents that's why aqueous extract produced inhibitory effects against bacterial isolates in less concentration as compared to alcoholic extract. Moreover, similar results were also reported by Pandey and Kim (2011) about the antibacterial properties of *S. aromaticum* extracts against food-borne pathogens.

Commercially available antibiotics were evaluated for their antimicrobial activity against the prevailing bacterial isolates. For this purpose, a disc diffusion assay was performed to draw antibiotics susceptibility profiles for the isolated bacteria (Tables 2 and 3). The results of the experiments revealed that meropenem was the most potent antibiotic against most of the Gram-negative and Gram-positive bacteria and ciprofloxacin was found to be the second most effective antibiotic. Magnet *et al.* (2013) also conducted a similar study and found that ciprofloxacin was the most sensitive antibiotic against both Gram-positive and Gram-negative bacteria isolated from the skin of burn patients. In our study, *Pseudomonas* sp. showed sensitivity against ciprofloxacin, followed by meropenem, cefepime, amikacin, azithromycin, ceftazidime, and gentamicin, whereas showed resistance to clindamycin and ampicillin. Ahmad and Al-Kafri (2016) also evaluated the potency of commercially available antibiotics against *Pseudomonas* sp. isolated from the skin of burned patients. They reported that bacterial isolates had highest sensitivity to imipenem (47.92%), meropenem (50%), and cefepime (41.67%) and had resistance to ampicillin. Further, they also concluded that the possibility of bacterial infection increases due to nosocomial infection as the patient stays in the burn center for a long period. A possible reason for antibiotic resistance might be the irrational use of antibiotics. Therefore, it was suggested that antibiotics must be used in burn wards after performing susceptibility tests. Furthermore, Magnet *et al.* (2013) also described that genetic variation among bacterial isolates was the most significant reason for antibiotic resistance and suggested that all these complications would be minimized up to some extent by maintaining hygienic conditions within burn wards.

Further, silver sulphadiazine ointment was also assessed in the current study to evaluate their antibacterial activity against bacterial isolates because it is a sulfonamide containing antibacterial; however, unlike other sulfa drugs, this does not inhibit folic acid synthesis. Its antibacterial effects are due to the silver ions. As such, the silver ions only act superficially, and there is limited eschar penetration. The exact mechanism of action of silver sulfadiazine is currently unknown, but the drug hypothetically produces its bactericidal effects by increasing cell wall permeability through the impairment of DNA replication, the direct modification of the lipid cell membrane, and/or the formation of free radicals (Ullah *et al.*, 2019). Ordinary results were observed while using silver sulphadiazine ointment towards isolates and this might be due to nosocomial infections or the microorganisms become less sensitive or resistant to silver compounds. The maximum antibacterial activity of silver sulphadiazine ointment was apparent against *Streptococcus* sp. (16.0 ± 1.8 mm), isolated from unburned skin samples while the remaining isolates also showed sensitivity to silver sulphadiazine ointment. On the other hand, in case of bacterial isolates from infected burned skin samples, excellent activity was observed. It was reported that silver compounds had been used for centuries for their therapeutic properties and were considered as best topical burn treatment in burn wards. Silver sulphadiazine ointment had strong antibacterial activity as it contained silver as a constituent to heal the burn wounds (Atanasova-Pancevska *et al.*, 2017). However, nowadays through quorum sensing, microorganisms can communicate with each other and transfer genetic information through horizontal gene transfer as a result they displayed resistance to silver compounds (Rajbahak *et al*., 2014; Moissl-Eichinger *et al.*, 2017).

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

CONCLUSION

From the present study, it shows that burned patients were at high risk to different microbial infections. It was concluded that both methanolic and aqueous extracts showed excellent activity at all concentrations against all the tested bacteria and likewise, all tested antibiotics expressed potent antimicrobial activity against the majority of tested pathogenic bacteria and few of them had shown resistance. The most effective antibiotic against bacterial isolates was meropenem followed by ciprofloxacin. Consequently, it was concluded that meropenem and ciprofloxacin were the choice of antibiotics for the burn wound infections. Moreover, the isolated pathogenic bacteria were also sensitive to silver sulphadiazine ointment but comparatively, the zone of inhibition produced by antibiotics and methanolic extract of *S. aromaticum* was better than silver sulphadiazine ointment. Overall, it was suggested that proper precautionary measured should be accomplished in burn wards and empirical therapy should be avoided to reduce the chances of multi-drug resistant bacterial infections. Besides, culture sensitivity test must be encouraged to choose effective antibiotic's therapy in burn wards.

ETHICAL APPROVAL

Not Required.

ACKNOWLEDGEMENTS

We are thankful to the Dean of Faculty of Life Sciences, Abasyn University Peshawar, Khyber Pakhtunkhwa, Pakistan, for providing all possible facilities to accomplish this research work.

CONFLICT OF INTEREST

The authors have no potential conflict of interest regarding the manuscript.

REFERENCES

Ahmad, I. and Aqil, F. (2007). *In vitro* efficacy of bioactive extracts of 15 medicinal plants against ESβL-producing multidrug-resistant enteric bacteria. *Microbiological Research* **162(3), 264-275.**

Ahmad, R. and Al-Kafri, A. (2016). Pseudomonal infections in patients with burns in Al-Mouasat Hospital in Damascus-Syria. *Journal of Chemical and Pharmaceutical Sciences* **9(4), 2929-2932.**

Ali, N. H., Faizi, S. and Kazmi, S. U. (2011). Antibacterial activity in spices and local medicinal plants against clinical isolates of Karachi, Pakistan. *Pharmaceutical Biology* **49(8), 833-839.**

Allegranzi, B., Nejad, S. B., Combescure, C., Graafmans, W., Attar, H., Donaldson, L. and Pittet, D. (2011). Burden of endemic health-care-associated infection in developing countries: Systematic review and meta-analysis. *The Lancet* **377(9761), 228-241.**

Alwan, A. (2011). Global status report on non-communicable diseases 2010. World Health Organization **pp. 176.**

Atanasova-Pancevska, N., Bogdanov, J. and Kungulovski, D. (2017). *In vitro* antimicrobial activity and chemical composition of two essential oils and eugenol from flower buds of *Eugenia caryophyllata*. *Open Biological Sciences Journal* **3(1), 16-25.**

Azimi, G., Leion, H., Mattisson, T. and Lyngfelt, A. (2011). Chemical-looping with oxygen uncoupling using combined Mn-Fe oxides, testing in batch fluidized bed. *Energy Procedia* **4, 370-377.**

Benchamkha, Y., Dhaidah, O., Dahazze, A., Meriem, Q., Elamrani, M. D. and Ettalbi, S. (2017). The bacteriological profile of the burned patients in the center of burns in CHU Mohamed VI Marrakech (about 123 cases). *International Journal of Burns and Trauma* **7(6), 72.**

Chen, Y. E., Fischbach, M. A. and Belkaid, Y. (2018). Skin microbiota–host interactions. *Nature* **553(7689), 427-436.**

CLSI, Clinical Laboratory Standards Institute (2017). Performance Standards for Antimicrobial Susceptibility Testing**.** Wayne, PA, USA.

Cong, J. and Zhang, X. (2018). How human microbiome talks to health and disease. *European Journal of Clinical Microbiology and Infectious Diseases* **37(9), 1595-1601.**

Cressy, H. K., Jerrett, A. R., Osborne, C. M. and Bremer, P. J. (2003). A novel method for the reduction of numbers of Listeria monocytogenes cells by freezing in combination with an essential oil in bacteriological media. *Journal of Food Protection* **66(3), 390-395.**

Dai, T., Huang, Y. Y., Sharma, S. K., Hashmi, J. T., Kurup, D. B. and Hamblin, M. R. (2010). Topical antimicrobials for burn wound infections. *Recent Patents on Anti-Infective Drug Discovery* **5(2), 124-151.**

Darlenski, R. and Fluhr, J. W. (2017). Measurement of skin surface acidity. *In:* Agache's Measuring the Skin. Humbert, P., Fanian, F., Maibach, H. I. and Agache, P. (eds.). 2nd edn. Springer, Cham. **pp. 113-120.**

Espiritu, A. A., Lao, S. N. L. and Guerrero, J. J. G. (2016). Burn wound healing potential of *Bixa orellana* Linn [*Bixaceae*] leaf extracts on albino mice. *Journal of Medicinal Plants* **4, 84-87.**

Gallo, R. L. and Nakatsuji, T. (2011). Microbial symbiosis with the innate immune defense system of the skin. *Journal of Investigative Dermatology* **131(10), 1974-1980.**

Grice, E. A., Kong, H. H., Conlan, S., Deming, C. B., Davis, J., Young, A. C. and Turner, M. L. (2009). Topographical and temporal diversity of the human skin microbiome. *Science* **324(5931), 1190-1192.**

Im, A., Kim, H. S., Hyun, J. W. and Chae, S. (2016). Potential for tyndalized *Lactobacillus acidophilus* as an effective component in moisturizing skin and anti-

wrinkle products. *Experimental and Therapeutic Medicine* **12(2), 759-764.**

Kim, A. L., Labasi, J. M., Zhu, Y., Tang, X., McClure, K., Gabel, C. A. and Bickers, D. R. (2005). Role of p38 MAPK in UVB-induced inflammatory responses in the skin of SKH-1 hairless mice. *Journal of Investigative Dermatology* **124(6), 1318-1325.**

Magnet, M. D. M. H., Arongozeb, M. D., Khan, G. M. and Ahmed, Z. (2013). Isolation and identification of different bacteria from different types of burn wound infections and study their antimicrobial sensitivity pattern. *International Journal of Research in Applied, Natural and Social Sciences* **1(3), 125-132.**

Moissl-Eichinger, C., Probst, A. J., Birarda, G., Auerbach, A., Koskinen, K., Wolf, P. and Holman, H. Y. N. (2017). Human age and skin physiology shape diversity and abundance of Archaea on skin. *Scientific Reports* **7(1), 1-10.**

Mostafa, N. E. S., Hamed, E. F. A., Rashed, H. E. S., Mohamed, S. Y., Abdelgawad, M. S. and Elasbali, A. M. (2018). The relationship between toxoplasmosis and different types of human tumors. *The Journal of Infection in Developing Countries* **12(2), 137-141.**

Nagoba, B. S., Gandhi, R. C., Hartalkar, A. R., Wadher, B. J. and Selkar, S. P. (2010). Simple, effective and affordable approach for the treatment of burns infections. *Burns* **36(8), 1242-1247.**

Oaks, R. J. and Cindass, R. (2020). Silver Sulfadiazine. Treasure Island (FL), StatPearls Publishing: https://www.ncbi.nlm.nih.gov/books/NBK556054/ [Retrieved on 1 August 2020].

Oncul, O., Yüksel, F., Altunay, H., Açikel, C., Çeliköz, B. and Çavuşlu, Ş. (2002). The evaluation of nosocomial infection during 1-year-period in the burn unit of a training hospital in Istanbul, Turkey. *Burns* **28(8), 738-744.**

Pahlow, S., Meisel, S., Cialla-May, D., Weber, K., Rösch, P. and Popp, J. (2015). Isolation and identification of bacteria by means of Raman spectroscopy. *Advanced Drug Delivery Reviews* **89, 105-120.**

Pandey, M. P. and Kim, C. S. (2011). Lignin depolymerisation and conversion: A review of thermochemical methods. *Chemical Engineering and Technology* **34(1), 29-41.**

Pappas, G., Roussos, N. and Falagas, M. E. (2009). Toxoplasmosis snapshots: Global status of *Toxoplasma gondii* seroprevalence and implications for pregnancy and congenital toxoplasmosis. *International Journal for Parasitology* **39(12), 1385-1394.**

Proksch, E., Brandner, J. M. and Jensen, J. M. (2008). The skin: An indispensable barrier. *Experimental Dermatology* **17(12), 1063-1072.**

Rajbahak, S., Shrestha, C. and Singh, A. (2014). Bacteriological changes of burn wounds with time and their antibiogram. *Scientific World* **12(12), 70-76.**

Shoaib, A., Saeed, G. and Ahmad, S. (2014). Antimicrobial activity and chemical analysis of some edible oils (Clove, Kalonji and Taramira). *African Journal of Biotechnology* **13(46), 4347-4354.**

Simard-Bisson, C., Parent, L. A., Moulin, V. J. and Fruteau de Laclos, B. (2018). Characterization of epidermal lipoxygenase expression in normal human skin and tissue-engineered skin substitutes. *Journal of Histochemistry and Cytochemistry* **66(11), 813-824.**

Ullah, S., Hashmi, M., Kharaghani, D., Khan, M. Q., Saito, Y., Yamamoto, T. and Kim, I. S. (2019). Antibacterial properties of *in situ* and surface functionalized impregnation of silver sulfadiazine in polyacrylonitrile nanofiber mats. *International Journal of Nanomedicine* **14, 2693.**

Yoo, S. M. and Lee, S. Y. (2016). Optical biosensors for the detection of pathogenic microorganisms. *Trends in Biotechnology* **34(1), 7-25.**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538