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Antimicrobial Activities of Unconventional Compounds against Some Bacteria Associated with Skin Infections in Humans, Sheep and Goats

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Abstract: Bacterial skin infections are a common problem encountered in clinical practice and causing great economic losses for sheep and goats producers. Increasing multidrug resistance of pathogens paves the way for reconsidering alternative medicine. The present study was carried out to explore the antibacterial activities of different volumes; 5, 25, 50 and 100 µL of gold nanoparticles (NPS) and the aqueous and ethanolic extracts of garlic, turmeric and cinnamon at different concentrations (20, 40, 80 and 100%) against molecularly confirmed *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Escherichia coli* isolated from skin pyogenic lesions in humans, sheep and goats using disc diffusion assay. The results compared with ciprofloxacin (5 µg mL^{-1}). Gold nanoparticles (NPS) 100 µL were highly effective against *Pseudomonas aeruginosa* and *Escherichia coli* in comparing with ciprofloxacin (5 µg mL^{-1}). Garlic has shown better activity against *Staphylococcus aureus* and *Streptococcus pyogenes* in aqueous solution while the ethanolic extract of cinnamon and turmeric was more efficient than the aqueous extract. Among the three tested spices, turmeric was the least effective against tested bacteria. The proven activity of 100 µL Gold nanoparticles (NPS) and 100% aqueous garlic extract compared with ciprofloxacin (5 µg mL^{-1}), suggests their use in clinical trials as an alternative medicine to reduce the side effects and progressively increasing drug resistance of pathogens.

Keywords: *S. aureus*, *S. pyogenes*, *Ps. aeruginosa*, *E. coli*, GNPS, Spices

Introduction

Bacterial skin infections are very common and they can range from merely annoying to deadly. Most bacterial infections of the skin are caused by gram-positive bacteria (Streptococcus, Staphylococcus) and gram-negative (e.g., Klebsiella, *Escherichia coli,* Pseudomonas) (AFHSC, 2013). Pyogenic skin infections of sheep and goats are of worldwide distribution, especially in developing countries. It causes severe economic losses to sheep and goats producers due to decrease live animal sales, condemnation and downgrading of carcasses and skin in abattoirs as well as the reduction in wool growth (Koutinas *et al*., 2007). Bacterial skin infections are treated with antibiotics, but there is a concern that widespread antibiotic use might lead to antibiotic resistance. Antibiotics resistance are an increasing public health problem. Multiple drug resistance has been developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. In addition, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression, allergic reactions. Certain strains of bacteria are able to produce substances which block the action of antibiotics or change their target or ability to penetrate cells (Aly, 2013). This situation forced scientists to search for a new treatment that does not generate resistance and present a good bactericidal property. Gold-nanoparticles have a great bactericidal effect on a wide range of bacteria; its bactericidal effect depends on the size and the shape of the particle (Nirmala *et al*., 2011). Nanoparticles can act as antibacterial and antifungal agents, due to their ability to interact with

microorganisms (Eby *et al*., 2009; Panacek *et al*., 2009). Nanoparticles attach to the surface of the cell, this interaction causes structural changes and damage; markedly disturbing vital cell functions such as permeability, causing pits and gaps, depressing the activity of respiratory chain enzymes and finally leading to cell death (Li *et al*., 2010). Several species of plants have been used for centuries as remedies for human diseases because they contain components of therapeutic values (Kaushik *et al*., 2011). Recently, the acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics, have led researchers to investigate the antimicrobial activity of medicinal plants (Samie *et al*., 2009). Medicinal plants produce certain bioactive molecules which show both antibacterial and antifungal activities (Violante *et al*., 2012). Many medicinal plants produce antioxidant and antimicrobial properties which protect the host from cellular oxidation reactions and other pathogens highlighting the importance of looking for natural antimicrobial drugs (Farzaei *et al*., 2014; Bajpai *et al*., 2005). Garlic has generated a lot of interest as a medicinal plant. A broad range of microorganism including bacteria, fungi, protozoa and viruses has been found to be responsive to crushed garlic preparations. Chemical analysis of garlic has revealed an unusual concentration of sulfur-containing compounds which have antibiosis effect (Shaheen *et al*., 2015). Cinnamon has a potential medical use with regards to its antimicrobial properties, especially antibacterial activity (Nabavi *et al*., 2015). Turmeric is known to possess multifunctional properties, including antibacterial activity (Izui *et al*., 2015).

The aim of the present study was to identify different etiological pathogens which inhabit the pyogenic skin lesions in humans, sheep and goats and find the inhibitory activity of gold nanoparticles and different concentrations of the aqueous and ethanolic extracts of garlic, cinnamon, turmeric against *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa* and *Escherichia coli* isolates, associated with pyogenic skin infections in comparing with ciprofloxacin (5 μg mL^{-1}).

Materials and Methods

Bacterial Isolates

Swabs from pyogenic skin lesions were collected from patients in King Fahd hospital at Al-Madinah Al-Munawarah, sterile cotton swabs which were moistened with sterile saline to prevent drying were used for sample collection. A swab from the affected area or the exudates of the lesions were collected from the affected sheep and goats. The specimens were brought to the laboratory in a sterile container within one hour after the collection and processed immediately and inoculated on MaCconkey agar and blood agar plates for isolating the pathogens. The inoculated plates were incubated at 37°C overnight. After incubation, the plates were observed for growth and the isolated colonies were identified by morphological and biochemical characteristics. The tests performed include Gram staining, Motility, Catalase, Oxidase, Indole, Methyl Red, Voges-Proskauer, Citrate Utilization production, Urease production, Triple Sugar Iron, Mannitol, Phenylalanine (Koneman *et al*., 2005). The pure cultures were refreshed on Nutrient agar slant and kept in the incubator for 24 h at 37°C then stored at 4°C.

Molecular Identification of Isolates

Template DNA Preparation

DNA templates for PCR were obtained from overnight bacterial cultures that were suspended in 200 mL of sterile distilled water and boiled for 15 min (Usein *et al*., 2009).

Polymerase Chain Reaction (PCR)

Detection of a species-specific gene (*uidA*) in *E. coli*, (ecfX) in *Pseudomonas areuginosa* and 16s rRNA in *staphylococcus aureus* and *streptococcus pyogens* were performed by conventional PCR (Moyo *et al*., 2007; Al-Talib *et al*., 2009; Clifford *et al*., 2012). Primer sequences and PCR conditions used for the study are listed in Table 1. PCR was performed in the Takara thermal cycler (Bio-Rad). PCR products were separated and visualized by gel electrophoresis in 1.5% agarose (Wako, Japan) in Tris-Acetate-Edta (TAE) buffer at 100 V. A 100 or 500 bp DNA ladder (one-step ladder, Wako) was included in each agarose run, according to the amplified product.

Gold Nanoparticles Preparation

Gold colloids 5, 25, 50 and 100 μL were prepared by citrate thermal reduction method (Yang *et al*., 2005). In this method, a gold solution was prepared by adding 1 mL of 1% hydrogen tetra-chloroauric (HAucl4.3H2O) aqueous solution and 1.5 mL of 38.8 mM sodium citrate aqueous solution into 90 mL boiling water. After the solution had turned purple-red within the 30s, the solution was cooled quickly in an ice bath. This indicated the dispersion of gold particles with an average diameter of around 12 mm width and 40 mm length. 0.2 mL of 0.1 M freshly prepared acetyl Trimethyl Ammonium Bromide (CTAB) aqueous solution was added to 20 mL of prepared gold colloid at room temperature. Finally, 1 mL of 0.5 mM of 11-Mercaptoundecanoic (MUA) aqueous solution was added to the gold colloid-modified by 0.1 mM of CTAB in order to restrain the over much aggregation process.

Table 1. PCR primers and conditions for amplification of virulence genes

Target gene	Primer designation	Primer sequence (5'-3')	Length (bp) PCR conditions *	PCR product	Reference
UidA (*E. coli*)	*UidA-F*	CCAAAAGCCAGACAGAGT	95°C, 30 s; 57°C, 30 s; 72°C,30 s	623	42
	UidA-R	GCACAGCACATCAAAGAG			
16S rRNA	*16S rRNA-F*	GCAAGCGTTATCCGGATTT	95°C, 30 s; 57°C,30 s; 72°C,30 s	597	43
(*S. aureus)*	*16S rRNA-R*	CTTAATGATGGCAACTAAG			
ecfX	*ecfXRT-F*	AGCGTTCGTCCTGCACAAGT	95°C, 30 s;52°C,30 s; 72°C,30 s	81	44
(*Ps. aeruginosa*)	*ecfXRT-R*	TCCACCATGCTCAGGGAGAT			
16S rRNA	*16S rRNA-F*	CAGTTCGGATTGTAGGCTGC	95°C, 30 s; 52°C, 30 s; 72°C, 30 s	194	The current
(*S. pyogenes*)	*16S rRNA-R*	ACCCCAATCATCTATCCCACC			study

Extract and Preparation of Plant Materials

The spices including Garlic (*Allium sativum*), Turmeric (*Curcuma longa*) and Cinnamon (*Cinnamomum zeylanicum*) were purchased from local market. The species were washed with distilled water thoroughly. Garlic (100 mg each) were washed by distilled water, homogenized using sterile mortar or blender and then saved through double layer of sterile line mesh cloth to make 100% extract. Turmeric, Cinnamon (100 mg each) was crushed and sieved through mesh cloth to get the fine powder. Powdered spices were soaked in 200 mL of sterile distilled water and kept at room temperature for 24 h, then were filtered using Whatman filter paper. The filtrate was kept at room temperature until dry and thick layer formed. The drying thick layer was considered as 100% extract. This extract was stored at 4°C, further diluted to make different concentrations such as 80, 40 and 20% by mixing with appropriate volumes of distilled water. The ethanolic extract was prepared following the same procedure with the exception of solvent which was 95% ethanolic instead of sterilized distilled water.

Antimicrobial Activity Testing

Standard well agar diffusion method was carried out to detect the activity of gold nanoparticles and garlic, cinnamon, turmeric in aqueous and ethanolic extract against pathogenic bacterial isolates according to (Cheesbrough, 2000). Only 100 mL of the overnight 0.5 McFarland suspension of each isolate were inoculated into 100 mL warm nutrient agar medium (45-55°C). The media were poured in sterile plates and left for solidification. Each plate is called a seeded plate. The seeded plates with the isolated bacteria were cut by sterile cork borer to make holes (8 mm in diameter). Each hole was saturated (100 mL) with different volumes of gold nanoparticles (5, 25, 50 and 100 µL), 100 mL of the ethanol extract and aqueous extract of garlic, cinnamon and turmeric were transferred into each hole at all dilutions and 50 µL ciprofloxacin (5 µL) under aseptic conditions. Then, the plates were kept in the refrigerator for 2 h before incubation to permit diffusion of the extract before the growth of the tested isolates takes place. The plates were incubated at 37°C for 24 h and then examined for antibacterial activity. Duplicate plates were used for each isolate. The detection of clear inhibition zones around the wells on the inoculated plates is an indication of antimicrobial activity.

Antimicrobial Activity of Ciprofloxacin (5 µg mL^{-1})

Antimicrobial susceptibility testing of the isolates to ciprofloxacin (5 µg mL^{-1}) was performed using the standard disc diffusion method; using commercially available antimicrobial susceptibility discs (Kirby-Bauer SN DISC, Nissui Pharmaceuticals, Tokyo, Japan) according to Clinical and Laboratory Standards Institute (CLSI) instructions (Wikler, 2006).

Results

Bacterial Isolates

Molecularly confirmed *Staphylococcus aureus, Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Escherichia coli* were obtained from pyogenic skin lesions.

Characterization of Gold Nanoparticles

Photo (1) shows TEM image of the obtained gold nanoparticles. The prepared gold nanoparticles were almost rod shape and separated from each other. The particle size mainly was 12 nm width and 40 nm length. The absorption spectrum of these gold rods has two characteristic absorption bands, one at 523 nm for transverse surface Plasmon resonance and the other at 753 nm for the longitudinal surface Plasmon band (Fig. 1).

Antimicrobial Activity of Gold Nanoparticles

Antibacterial activities of gold nanoparticles increased with higher volume; 100 > 50 > 25> 5 µL. Gold nanoparticles (vol. 100 µL) showed great antimicrobial activities with the best inhibition zone against *Ps. areuginosa* (27 mm), *E. coli* (25.5 mm) and *S. pyogens* (25 mm) and *S. aureus* (24 mm) as shown in Fig. 2 and Table 2. The effect of ciprofloxacin antibiotics was also studied, the best results were against *S. aureus* with an inhibition zone of (25 mm).

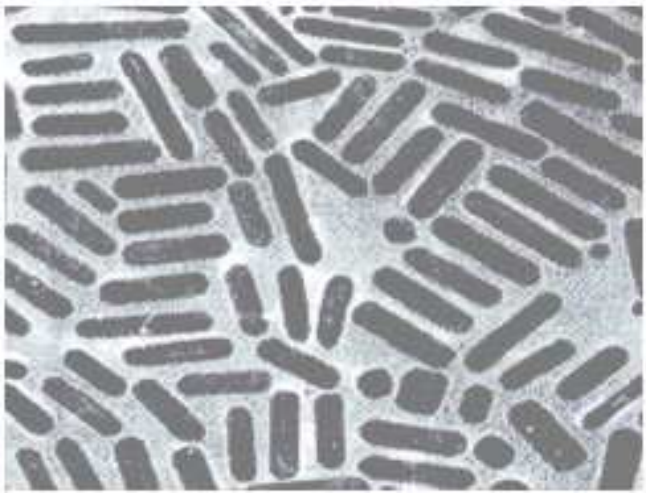

Fig. 1. TEM image of gold nanoparticles

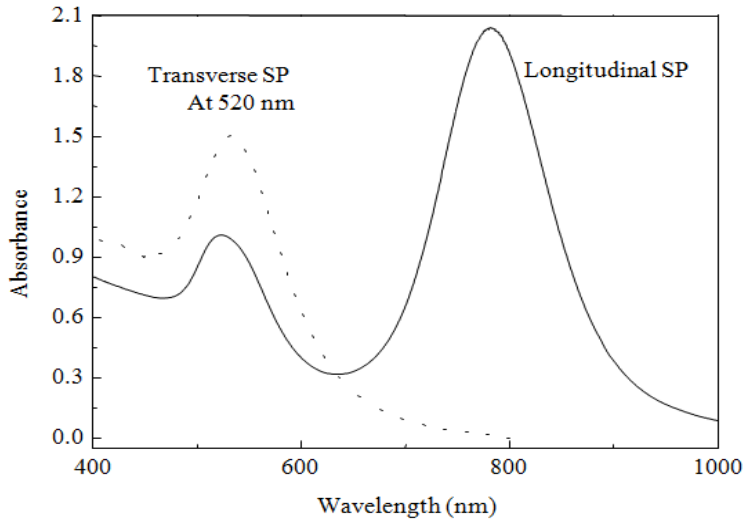

Fig. 2. Absorption spectra of gold nanoparticles

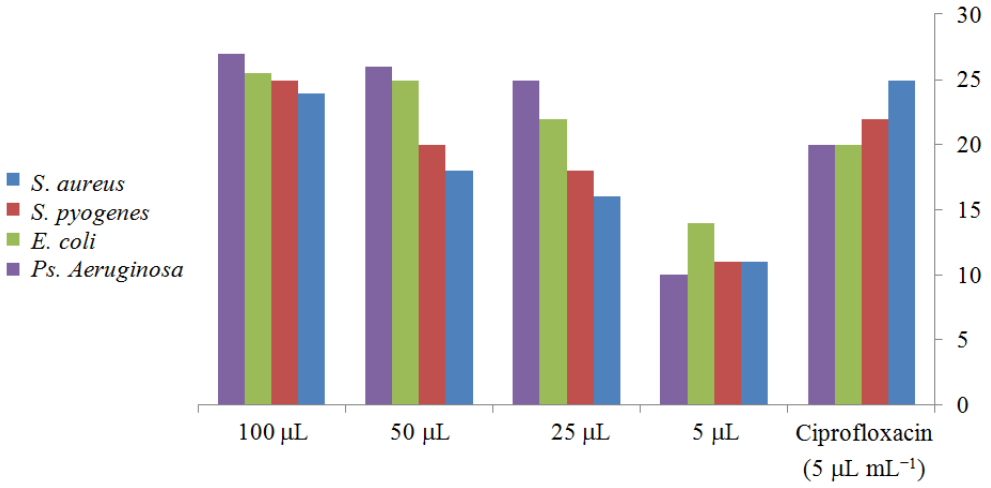

Fig. 3. Antibacterial activity of different volumes of gold nanoparticles against bacterial isolates compared with Ciprofloxacin (5 µg mL^{-1})

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Antimicrobial Activity of Garlic, Cinnamon and Turmeric Solution

Among the three tested spices, garlic has shown the best activity at all concentrations both in aqueous and ethanolic solution. Garlic has shown better activity against *S. aureus* (28 mm) in aqueous solution as compared to the other tested bacteria. Aqueous extract of garlic was more effective as compared to ethanolic extract. The activity of 100% garlic extract was comparatively more than of ciprofloxacin (5 µg mL^{-1}) (Fig. 3 and Table 3). The cinnamon ethanolic (100%) extract was also effective against *E. coli* (17 mm). The ethanolic extract of cinnamon was more efficient in its antibacterial activity as compared to the aqueous extract (Fig. 4 and Table 4). Turmeric was less effective against tested bacteria among the three tested spices. The ethanol extract of turmeric showed better results as compared to the aqueous ones showing the largest inhibition zone against *S. pyogens* (14.1 mm) at 100% ethanolic extract (Fig. 5 and Table 5).

Antimicrobial Activity of Ciprofloxacin (5 µg mL^{-1})

Ciprofloxacin showed high activity against the tested isolates, the largest inhibition zone (25 mm) was around *S. aureus*, followed by *S. pyogenes, E. coli*, *Ps. aeruginosa* (22, 20 and 20 mm respectively).

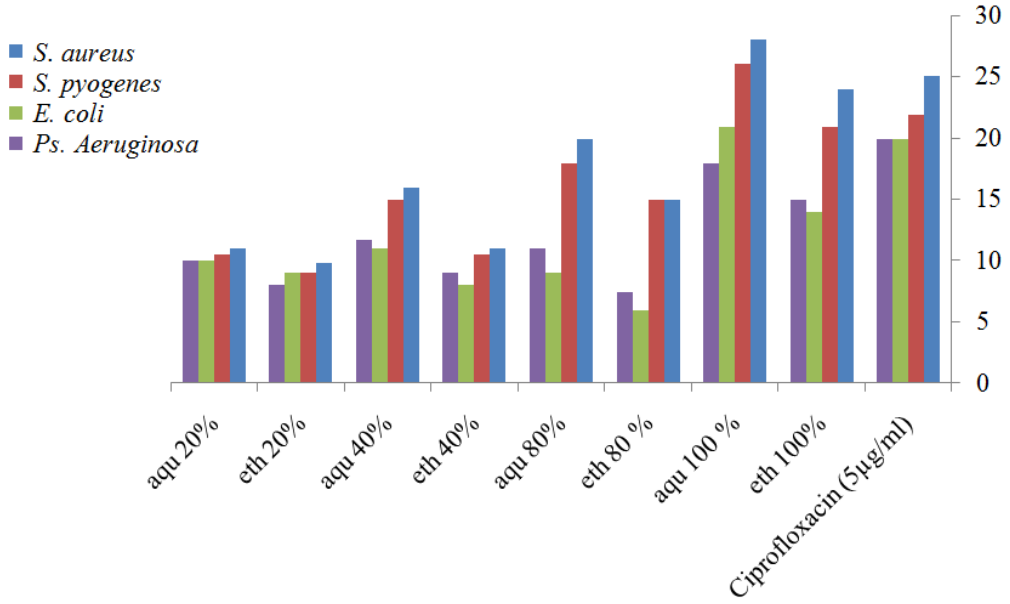

Fig. 4. Antibacterial activity of different concentrations of garlic against bacterial isolates compared with Ciprofloxacin (5 µg mL^{-1})

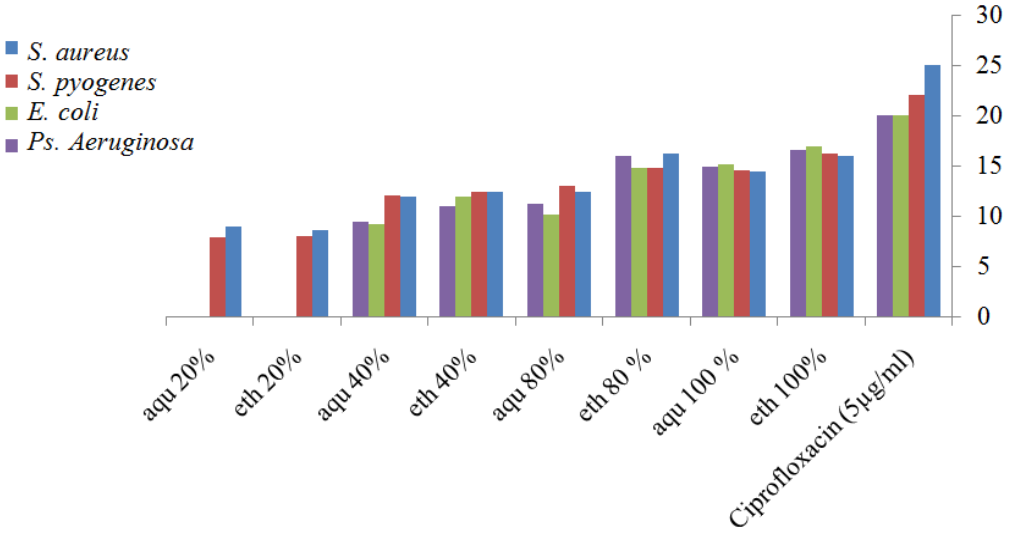

Fig. 5. Antibacterial activity of different concentrations of cinnamon against bacterial isolates compared with Ciprofloxacin (5µg mL^{-1})

Table 2. Antibacterial activity of different volumes of gold nanoparticles against bacterial isolates compared with Ciprofloxacin (5 μg mL^{-1})

	Volume of gold nanoparticles (μL)				
Bacterial isolates	5 μL	25 μL	50 μL	100 μL	Ciprofloxacin (5μg mL^{-1})
S. aureus	11*	16	18	24	25
S. pyogenes	11	18	20	25	22
E. coli	14	22	25	25.5	20
Ps. aeruginosa	10	25	26	27	20

* :Mean value of inhibition zone (mm)

Table 3. Antibacterial activity of different concentrations of garlic against bacterial isolates compared with Ciprofloxacin (5 μg mL^{-1})

	Concentration								
	20%		40%		80%		100%		
Bacterial isolates	aqaous	ethanol	aqaous	ethanol	aqaous	ethanol	aqueous	ethanol	Ciprofloxacin (5μg mL^{-1})
S. aureus	11*	9.8	16.0	11.0	20	15.0	28	24	25
S. pyogenes	10.5	9.0	15.0	10.5	18	15.0	26	21	22
E. coli	10.0	9.0	11.0	8.0	9	6.0	21	41	20
Ps. aeruginosa	10.0	8.0	11.7	9.0	11	7.5	18	15	20

* :Mean value of inhibition zone (mm)

Table 4. Antibacterial activity of different concentrations of cinnamon against bacterial isolates compared with Ciprofloxacin (5 μg mL^{-1})

	Concentration								
	20%		40%		80%		100%		
Bacterial isolates	aqaous	ethanol	aqaous	ethanol	aqaous	ethanol	aqueous	ethanol	Ciprofloxacin (5 μg mL^{-1})
S. aureus	9*	8.6	12.0	12.5	12.4	16.3	14.5	16.0	25
S. pyogenes	8	8.1	12.1	12.5	13.0	14.8	14.6	16.3	22
E. coli	0	0.0	9.3	12.0	10.2	14.8	15.2	17.0	20
Ps. aeruginosa	0	0.0	9.5	11.0	11.3	16.0	14.9	16.6	20

* :Mean value of inhibition zone (mm)

Table 5. Antibacterial activity of different concentrations of tumeric against bacterial isolates compared with Ciprofloxacin (5 μg mL^{-1})

	Concentration								
	20%		40%		80%		100%		
Bacterial isolates	aqaous	ethanol	aqaous	ethanol	aqaous	ethanol	aqueous	ethanol	Ciprofloxacin (5 μg mL^{-1})
S. aureus	6.4*	8.0	9.5	10.5	10.1	13.0	11.6	13.5	25
S. pyogenes	7.0	9.9	10.6	13.0	11.0	13.5	12.6	14.1	22
E. coli	8.0	8.8	9.0	9.5	9.6	10.3	9.7	10.5	20
Ps. aeruginosa	8.3	8.5	9.0	9.3	9.5	10.1	9.7	10.4	20

Discussion

Escherichia coli, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Pseudomonas aeruginosa* are the most commonly isolated bacteria from pyogenic skin lesions (Lorrot *et al*., 2014; Zhang *et al*., 2014). The tested isolates in the current study were sensitive to ciprofloxacin, which is so far effective against the bacterial infections except in the case of the development of antimicrobial resistance (Sedláková *et al*., 2014). Antibiotic resistance poses a growing threat to health. Complementary and Alternative Medicine (CAM) therapies may provide a safer and a more effective treatment (MacKay, 2003). Antimicrobial activities of gold nanoparticles or plant extract differ according to the volume or concentration used and the tested isolates. The antimicrobial ability of gold nanoparticles might be referred to their size which is smaller than the bacterium and fungal cells (Nirmala *et al*., 2011). This makes them easier to adhere with the cell wall of the microorganisms

causing its destruction and leading to the death of the cell. Metal nanoparticles are harmful to bacteria and fungi (Chwalibog *et al*., 2010). Gold nanoparticles stimulate biofilm production and aggregate within a biofilm, they bind closely to the surface of microorganisms causing visible damage to the cells and demonstrating good self-assembling ability. Gold nanoparticles possess well-developed surface chemistry, chemical stability, appropriate smaller size, which make them easier to interact with the microorganisms (Nirmala *et al*., 2011). The particles interact also with the building elements of the outer membrane and might cause structural changes; degradation and finally cell death. The antibacterial activities of the synthesized gold nanoparticles could be due to the susceptibility of pathogens cell wall and toxicity of metallic gold. Gold nanoparticles exert their antimicrobial action mainly changing the membrane potential and inhibiting ATP syntheses activities to decrease the ATP level, indicating a general decline in metabolism and it also inhibits the ribosome subunit for tRNA binding, indicating a collapse of a biological process. Gold nanoparticles also enhance chemotaxis in the early-phase reaction (Cui *et al*., 2012). Gold was tested by the well diffusion method against *Microsporum gypsum* (10 mm) and *Trichophyton rubrum* (13 mm) (Karthik *et al*., 2013). The accumulation of positively charge gold nanoparticles (Au+) on the negatively charged cell membrane of microorganisms leads to conformational changes in the membrane, which loses permeability control which in turn cause the cell death (Chwalibog *et al*., 2010). This may be the possible mechanism of gold nanoparticles which Perform *in vitro* free radical quenching property; on the other hand, it induces death of the microorganism. However, further studies are required to know more about the biological activity of nanoparticles.

Garlic has shown the best activity and its aqueous extract was more effective as compared to ethanolic extract. Garlic has better activity against *B. subtilis* as compared to *E. coli* and aqueous extract of garlic was more effective as compared to ethanolic extract (Gull *et al*., 2012). Garlic was more effective against Gram-positive than Gram-negative bacteria (Srinivasans *et al*., 2009). The Gram-negative *E. coli* was comparatively resistant to Gram-positive bacteria, this may be due to the structure differences in cell membrane and cell wall structure, Gram-negative has outer membrane as well which further block the penetration of antibiotics including the extracts of spices making them resistant. The garlic extract is effective against different serotype of *E. coli* and also *S. aureus* (Naveed *et al*., 2013). Fresh garlic concentration 0.5-5.0% was sufficient to inhibit the growth of *E. coli* O157: H7 (Tessema *et al*., 2006).

The ethanolic extract of cinnamon was more efficient than its aqueous extract, as the antimicrobial component of the cinnamon bark is more soluble in ethanol as compared to water, but its activity was reported less as compared to the garlic (Naveed *et al*., 2013). Cinnamon extract possesses effective antibacterial properties against *B. subtilis* and *E. coli*, Gram-positive bacteria are more sensitive to essential oil of *Cinnamon zeylanicum* than Gram-negative bacteria (Buru *et al*., 2014). Some molecules of cinnamon oil (cinnamaldehyde and cinnamyl) bind to membrane proteins and inhibit peptidoglycan synthesis, the essential component of the bacterial cell wall, thereby increasing their antibacterial effect (Al-Mariri and Safi, 2014). The antimicrobial activity of cinnamon might be due to the presence of cinnamaldehyde compound which inhibits the amino acid decarboxylation activity in the cell which leads to energy deprivation and microbial cell wall (Ooi *et al*., 2006). These phenolic compounds are capable of further cellular destruction and inhibition by establishing the hydrophobic and hydrogen bonding of theses degradative phenolic compounds to membrane proteins resulting in portioning of the lipid bilayer (Juven *et al*., 1994). The antimicrobial activity at aqueous extracts could be due to anionic components such as thiocyanate, nitrate, chlorides and sulfates in addition to many other compounds naturally present in plants (Hill *et al*., 2014). The ethanolic extracts showed better results as compared to aqueous as being organic dissolves more organic compounds resulting in the release of a greater amount of active antimicrobial components (Cowan, 1999).

Turmeric extract was effective against all tested bacterial isolates in both aqueous and ethanolic solution; Turmeric was effective against *E. coli*. *b. subtilus* and *s. aureus* due to the presence of a phenolic compound, curcuminoid, the presences of essential oil, an alkaloid, curcumins, turmerol and veleric acid are responsible for the antibacterial activity of turmeric (Moghadamtousi *et al*., 2014). Chloroform and isoamyl alcohol extracts of *Cuminum cyminum* showed a significant effect against *Ps. aeruginosa*, *S. marcesnces* and *S. pyogenes* (Awan *et al*., 2013). Aqueous and ethanolic extract of turmeric showed antibacterial activity against all bacterial cells isolated and tested at concentrations of 40, 80 and 100 %, but an aqueous and ethanolic extract of cinnamon has no effect on *E. coli* and *Ps. aeuginosa* at 20% concentration (Fig. 6). Curcumin inhibits bacterial cell division (Rai *et al*., 2008). Curcumin can be used safely in a wide range of concentrations without toxicity (Yeon *et al*., 2010).

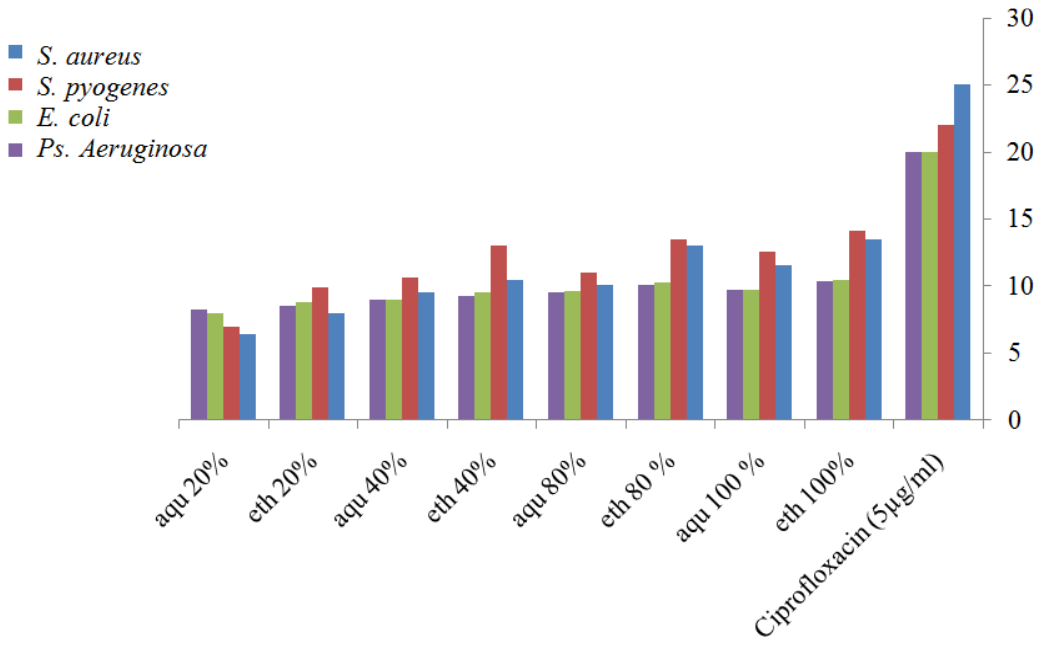

Fig. 6. Antibacterial activity of different concentrations of tumeric against bacterial isolates compared with Ciprofloxacin (5 µg mL^{-1})

Conclusion

The present study concluded that gold nanoparticles and plant extract can be used either in aqueous or ethanolic solution to produce new therapeutics. Further research is required for possible future use of these extracts as alternatives to common antibiotics.

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Author's Contributions

Iman Shabana: Contributed to the design and the establishment of experiments scheme. Sample collection, isolation and identification of the bacterial isolates. The performance of the disc diffusion assay. Data modeling, analysis and interpretation. The orientation of statistical graphics. Contributed to the writing of the manuscript. Important review contributions.

Amira El-Adly: Contributed to the design and the establishment of experiments scheme. Preparation of extracts and gold nanoparticles. The performance of the disc diffusion assay. Data modeling, analysis and interpretation. Contributed to the writing of the manuscript. Important review contributions.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

References

Al-Mariri, A. and M. Safi, 2014. *In vitro* antibacterial activity of several plant extracts and oils against some gram-negative bacteria. Iran J. Med. Sci., 39: 36-43. PMID: 24453392

Al-Talib, H., C.Y. Yean, A. Al-Khateeb, H. Hassan and K.K. Singh *et al*., 2009. A pentaplex PCR assay for the rapid detection of methicillin-resistant *Staphylococcus aureus* and Panton-Valentine Leucocidin. BMC Microbiol., 9: 113-113. DOI: 10.1186/1471-2180-9-113

Aly, S.M., 2013. Risk of antimicrobial misuse. Int. J. Health Sci., 7: 5-7. DOI: 10.12816/0006014

AFHSC, 2013. Bacterial skin infections, active component, U.S. Armed Forces, 2000-2012. MSMR., 20: 2-7. PMID: 24428536

Awan, U.A., S. Andleeb, A. Kiyani, A. Zafar and I. Shafique *et al*., 2013. Antibacterial screening of traditional herbal plants and standard antibiotics against some human bacterial pathogens. Pak. J. Pharm. Sci., 26: 1109-1116. PMID: 24191314

Bajpai, M., A. Pande, S.K. Tewari and D. Prakash, 2005. Phenolic contents and antioxidant activity of some food and medicinal plants. Int. J. Food Sci. Nutr., 56: 287-291. DOI: 10.1080/09637480500146606

Buru, A.S., M.R. Pichika, V. Neela and K. Mohandas, 2014. *In vitro* antibacterial effects of Cinnamomum extracts on common bacteria found in wound infections with emphasis on methicillin-resistant *Staphylococcus aureus*. J. Ethnopharmacol., 153: 587-595. DOI: 10.1016/j.jep.2014.02.044

Cheesbrough, M., 2000. District Laboratory Practice in Tropical Countries, Part 2. 2nd Edn., Cambridge University Press, Cambridge, ISBN-10: 0521676312, pp: 440.

Chwalibog, A., E. Sawosz, A. Hotowy, J. Szeliga and S. Mitura *et al*., 2010. Visualization of interaction between inorganic nanoparticles and bacteria or fungi. Int. J. Nanomed., 5: 1085-1094. DOI: 10.2147/IJN.S13532

Clifford, R.J., M. Milillo, J. Prestwood, R. Quintero and D.V. Zurawski *et al*., 2012. Detection of bacterial 16S rRNA and identification of four clinically important bacteria by real-time PCR. PLoS One., 7: e48558-e48558. PMID: 23139793

Cowan, M.M., 1999. Plant products as antimicrobial agents. J. Clin Microbiol. Rev., 12: 564-582. PMID: 10515903

Cui, Y., Y. Zhao, Y. Tian, W. Zhang and X. Lux, 2012. The molecular mechanism of action of bactericidal gold nanoparticles on *Escherichia coli*. Biomaterials., 33: 2327-2333. DOI: 10.1016/j.biomaterials.2011.11.057

Eby, D.M., N.M. Shaeublin, K.E. Farrington, S.M. Hussain and G.R. Johnson, 2009. Lysozyme catalyzes the formation of antimicrobial silver nanoparticles. ACS. Nano., 3: 384-394. DOI: 10.1021/nn900079e

Farzaei, M.H., R. Rahimi, F. Attar, F. Siavoshi and P. Saniee *et al*., 2014. Chemical composition, antioxidant and antimicrobial activity of essential oil and extracts of *Tragopogon graminifolius*, a medicinal herb from Iran. Nat. Prod. Commun., 9: 121-124. PMID: 24660479

Gull, I., M. Saeed, H. Shaukat, S.M. Aslam and Z.Q. Samra *et al*., 2012. Inhibitory effect of *Allium sativum* and *Zingiber officinale* extracts on clinically important drug resistant pathogenic bacteria. Ann. Clin. Microbiol. Antimicrob., 27: 11-18. DOI: 10.1186/1476-0711-11-8

Hill, E.H., H.C. Pappas and D.G. Whitten, 2014. Activating the antimicrobial activity of an anionic singlet-oxygen sensitizer through surfactant complexation. *Langmuir*, 30: 5052-5056. DOI: 10.1021/la501230m

Izui, S., S. Sekine, K. Maeda, M. Kuboniwa and A. Takada *et al*., 2015. Antibacterial activity of curcumin against periodontopathic bacteria. J. Periodontol., 8: 1-18. PMID: 26447754

Juven, B.J., J. Kanner, F. Sched and H. Weissslowic, 1994. Factors that interact with the antibacterial action of thyme essential oil and its active constituents. J. Applied Microbiol., 76: 626-631. PMID: 8027009

Karthik, L., G. Kumar, T. Keswani, A. Bhattacharyya and B.P. Reddy *et al*., 2013. Marine actinobacterial mediated gold nanoparticles synthesis and their antimalarial activity. Nanomedicine., 9: 951-960. PMID: 23434675

Kaushik, D., J. Yadav, P. Kaushik, D. Sacher and R. Rani, 2011. Current pharmacological and phytochemical studies of the plant Alpinia galanga. J. Chin. Integr. Med., 9: 1061-1065. DOI: 10.3736/jcim20111004

Koneman, W.K., S.D. Allen, W.M. Janda, P.C. Schreckenberger and G.W. Procop *et al*., 2005. Jr. Philadelphia color atlas and textbook of diagnostic microbiology. Lippincott-Raven Publisher.

Koutinas, A.F., M.N. Saridomichelakis, S. Argyroudis, C.K. Koutinas and P. Karatzanos *et al*., 2007. Clinical, histopathological and therapeutic considerations in a flock of sheep with facial staphylococcal-associated dermatitis. Vet. Dermatol., 18: 211-6.

Li, W.R., X.B. Xie, Q.S. Shi, H.Y. Zeng and Y.S. Ou-Yang *et al*., 2010. Antibacterial activity and mechanism of silver nanoparticles on *Escherichia coli*. Applied Microbiol. Biotechnol., 85: 1115-1122. DOI: 10.1007/s00253-009-2159-5

Lorrot, M., E. Bourrat, C. Doit, S. Prot-Labarthe and S. Dauger *et al*., 2014. Superficial skin infections and bacterial dermohypodermitis. Arch Pediatr., 14: 162-166. PMID: 24957981

MacKay, D., 2003. Can CAM therapies help reduce antibiotic resistance? Altern Med. Rev., 8: 28-42. PMID: 12611559

Moghadamtousi, S.Z., H.A. Kadir, P. Hassandarvish, H. Tajik and S. Abubakar *et al*., 2014. A review on antibacterial, antiviral and antifungal activity of curcumin. Biomed. Res. Int. DOI: 10.1155/2014/186864

Moyo, S.J., S.Y. Maselle, M.I. Matee, N. Langeland and H. Mylvaganam, 2007. Identification of diarrheagenic *Escherichia coli* isolated from infants and children in Dar es Salaam, Tanzania. BMC Infect. Dis., 9: 7-92. PMID: 17688682

Nabavi, S.F., A. Di Lorenzo, M. Izadi, E. Sobarzo-Sánchez and M. Daglia *et al*., 2015. Antibacterial effects of cinnamon: From farm to food, cosmetic and pharmaceutical industries. Nutrients, 7: 7729-7748. PMID: 26378575

Naveed, R.1., I. Hussain, A. Tawab, M. Tariq and M. Rahman, 2013. Antimicrobial activity of the bioactive components of essential oils from Pakistani spices against Salmonella and other multi-drug resistant bacteria. BMC Complement Altern Med., 14: 265-265. PMID: 24119438

Nirmala, R., H.M. Park, D. Kalpana, H.S. Kang and R. Navamathavan *et al*., 2011. Bactericidal activity and *in vitro* cytotoxicity assessment of hydroxyapatite containing gold nanoparticles. J. Biomed. Nanotechnol., 7: 342-350. DOI: 10.1166/jbn.2011.1292

Ooi, L.S., Y. Li, S.L. Kam, H. Wang and E.Y. Wong *et al*., 2006. Antimicrobial activities of cinnamon oil and cinnamaldehyde from the Chinese medicinal herb *Cinnamomum cassia* Blume. Am. J. Chin. Med., 34: 511-522. DOI: 10.1142/S0192415X06004041

Panacek, A., M. Kolar, R. Vecerova, R. Prucek and J. Soukupov ل *et al*., 2009. Antifungal activity of silver nanoparticles against *Candida* spp. Biomaterials, 31: 6333-6340. DOI: 10.1016/j.biomaterials.2009.07.065

Rai, D., J.K. Singh, N. Roy and D. Panda, 2008. Curcumin inhibits fast assembly: An attractive mechanism for it antibacterial activity. Biochem. J., 410: 147-155. DOI: 10.1042/BJ20070891

Samie, A., C.L. Obi, N. Lall and J.J. Meyer, 2009. *In-vitro* cytotoxicity and antimicrobial activities, against clinical isolates of Campylobacter species and *Entamoeba histolytica*, of local medicinal plants from the Venda region, in South Africa. Ann. Trop. Med. Parasitol., 103: 159-170. DOI: 10.1179/136485909X384992

Sedláková, M.H., K. Urbánek, V. Vojtová, H. Suchánková and P. Imwensi *et al*., 2014. Antibiotic consumption and its influence on the resistance in Enterobacteriaceae. BMC Res Notes., 7: 454.

Shaheen, A.Y., A.A. Sheikh, M. Rabbani, A. Aslam and T. Bibi *et al*., 2015. Antibacterial activity of herbal extracts against multi-drug resistant *Escherichia coli* recovered from retail chicken meat. Pak. J. Pharm. Sci., 28: 1295-1300. PMID: 26142503

Srinivasans, D., S. Nathan, T. Suresh and P.L. Perumalsamy, 2009. Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. J. Ethnopharm., 74: 217-220. DOI: 10.1016/S0378-8741(00)00345-7

Tessema, B., A. Mulu, A. Kassu and G. Yismaw, 2006. An *in vitro* assessment of the antibacterial effect of garlic (*Allium sativum*) on bacterial isolates from wound infections. Ethiop Med. J., 44: 385-389. PMID: 17370439

Usein, C.R., D. Tatu-Chitoiu, S. Ciontea, M. Condei and M. Damian, 2009. *Escherichia coli* pathotypes associated with diarrhea in Romanian children younger than 5 years of age. Jpn. J. Infect. Dis., 62: 289-293. PMID: 19628907

Violante, I.M., L. Hamerski, W.S. Garcez, A.L. Batista and M.R. Chang *et al*., 2012. Antimicrobial activity of some medicinal plants from the cerrado of the central-western region of Brazil. Braz. J. Microbiol., 43: 1302-1308. DOI: 10.1590/S1517-83822012000400009

Wikler, M.A., 2006. Performance Standards for Antimicrobial Disk Susceptibility Tests: Approved Standard. 9th Edn., Clinical and Laboratory Standards Institute, Wayne, PA., ISBN-10: 1562385860, pp: 37.

Yang, Y., M. Nogami, J. Shi, H. Chen and G. Ma *et al*., 2005. Enhancement of third-order optical nonlinearities in 3-dimensional films of dielectric shell capped Au composite nanoparticles. J. Phys. Chem. B., 109: 4865-4871. DOI: 10.1021/jp045854a

Yeon, K.Y., S.A. Kim, Y.H. Kim, M.K. Lee and D.K. Ahn *et al*., 2010. Curcumin produces an antihyperalgesic effect *via* antagonism of TRPV1. J. Dent. Res., 89: 170-174. DOI: 10.1177/0022034509356169

Zhang, S., L. Ren, Y. Li, J. Wang and W. Yu *et al*., 2014. Bacteriology and drug susceptibility analysis of pus from patients with severe intra‐abdominal infection induced by abdominal trauma. Exp. Ther. Med., 7: 1427-1431. DOI: 10.3892/etm.2014.1609