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Antibacterial activity of Vitellaria paradoxa seed oil extract (shear butter) and Honey against Bacterial Pathogens Causing wound infection

Adegoke C.O.[1]*, Laoye, O.O. [1], Folorunso, O.A.[2], Ogunbanwo, S.T.[3]

Ladoke Akintola University of Technology, Ogbomoso, Nigeria Department of Medical Laboratory Science [1], Department of Microbiology, University of Ibadan, Oyo State, Nigeria Department of microbiology university of Ibadan [2], Department of Biological Sciences and Biotechnology Caleb University, Imota, Lagos State, Nigeria [3].

Author for Correspondence*: coadegoke25@lautech.edu.ng/+234-803-485-3390/https://dx.doi.org/10.4314/sokjmls.v9i3.30

Abstract

This research was carried out to evaluate the antibacterial activity of *Vitellaria paradoxa* seed oil extract (shear butter) and honey against bacterial pathogens causing wound infection. The pathogens used for this study were *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli, Streptococcus pyogenes* and *Proteus mirabili*s. Wound swaps were collected from Ladoke Akintola University Teaching Hospital Ogbomosho (LTH) and processed for microbiological assessment. Actively growing culture (0.1 ml) containing 1 x 10^6 cfu/ml of each bacterium pathogen used was introduced into Petri dishes and 20 ml of molten agar added. The antibiotic sensitivity discs (Abtek Biological Ltd, UK) consisting of different antibiotics namely, ampicillin (10µg), amplicox (10µg), augmentin (30µg), gentamycin (10µg), metronidazole (10µg), ofloxacin (5µg) and tetracycline (10 µg) were placed on the solidified agar surface. The plates were incubated aerobically at 37°C for 24 hours. After this period, the diameter of the zone of inhibition of each antibiotics disc and agar well diffusion of Honey was measured. Data were analyzed using ANOVA at $p<0.05$. Honey produced clear zones of inhibition ranging from 12 to 36 mm against the tested bacterial isolates, while *Vitellaria paradoxa* seed oil extract (shea butter) also produced clear zones of inhibition ranging from 6 to 12 mm against the tested bacterial isolates. The Minimum inhibitory concentration of honey ranged from 12.5 to 25µg/ml on the bacterial isolates for different honey samples which were obtained from various markets (Owode honey, Saki honey, Tede honey and Ojaoba honey), while the Minimum inhibitory concentration of shea butter ranged from 25 to 100% on the bacterial isolates for different shea butter samples gotten from divers sources (Owed shea butter, Saki shea butter, Tede shear butter and Ojaoba shea butter). The minimum bactericidal *concentration* of the honey samples on the tested wound isolates ranged from 25 to 100 µg/ml, while the minimum bactericidal concentration of the shea butter samples ranged from 50 to 100 µg/ml. The seed oil extracts of *Vitellaria paradoxa* and honey samples used in this study have demonstrated antimicrobial activities against the tested clinical isolates thus justifying their use in traditional medicine for treating different diseases especially wound infections associated with the tested organisms, as they also serve as new and cheaper alternative for treatment for wounds.

Keywords: Antibacterial activity, (*Vitellaria paradoxa)* Shear butter, Honey, Wound infection and Microorganisms.

Introduction

A wound is a disruption in the structure of the skin leading to the exposure of the subcutaneous tissue (Canedo and Canedo, 2019) and providing a condition suitable for microbial colonization and proliferation (Wada *et al*., 2019). The proliferation of microorganisms either from the body (endogenous) or from the environment (exogenous) in a wound causes wound infection (Omeke *et al*., 2019). The commonest pyogenic bacteria often associated with infected wounds infections are *Staphylococcus aureus*,

Streptococcus pyogenes, Pneumococcus species and *coliform bacilli*, such as *Escherichia coli, Proteus* species*, Pseudomonas aeruginosa*, other enteric bacilli and some fungi (Kronen, 2017).

In chronic infections that are slow to heal and in pus showing no other microbes, there is a high possibility of infection with *Mycobacterium tuberculosis* and other Mycobacteria (Omeke *et al*., 2019). Due to the distinctive biological, non-sterile wound environment and the extremely intricate system of wound healing, effective and targeted cures are still needed. Hence, research is currently motivated to find (Omokhua *et al*., 2016) more efficient therapeutics for both chronic and acute wounds infections (Vyas and Vasconez, 2014). For many centuries, medicinal plants have been used as medicine in the treatment of many infections and diseases either as whole plants or plant Extracts (Omokhua *et al*., 2016) because of bioactive compounds present in them which offers protection against microorganisms and insects, the leaves of the shea tree contain saponin which makes it flap in water and hence is used in washing. Medicinal plants are broadly used to cure various infections and also used as a precursor for the synthesis of natural drugs (Omokhua *et al*., 2016). The leaves are also used in a mixture with other leaves in a traditional mixture to produce vapor which is used to bath persons for the treatment of fevers and headaches.

The leaves when soaked in water turn to a soapy and frothy liquid which is used to bath the head of persons suffering from fever. Bioactive compounds responsible for therapeutic effects of plants are normally stored in plant cells as secondary metabolites and may vary in concentration depending on plant part, season, climate and growth phase (Arruda *et al.,* 2016). Herbal practitioners believe that their medicine is cheaper, more effective and causes less side effects as compared to synthetized drugs. In developing countries like Nigeria, poor people such as farmers, rural dwellers and native communities used traditional medicine for the treatment of common illness (Rojas *et al*., 2006). Major challenges encountered with antibiotics in clinical use are resistance to antibiotics which leads eventually to failure of the treatment. Over the years, there have been reports of the production of more potent antibiotics e.g. third and fourth generation of Cephalosporin by pharmaceutical companies which are not readily available, expensive and appearance of resistance genes in clinical isolates.

Therefore, there is need to find new herbal antimicrobial agents in this era of rapid global spread of resistance to commonly used antibiotics. The aim of this study is to determine the antimicrobial, susceptibility effect of honey and *Vitellaria paradoxa* (shear butter) seed oil extract on wound microorganisms.

Materials and Methods

Materials

Blood agar, Chocolate agar, Mannitol salt agar, MacConkey agar, Mueller-Hinton agar, Cystine Lactose Electrolyte Deficient (CLED) agar, Petri dish, Antibiotic discs, Clinical samples, Gloves, distilled water, Microscope slides, Biochemical tests, Microscope, Centrifuge, Autoclave, Cover slip.

Study site

The study was carried out in Ladoke Akintola University of Technology (LAUTECH) Teaching Hospital, the Department of Medical Microbiology and Parasitology, Ogbomosho, Oyo State, Nigeria.

Study Duration

This study was carried out between March 2021 and December 2022.

Preparation and Sterilization of Media

The media used in this study were Nutrient Agar (NA), Nutrient broth, antibiotics disc and Muller- Hinton Agar (MHA). The agars were weighed according to manufacturers specification. The media were autoclave at 121°C for 15mins before commencement of all laboratory work

Isolation of microorganisms

Isolation of the microorganisms was done by inoculating the collected samples on Blood, Chocolate and incubating for 72 hours (Osoba, 1979).

Preservation of culture medium

The pure cultures of the isolates were sub-cultured into maintenance medium. It was then incubated at 37°C; the stock culture was stored at 4°C for subsequent use.

Characterization of isolates

Characterization of isolates was carried out by employing macroscopic, microscopic and biochemical tests Sneath *et a*l. (2006).

Isolates Identification using Phenotypic Methods

The isolates were confirmed by sub-culturing in a freshly prepared Mannitol Salt Agar (Oxoid Ltd., United Kingdom) Blood agar, Chocolate agar Nutrient agar and incubated overnight at 37°C colonies presumptive of *S. aureus* appeared golden yellow colonies with a smooth glistening surface, 2-4mm in diameter, circular, convex, shiny and easily emulsifiable. Other organisms showed peculiar characteristics. These were confirmed using the Gram staining technique and conventional biochemical tests such as Gram staining, Catalase, coagulase, Citrate and pigmentation on Mannitol Salt Agar (Oxoid Ltd., United Kingdom) (Cheesbrough *et al.,* 2005).

Preparation of Honey and shear butter extracts:

Twenty-five milliliter/grams of each of the honey and shear butter were weighed separately using weighing balance. This was transferred into conical flasks containing 80 ml of ethanol. The different mixtures were placed on a mechanical shaker and allowed to mix for 24 hours, filtration was done using sterile filter cloth and the filtrate collected. The total filtrate collected was evaporated to dryness by pouring into sterile stainless plate and was kept in hot air oven at 50° C until whole moisture evaporates completely leaving powder behind (Orji *et al*., 2015).

Antibiotic Susceptibility Testing of the bacteria isolates

Antibiotic susceptibility test for each bacteria pathogen was performed using the disc diffusion method. Actively growing culture (0.1 ml) containing 1×10^6 cfu/ml of each bacterium pathogen used was introduced into Petri dishes and 20 ml of molten agar (Muller- Hinton Agar - MHA) added. The antibiotic sensitivity discs (Abtek Biological Ltd, UK) consisting of different antibiotics namely Augmentin (30µg), Gentamycin (10µg), ofloxacin (5µg), Tetracycline (10µg), Metronidazole (10µg), Ampiclox (10µg), Ampicillin (10µg), Nalidixic (30µg), Amoxicillin (30µg), Nitrofurantoin (300 µg), Ceftazidime (30µg) and Cefuroxime (30µg) were placed on the solidified agar surface. The plates were incubated aerobically at 37°C for 18 hours. After this period, the diameter of the zone of inhibition of each disc was measured. The zone of inhibition corresponded to the antibiotic activity of each disc (Akinyemi *et al*., 2006). Resistance was defined by the absence of a zone of inhibition. The relative susceptibility of each isolate to each antibiotic was shown by a clear zone.

Antimicrobial Susceptibility Studies of Extracts of Honey and shear butter by Agar Well Diffusion

Preparation of Leaf Extracts: This was carried out to determine the effects of Ethanol extract of honey and shear butter against wound pathogens organisms by methods described by Orji *et al.* (2015). A standardized inoculum of 0.5 McFarland standard containing 1.0×10^6cfu/ml was introduced onto the surface of sterile agar plates (MHA), and a sterile glass spreader was used for even distribution of inoculums. Well, was dug on each of the plate with a sterile 6 mm diameter cork borer, and 100 µL of the crude extracts were introduced into the wells, allowed to stand at room temperature for about 30 minutes. The standardized drugs gentamycin and Cotrimoxazole were used as positive control and DMSO solution only as negative control. The plates were incubated aerobically at 37° C and examined for zone of inhibition after 24 hour. Each zone of inhibition was measured with a ruler and compared with the control in accordance to the method of Akinyemi *et al*. (2015).

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of honey and shear butter Extracts by Tube Technique

Doubling dilution of 256µg/ml **of** honey and shear butter Extracts solution was made in 1ml volume of broth to 0.125µg/ml. One row of the test was inoculated with 0.02ml of 1 in 100 dilution of the overnight broth culture of the test organism of 0.5 McFarland standard equivalent to 0.1ml actively growing culture containing 1.0×10^6 cfu/ ml of each bacterium pathogen used. The test was incubated aerobically at 37°C for 18 hours. Two control tubes were maintained for each test batch which include tube containing extracts together with the growth medium

without inoculum (antibiotic control) and the tube containing the growth medium, physiological saline with the inoculums (organism control). MIC was determined as the lowest concentration of the extracts with no visible growth (no turbidity) when compared with the control tubes. The MBC was determined by sub-culturing the test dilution on fresh solid medium (MHA) and further incubated at 37°C for 18 - 24 h. The lowest concentration of MIC tubes with no visible bacterial growth on solid medium was regarded as MBC as described by Udu-ibiam *et al*. (2015).

Statistical Analysis

One-way analysis of variance (ANOVA) was used to find out the significance of the treatments. The treatments were separated by least significance different (LSD) at $p < 0.05$ level.

Results

The physical characteristics of the various Shea butter samples are shown in Table 1. The Nature column shows that all the samples were crude for Ows to Ojs samples while the colour shows that Ows and Ojs were ivory in colour and SS is pale in colour and TS is Yellow. The odour column indicates that OWS and OJS samples had aromatic scent while SS and TS had no scent. While consistency shows the sample were all in solid form.

The physical characteristics of various Honey Samples were shown in Table 2. The honey is amber in colour, i.e. OWH and OJH while SH and TH are light and dark amber respectively. Odour had a sweet aroma, Consistency is syrupy similarly its taste was sweet, all were organic in nature and opacity were opaque.

From Table 3, the burns sites yielded three organisms namely *Escherichia coli, Staphylococcus aureus* and *Strep pyogenes* while *Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumonia* and *Strep. pyogenes.* Also, from Gluteal wound *Klebsiella pneumonia and Proteus mirabilis* were *isolated* and from avulsion wound similarly from cuts and boils only *Staphylococcus aureus Escherichia coli were* isolated respectively. From Accident and Sore *Staphylococcus aureus Kleb pneumonia* and *Streptococcus pyogenes* and *Streptococcus* pyogenes.

Antibiotics susceptibility testing is shown on Table 4 *Staphylococcus aureus, showed* highest zone of inhibition to cefuroxime which was 25 mm and recorded lowest zone of inhibition to amoxicillin, ampicillin and ampiclox which had 10mm each. Also, *Streptococcus pyogenes* had the highest zone of inhibition to ceftazidime and cefuroxime of 22mm each and had lowest zone to ampiclox with 9 mm zone of inhibition. On the other hand, K. *pneumoniae* had the highest zone of inhibition of 26mm compared to cefuroxime followed by ampiclox with 9mm zone of inhibition as the lowest. Also *E. coli* had the highest zone of inhibition of 18 mm to Augmentin with resistance to other antibiotics used. More so, *Proteus mirabilis* had highest zone of inhibition with 22mm as against Augmentin followed by 18 mm against ofloxacin while the lowest zone was cefuroxime Gentamicin and ampliclox which all had 11mm zone of inhibition and finally, *Pseudomonas aeruginosa* had highest Zone of inhibition of 18mm against cefuroxime followed by which had 17 mm zone of inhibition and resistant to other antibiotic used.

The class of antibiotics and their percentage resistance to wound isolates species is shown in Table 5. All the isolates were resistant to the antibiotics with 100 percent. Ofloxacin belongs to Quinolones as a group of antibiotics and had resistance against those six isolates with 66.6% while aminoglycosides which have amoxicillin, and gentamycin were 100 percent resistant to all the wound pathogens. Equally beta lactam, as a group of antibiotics including ceftazidime, cefuroxime ampicillin and ampiclox, however ceftazidime and cefuroxime has 66.66 percent resistance, while ampicillin and ampiclox had 100 percent resistance. Another class of antibiotics is beta lactam inhibitor that is made up of Augmentin and tetracycline which had 66 percent and 100 percent respectively. Also, nitrofurantoin was resistant to five of the isolates out of six organisms and was translated to 83.33 percent

Minimum inhibitory Concentration (MIC µg/ml) and Minimum Bactericidal Concentration (MBC %) of Shea butter and honey on wound isolates is shown on Table 6.

Minimum inhibitory Concentration (MIC µg/ml) and Minimum inhibitory Concentration (MIC µg/ml) is shown on Table 4.5. Staphylococcus aureus had 8 µg/ml MIC against shear butter and MBC of 16 µg/ml against shear butter. Also, *Staphylococcus aureus* had MBC of 16 µg/ml and 32 µg/ml MBC against and honey. Also *S. pyogenes* had equal value of 32 µg/ml of MIC and MBC against shear butter and 16 µg/ml MIC with 32 µg/ml MBC against honey. In the same way, *K. pneumonia* had MIC of 64 µg/ml and MBC of 32 µg/ml against shear butter also had 64MIC and MBC of 32 µg/ml against honey. In the same manner, *E. coli* had MIC of 64 µg/ml and MBC of 32 µg/ml against shear butter and MIC of 128 µg/ml with 64 µg/ml against honey. Also *P. mirabilis* had equal value MIC and MBC against shear butter and MIC OF 128 µg/ml and MBC of 64 µg/ml against honey and lastly, *Pseudo. Aeruginosa* had equal value of MIC and MBC of 64 against shear butter and MIC OF16 µg/ml and MBC of 32 µg/ml against honey.

Antagonistic activity of Shear butter and Honey extract against selected wound pathogens is shown on Table 7. *S. aureus* has 20.0±00 mm zone of inhibition against shear butter and 19.0±01mm zone of inhibition against honey. Also, *S. pyogenes* has 22.0±0.02 zone of inhibition against shear butter and 18.0±0.00 against honey. Equally, *Kleb pneumonia* has an equal value of 17±0.00 zone of inhibition against shear butter and honey.

Table 1. The physical characteristics of the various Shea butter Samples

Shear butter sample	Nature	colour	consistency
OWS	Crude	Ivory	Solid
SS - SAKI	Crude	Pale Yellow	solid
TS -TEDE	Crude	Yellow	solid
OJS OJA OBA	Crude	Ivory	solid

Key: Ows- Owode shear butter, SS-Saki butter, TS- Tede Saki, Ojs-Oja oba shear butter

Table 2 The Physical characteristics of various Honey Samples

Honey Sample	Colour	Odour	Consistency	Taste	Nature	Opacity
OWH	Amber	Sweet aroma	syrupy	Sweet	Organic	Opaque
SH	Light amber	Sweet aroma	syrupy	Sweet	Organic	Opaque
TH	Dark amber	Sweet aroma	syrupy	Sweet	Organic	Opaque
OJH	Amber	Sweet aroma	syrupy	sweet	Organic	Opaque

Key; OWH-Owode honey, SH □Saki honey, TH-Tede honey, OJH -Oja oba honey

Table 3: Isolated bacteria and its respective sites of collection

Site of wound	Organisms' Isolates
Burn	*Escherichia coli, Staphylococcus aureus and Strep pyogenes*
Leg Ulcer	*Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumonia* and *Strep. Pyogenes*
Gluteal wound	*Klebsiella pneumonia and Proteus mirabilis*
Avulsion wound	*Klebsiella pneumonia and Proteus mirabilis Klebsiella pneumonia and Proteus mirabilis*
Cuts	*Staphylococcus aureus*
Boils	*Escherichia coli*
Accident	*Staphylococcus aureus Kleb pneumonia* and *Streptococcus pyogenes Staphylococcus aureus Kleb pneumonia* and *Streptococcus pyogenes*
Sore	*Streptococcus pyogenes*

Table 4 Antibiotics Susceptibility pattern of organisms isolated from wound sample.

Microorganisms	Antibiotic sensitivity pattern of microorganism isolated from wound of patient attendin g LTH Hospital with the zone of inhibition (mm)									
	Aug 30µg	Tet10µg	Oflo30 µg	Genta 10µg	AAMO XYI10µg	Nitro 30µg	Ceftaz 30µg	Cefro x30µg	Ampicil li 10µg	Ampiclo x 10µg
S. aureus	12	12	16	13	10	24	24	25	10	10
S. pyogenes	14	13	12	11	19	11	22	22	11	09
k. pneumoniae	12	14	10	10	10	13	11	26	11	09
E. coli	18	14	10	13	11	14	12	13	12	12
P. mirabilis	22	14	18	11	12	14	19	11	12	11
Pseudo. Aeruginosa	12	13	17	09	09	11	11	18	13	13

CLSI (2012)

Table 5: Classes of antibiotics that shows resistance to wound isolates

Class of antibodies	Group of antibiotics	no of isolates tested	No resistance	Percentage resistance
Quinolone	Ofloxacilin	06	04	66.66
Aminoglycosides	Amoxicillin	06	06	100
	Gentamycin	06	06	100
	Ceftazidime	06	04	66.66
β-Lactam penicillin	Cefoxitin	06	04	66.66
	Ampicillin	06	06	100
	Ampiclause	06	06	100
β-Lactam inhibitor	Augmentin	06	04	66.66
Tetracycline	Tetracycline	06	06	100
Nitrofuran	Nitrofurantoin	06	05	83.33

CLSI (2012)

Table 6: Minimum inhibitory Concentration (MICµg/ml) and Minimum Bactericidal Concentration (MBC µg/ml) of Shea butter and wound against indicator organisms

Test organisms	Shear Butter		Honey	
	MIC (µg /ml)	MBC (µg /ml)	MIC (µg /ml)	MBC (µg/ml)
S. aureus	8	I6	32	`16
S. pyogenes	32	32	16	32
k. pneumonia	64	32	64	32
E. coli	64	32	128	64
P. mirabilis	32	32	8	16
Pseudo. Aeruginosa	64	64	16	32

CLSI (2012)

Table 7: Antagonistic activity of Shear butter and Honey extract against selected wound pathogens

Microorganisms	Zone of inhibition (mm)	
	Shear butter	Honey
S. aureus	20.0±00	19.0±01
S. pyogens	22.0±0.02	18.0±0.00
Kleb pneumoniae	17.0±0.00	17.0±0.01
E. coli	25.0±0.01	17.0±0.00
P. mirabilis	26.0±0.02	21.0±0.02
Pseudo. Aeruginosa	17.0±0.01	20.0±0.00

CLSI (2012)

Discussion

In this study, the organisms isolated from the wound samples are identified as *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Streptococcus pyogenes* and *Proteus mirabilis.* The organisms were identified based on morphological and biochemical test and identified using Bergey's manual of systematic bacteriology (1986).

The physical property of honey and shea butter (V. paradoxa seed oil extract) depends on environmental factors, the season, and the species of bee producing them. The physical characteristics of this study aligned with the findings of Taati *et al*. (2020) who noted various physical property of honey and shear butter while working on it and *Karika papaya* on some clinical isolates and so the properties exhibited by these extract and honey agreed with afore mentioned author.

The most common bacterial species that cause wound infections are mostly Gram negative and positive and distributed all over the body according to (Taati *et al.,* 2020) especially *S. aureus* that appears to be the most colonizer, so this study agrees with Taati *et al*. (2020) as the microorganisms were distributed all over the body. In this study, the distribution of drug resistance among various species of Gram

negative is of much public health concern because it can lead to multiorgan failure according to Thornton *et al*. (1997) who reported complications of wounds that led to systemic infections and organ failure. In this study, the distribution of drug resistance among various wound isolates is of much public health concern because the research indicates the appearance of wound isolates is of main human pathogens and so their presence in and the wound should be recognized as an important public health hazard. All isolates were significantly resistant to common antibiotic used in various homes. The antibiotics were further grouped into six classes thus the quinolones (ofloxacin) the aminoglycoside (gentamycin) the (β) beta lactam penicillin (ampicillin and penicillin). The β (beta) lactam inhibitor (Augmentin) the tetracyclines (tetracyclines) and others which is the nitrofurantoin in this class. A very high percentage of wound species show resistance to ofloxacin, gentamycin, Augmentin, penicillin, tetracycline and while they were partially susceptible to Nitrofurantoin in research work.

The pattern of antimicrobial activities of seed oil extract of Shea butter (*Vitellaria paradoxa*) and Honey with their zones of inhibitions (mm) were significantly cleared which was in agreement with the work of Akujobi *et al*. (2004) who revealed varying degree of inhibitions with shear butter and honey with gram negative bacteria. The extract and honey had an appreciable activity against the bacterial isolates. The antagonistic activity of the extract and the honey was observed to exert strong antimicrobial activities by inhibiting the growth. This was similar to the study conducted by (Nwankwo and Daodu, 2021) who carried out a similar study on wound isolates. This particular findings was also revealed by Emeruwa (2000) in his study on the antimicrobial substance of Carica papaya fruit extract on *Escherichia coli, Klebsiella pneumonia* and *Staphylococcus aureu*s were noted to be susceptible to both the Shear butter (*V. paradoxa* seed oil extract) and honey at all Concentrations. This validated the claims of Esimone *et al*. (2008) that wider range of susceptibility are usually recorded by gram negative bacteria. The difference in the resistance profile reported by different researchers was due to a different site of isolation and different species. More so due to uncontrolled use of antibiotics for the treatment of bacterial infection, these organisms might have developed resistance towards several antibiotics and so potential danger in the antimicrobial therapy for such infections (Grim *et al*., 2013) Almost all isolates were resistance to all antibiotics used in this study aligned with the findings of Adegoke and Ogunbanwo (2018) who reported resistance of Aeromonas species to a significant number of antibiotics used.

The minimum inhibitory concentration Minimum inhibitory concentration (MIC µg/ml) of µg/ml recorded for *E. coli* and *Staphylococcus aureus* (8 to and 64) µg/ml for the oil extracts were similar to the result of Bartelt *et al*. (2003) who reported that MIC and MBC interrelated with the agar diffusion method. However, MIC result could help a physician in choosing from among a group of similar drugs for treatment.

The percentage by volume of honeys to completely prevent growth of *S. aureus*, *Streptococcus pyogenes*, and *P. mirabilis* was in the range of 32 µg/ml to 64 µg/ml and for *P. aeruginosa, Klebsiella pneumoniae*, and *Escherichia coli* 64 µg/ml. In contrary to this, a study conducted in Ethiopia has shown that the concentration meant to prevent total growth was in the ranges of 32 to 64 µg/ml) honey that completely prevent growth of *E. coli, S. aureus and P. mirabilis* to be 16 and 32 µg/ml) and for *P. aeruginosa* 7.5 % v/v (Ahmed *et al*., 2014) which is lower concentration than the result of this study. Another study by Willix has also found that the percentage concentration 16 to 64 µg/ml) of Manuka honey to completely prevent growth for *S. aureus, S. pyogenes, E. coli, P. mirabilis and P. aeruginosa* 8 to 64 µg/ml was respectively (Willix *et al.,* 2002). This difference in the antibacterial activity of honeys over place might be due to the difference in the species of bees (Ashenafi, 1994) and the differences in the test methods used and test organisms, where in this case, bacteria from wound isolates were used. All *V. paradoxa* extracts exhibited bacteriostatic effects) on all tested clinical isolates but showed different minimum inhibitory concentration (MIC) which was also

different with respect to each organism tested in the experiment. The bacteriostatic effect of these extracts could possibly be due to the presence of certain phytochemicals such as saponins, which demonstrate remarkable physiological activity and forms and responsible for wound and skin protection (Olawuyi *et al.,* 2010) which specifically saponins have been suggested to exhibit greater antimicrobial effect and could serve as a precursor of steroidal substances. The MIC µg/ml of the oil extracts ranged from 8 µg/ml to one 64 µg/ml against all the organisms, except for Tede Shea butter which had an MIC of 32 µg/ml for *Pseudomonas aeruginosa*. However, at higher concentration of 64 µg/ml), all *V. paradoxa* extracts showed antibacterial activity against all the clinical isolates. The variation in the MIC may be due to the phytochemical composition of the respective crude oil extracts and the genetic make-up of each test organism as reported by Philip *et al. (*2009). Different organisms have been shown to respond differently to different and same concentrations of a specific medicinal plant (Philip *et al.,* 2009).

This study revealed the content of honeys that can completely kill *Streptococcus pyogenes* and *Proteus mirabilis* was 16 µg/ml) while the (MBC) Minimum bactericidal concentration for *Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus and Escherichia coli* was in the range 16 to 32 µg/ml) which contradict the, study done in Ireland on Tazma honey have similar result 32 to 64 µg/ml of MBC for *S. aureus, E. coli and P. aeruginosa* (Cooper *et al.2019)* of Tazma honey on MRSA bacteria have shown from ten to eleven point five percent v/v. Similarly, a study done from Ethiopia (Getaneh *et al.,* 2013) on the antibacterial activity supports the idea that there is variation in the antimicrobial activities of honey by the source of flower and type of honey. Possible reasons for these variations between bacteria response to honeys might be due to difference in the cellular organization of the bacteria and the physical, types of bees and environmental variation of honeys. The bactericidal activity of the honeys for all tested isolates in this study was found to be between twenty-five and hundred percent v/v concentration. This was comparable to other researchers from other places (Kingsley, 2001; Ahmed *et al*., 2014). The bactericidal concentrations of honey against *S. aureus* in this study was 32µg/ml. This concentration was higher than the finding of other researchers (Molan and Betts 2000; Ahmed *et al.,* 2014). *Pseudomonas aeruginosa* was reported to be resistant to honey by Efem (2001); in contrary to this result however, the bacterium was sensitive to all honeys tested in this study (except Owode Honey sample from Osogbo), at which it was a bit at lower (25 µg/ml). This result was also supported by the study done in other part of Ethiopia (Ahmed *et al.,* 2014). *P. aeruginosa* resistance to honeys could be due to the low permeability of its cell wall, genetic capacity to express resistant mechanisms and mutation in chromosomal genes which regulate resistance genes (Allen *et al.,* 2000). Honeys from all areas (Ogbomoso, Ibadan, Osogbo and Offa) in this study have shown antibacterial activity against *P. mirabilis* and *K. pneumoniae*. This was in line with the report by other researchers (Allen *et al*., 2000; Anyanwu 2011). This result was however, in contrast with studies by Ahmed *et al.* (2014) and Olawuyi *et al.* (2010) who studied antibacterial activities of honey from different locations and reported no bactericidal activity against *K. pneumoniae*. The variations in sensitivity could be attributed to differences in growth rate and lower cell wall permeability of pathogen, nutritional requirements, temperature, inoculums size and difference in honeys and the test method used (Molan and Betts, 2000). The result of this study revealed that the minimum bactericidal concentration (MBC) of the crude oil extracts indicated higher concentrations than that of the MIC. This observation is based on the fact that the concentration of the crude extracts required to completely eliminate an organism must be higher (64 µg/ml) than the concentration required to inhibit the growth, as reported by Acheampong *et al*. (1984). Fifty percent µg/ml was recorded to be the lowest MBC for these crude oil extracts. For instance, 32 µg/ml was observed in Owode Shea butter for *Escherichia coli,* for *Klebsiella pneumoniae* and *Streptococcus pyogenes* for Saki Shea butter and also *Klebsiella pneumonia* for Tede and Oja oba Shea butter samples. This result agrees with the

findings of Ndukwe *et al.* (2007). In general *V. paradoxa* oil etracts and honey samples tested from the different areas showed varied bacteriostatic and bactericidal activities against the tested bacteria from infected wounds. However, pharmacological standardization and clinical evaluation on the effect of shea butter and honey samples are essential before using them as a preventive and curative measure to wound infections related to the tested bacterial species. However, all tested *V. paradoxa* oil extracts and honey samples showed antibacterial activities which is the essence of this study.

Conclusion

The seed oil extracts of Vitellaria paradoxa and honey samples used in this study have demonstrated antimicrobial activities against the tested clinical isolates *(Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa, Streptococcus pyogenes and Proteus mirabilis*), thus justifying their use in traditional medicine for treating different diseases especially wound infections associated with the tested isolates, as they could serve as new and cheaper alternative for antibiotic sources.

As *E. coli*, when taken orally in its pure form, honey may help speed up recovery from such infection thus justifying its recommendation.

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Competing Interests

Authors have declared that no competing interests exist.

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