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Antibacterial Activity of *Vitellaria Paradoxa* Seed Oil Extract and Honey Against Bacterial Isolates from Wound Infection

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ABSTRACT

The use of herbal medicines in Nigeria reveals a long evidence of human interactions with the nature. The medicinal important of herbal plants lies in some chemical compounds that produce a specific chemical action on the human body. These bioactive compounds of plants of medical importance include alkaloids, flavonoids, tannins and phenolic compounds. research has been focused on herbal and aromatherapy product. However, a number of their product such as honey has shown therapeutic promises The presence of honey in various inhibitions has been reported by several investigators. Honey was used to treat infected wound as long as 2000 years ago before bacterial were discovered to be the cause of infection. This research paper assesses the Antibacterial Activity of Vitellaria paradoxa seed oil extract and Honey value by determining their phytochemical and antimicrobial status against Escherichia coli, Staphylococcus aureus and Klebsiella pneumonia. Soxhlet apparatus was used for the extraction of the seed oil using Ethanol as the extraction solvent. phytochemical analysis of the extracts was also carryout. Agar well diffusion was used for the antimicrobial activity and broth dilution was used for MIC. The phytochemical results show the presents Saponins, Tannins, Alkaloids, Carbohydrates, Resins, Phenol steroid and the absence of Flavonoid in both plant extract and Honey. The antibacterial result showed clear inhibition in both the sample of oil extracted using the soxhlet apparatus and the honey.

1. Introduction

World Health Organization (WHO) investigation showed that more than 80% of the world's population use traditional medicine for treating illness, (Ammara, *et al*., 2009). Use of herbal medicines in Nigeria reveals a long evidence of human interactions with the nature. The medicinal important of herbal plants lies in some chemical compounds that produce a specific chemical action on the human body. These bioactive compounds of plants of medical importance include alkaloids, flavonoids, tannins and phenolic compounds, (Edeoga, *et al*., 2005). The use of botanicals as drugs is well known in rural areas of many developing countries, (Sandhu & Heinrich, 2005) and Gupta *et al.,* (2005). 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 use 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. Infectious diseases are known to be treated with herbal remedies throughout the history of mankind; even today, natural substances continue to play a major role in primary health care as therapeutic remedies in many developing countries 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 and expensive. Problems of various antibiotics include low efficacy, side effect which has lead investigations into natural and potent antibacterial seeming to be the right step to take. Current research has been focused on herbal and aromatherapy product. However, a number of their product such as honey has shown therapeutic promises The presence of

honey in various inhibitions has been reported by several investigators. Honey was used to treat infected wound as long as 2000 years ago before bacterial were discovered to be the cause of infection. Honey has been "good for all rotten and hollow ulcers" More recently, honey has been reported to have an inhibitory effect to around 60 species of bacterial including aerobes and anaerobes, Gram positive and Gram negative. The current prevalence of the therapeutic use of ancient remedies, include honey committee on science and technology.

Currently, the main therapy for infection is the use of synthetic antibiotics. The misuse and overuse of antibiotics has the key factor for the emergence of drug resistance strains of several groups of microorganisms. Plant based therapeutics are known to be easily biodegradable, having no or minimal adverse side effects, and been easily accessible at low prices at such, there is a high demand for it both in developing and developed countries. There is therefore, the need to find new herbal antimicrobial agents in this era of rapid global spread of resistant isolates to commonly used antibiotics. Some reports have indicated that herbal products may help alleviate this problem, in Africa most people use herbal products to cure ailments. However, there are few published reports of effectiveness of these products. It is certain that some of these herbal products will produce the solution to drug resistance. Therefore, there is need to investigate the antibacterial activity of medicinal plants such as *Vitellaria paradoxa* seed oil extract and honey against *Staphylococcus aures, Klebsiella pneumonia* and *Escherichia coli* from wound infection.

2. Methodology

2.1 Collection of Sample

Fresh ripened fruits were harvested from *Vitellaria paradoxa* tree in Federal Polytechnic Bauchi, they were collected in a sterile polythene bag and transported to the laboratory. Honey was purchased in a sterile bottle from Gwallamaji market and transported to the laboratory as well.

The test organisms: *Staphylococcus aureus, Klebsiella pneumonia* and *Escherichia coli* were isolated and obtained from the Microbiology Diagnostic Laboratory of Abubakrka Tafawa balewa University Teaching Hospital, Bauchi.

2.2 Identification of Test Organisms

The test organisms which were collected from the Microbiology Diagnostic Laboratory of Abubakar Tafawa Balewa Teaching Hospital were purity tested by sub-culturing the test organisms on fresh agar plates and carrying out bio-chemical test such as Gram staining, Indole test, Catalase and Coagulase test to identify the organisms.

2.3 Preparation of Sample

The collected ripened fruits were kept on the ground for 5days during which the fruits became very soft. The fruit cover was peeled off and the Shea nut was boiled for 40minuites.It was removed and sundried for one week after which shelling was done using a pestle and a mortar. The shelled kernels were winnowed to remove dirt and dried in an oven set at 70 ^{0}C to obtain a constant weight, it was pulverized and oven dried again. The obtained powder was then preserved in a sterile bottle until required.

2.4 Extraction Techniques

Soxhlet Extraction Method

Soxhlet apparatus was used for the extraction of the seed oil.70g of the powdered sample was weighed with the use of a weighing balance into a thimble whose weight was 5.5g. The thimble that contained the sample was placed in a soxhlet extractor.350Ml of ethanol (Solvent) was measured with a measuring cylinder into a distillation flask and the flask was connected to a distillation pipe. A reflux condenser and the extractor were connected to a heating mantle. As the temperature increased steadily, the solvent (Ethanol) began to boil and the boiling vapor passed through the condenser and condenses back to liquid. This condensed vapor falls back to the thimble leading to the formation of homogenous mixture of ethanol which was evaporated and the extract was collected after 6hours. The extract was filtered to remove impurities and the ethanol was evaporated using a hot water bath to isolate the free flow liquid from the solvent. The extracted oil was further kept in an oven at 150^{0}C to eliminate any moisture, residue, and solvent that may be present. Akpan, et al., (2005).

Gum Removal and Purification.

This was done according to the method of Akpan, et al., (2005). The extracted oil was subjected to 60^{0}C heating, followed by addition of Activated Carbon into the heated oil, this cause the oil to change color. The decolorized oil was mixed with standard distilled water thoroughly for 15miniutes, Sieved, cooled and the residue on filter paper was discarded. The oil finally obtained was transfer into a sterile bottle and was kept in a refrigerator until needed.

Test for an Unadulterated/Pure Honey
A dry matchstick was dips into the purchase honey; the matchstick was streaked against the match box which flamed. This shows that the honey was pure and does not contain any amount of moisture content that could contaminate it.

Phytochemical Screening
Phytochemical test was carried out on the seed oil extract and the honey sample for the presence of Phytochemicals.

Test for Phenol Steroids
To a volume of 1ml of the extract, five drops of the Concentrated H_2SO_4 was added, reddish brown coloration indicate the presence of steroids. The same procedure was carried out on the Honey sample as well.

Test for Alkaloids
0.5ml of the honey sample was added to 2ml of 1% hydrochloric acid (HCL). A few drop of Mayer's reagent was added. The same procedure was carried out for the seed oil extract. A brown precipitate indicates the presence of Alkaloids.

Test for Flavonoids.
0.5g of each of the sample was added to 2ml of diluted sodium hydroxide (NaOH) and a few drops of concentrated sulphuric acid (H_2SO_4) were added to it gently. Appearance of green color indicates the presence of Flavonoids.

Test for Saponnins
A few ml of each sample was taken in a test tube and shaken vigorously with small amount of sodium bicarbonate and water. If stable, characteristic honeycomb like froth indicates the presence of Saponnins.

Test for Tannins
2drops of 5% FeCl was added to 1ml of each of the sample. A dirty green precipitate indicates the presence of Tannins.

Test for Resin
5ml of copper acetate solution was added to 5ml of each of the sample; it was shaken vigorously and allowed to separate. A green colored solution indicates the presence of Resin.

Test for Carbohydrates
5-8 drops of each of the sample was heated with Benedict's reagent in a test tube, Change in color varying from yellow to brick red indicates the presence of carbohydrate**.**

Preparation of Media
Nutrient agar was prepared according to the manufacturer's instruction and sterilized with the use of the autoclave.

Determination Antimicrobial Activity
Concentrations of each of the sample was made (100, 75, 50, and 25) % appropriately. Each of the test organisms were diluted serially. Pour plate method was used to pour the organisms and the nutrient agar on the Petri dishes, it was allowed to solidify. Wells of 6.0mm in diameter were cut out on the seeded plates using a cork borer in four different places. The wells were labeled and sealed with 2drops of the prepared nutrient agar and allowed to solidify. Each of the labeled wells was filled with the appropriate concentration of the seed oil extract and the honey sample respectively. The samples were allowed to stay for 1hour to enter into the agar very well and each of the plates for each organism *(Staphylococcus aureus, Klebsiella pneumonia* and *Escherichia coli)* were incubated at 37^0C FOR 24hours.er which the diameter of zones of inhibitions were measured with a meter rule. (Levinson W. 2010).

Determination of Minimum Inhibitory Concentration (MIC)
The minimum inhibitory concentration of both the ethanol extract of *Vitellaria paradoxa* and the honey was determined using broth dilution method. Sahm and Washington, (1990). 1mi each of the concentrations (100%, 75%, 50%, and 25%) of the extract and honey were added to 9ml of sterile nutrient broth in different test tubes respectively, standardized culture of $1x10^8$cfu/ml was inoculated in each test tube. The tubes were incubated for at 37^0C for 24hours. The tube with the lowest concentration with no detectable growth was considered as the Minimum Inhibitory Concentration.

Determination of the Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration was determined by sub-culturing the seed oil extract and the honey which showed no detectable growth after incubation for 24hours from the minimum inhibitory concentration into a fresh nutrient agar plate (recovery medium) and incubated for 24hours after which the plates were observed for possible growth. The minimum concentration that did not show any growth after incubation was regarded as the minimum bactericidal concentration (MBC).

3.Results

Table 1: Phytochemical Screening of *Vitellaria paradoxa* Seed Oil Extract and Honey.

Phyto-Constituents	*V.paradoxa* Seed Oil Extract	Honey	Test Reaction	Colour Observed
Saponins	+	+	Frothing	_
Tannins	+	_	Precipitate	Dark green
Alkaloids	+	+	Precipitate	Reddish brown
Flavonoids	_	_	_	_
Phenol steroid	+	+	Precipitate	Light brown
Carbohydrate	_	+	Precipitate	Brick red
Resins	+	+	Foamimg	Brown

Key: (+) = Present (-) = Absent

Table 2: Effects of Different Concentrations of *Vitellaria paradoxa Seed* oil extract on *Staphylococcus aureus, Klebsiella. pneumoniae and Escherichia coli*

Concentrations (%)	Staphylococcus *aureus* (mm)	*Klebsiella pneumonia*	*Escherichia coli*
100	12.0	10.0	10.0
75	8.2	8.0	8.0
50	0	0	0
25	0	0	0

Key: (0) no clear zone.

Table 3: Effect of Different Concentration of Honey on *Staphylococcus aureus, Escherichia coli and Klebsiella pneumonia*

Concentrations (%)	*Staphylococcus. aureus (mm)*	*Klebsiella pneumonia*	*Escherichia coli*
100	8.0	6.2	6.2
75	6.0	4.2	6.0
50	3.0	3.0	2.0
25	0	0	0

Key: (0) no clear zone.

Table 4: Minimum Inhibitory Concentration (MIC) of *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia of the Seed Oil Extract of Vitellaria paradoxa*

Organisms	Concentrations(%)			
	100	75	50	25
Klebsiella pneumonia	7.0	6.0	6.0	0
Staphylococcus aureus	8.0	5.0	0	0
Escherichia coli	5.0	0	0	0

Table 5: Minimum Inhibitory Concentration (MIC) of Honey on *Escherichia coli, Klebsiella pneumonia* and *Staphylococcus aureus*

Organisms	Concentrations(%)			
	100	75	50	25
Escherichia coli	0	0	0	0
Staphylococcus aureus	3.2	3.0	0	0
Klebsiella pneumoniae	5.2	5.0	0	0

Table 6: Minimum Bactericidal Concentrations (MBC) of honey against *Escherichia coli*, *Klebsiella pneumon*ia and *Staphylococcus aureus*.

ORGANISMS	Concentrations(%)			
	100	75	50	25
Klebsiella pneumonia	5.2	5.0	0	0
Staphylococcus aureus	3.2	0	0	0
Escherichia coli	5.0	0	0	0

Key: (0) no clear inhibition.

Table 7: Minimum Bactericidal (MBC) Concentrations of *V.paradoxa* Seed Oil Extract against *Escherichia coli*, *Klebsiella pneumoni*a and *Staphylococcus aureus*.

ORGANISMS	Concentrations(%)			
	100	75	50	25
Klebsiella pneumonia	7.0	6.0	3.0	0
Staphylococcus aureus	8.0	0	0	0
Escherichia coli	5.0	0	0	0

Key: (0) no clear inhibition

4. Discussions

Analysis of the plant extract revealed the presence of Phytochemical such as Saponnins tannin, alkaloid, steroid and resin. In honey Phytochemical such as Saponnins, alkaloid, steroid, carbohydrate and resin were also present. The presence of tannin, alkaloid, Saponnins has been reported to possess antimicrobial activities on different organism, (Akujobi, *et al.,*2004) bioactive components of plant extracts and honey affects the cell membrane integrity microorganism, (Ibekwe*, et al*., 2013)

The Zone of inhibition with highest diameter as indicated in table 4.2 was obtained using 100% of the extract which gave 12.0mm and 8.0mm of 100% of honey and the lowest zone of inhibition in extract and honey as 2.0mm and 8.0mm at the

lowest concentration 50% and 75%. Decrease in different concentration of samples led to a direct reduction of inhibition zone around each test organism. Similar observation by (Akujobi, *et al.,*2004) had suggested that higher concentration of antimicrobial compounds gave appreciable antimicrobial officially.

The pattern of antimicrobial activities of the plant extracts Honey and the zones of inhibitions (mm) of the bacteria are in agreement with (Akujobi, *et al.,*2004) who revealed varying degree of gum negative bacteria. The plant and honey has both profound activities against the bacterial. The microbial substance appears to exert antimicrobial activity by inhibiting the growth and by killing the sensitive microbes. This particular findings was also encountered by Emeruwa, (2000) in his study on the antimicrobial substance from *Carica papaya* fruit extract *Escherichia coli, Klebsiella pneumonia* and *Staphylococcus aureus* were susceptible to both the *V.paradoxa* seed oil extract on honey at all the concentrations. This corrobointed the claims of Esimone *et al.,*(2008).That wider range of susceptibility are usually recorded by gram negative bacteria.

The minimum inhibitory concentration of 5.0mg/m and 3.0mg/m recorded for E. coli and staphylococcus aureus were similar to 12.5mg/m of *Cowbretum Spp* reported by Esimone *et al* (2008), inhibition against *salmonella typhyi.*
The lowest minimum bactericidal concentration of 6.0mg/ml and 3.0 mg/ml respectively of oil extract and honey against test organisms confirmed the findings of Anibijuwom and udeze, 2009 who posited that methanolic extracts of *Combretumade ogonium* showed activities against staphylococcus aureus. This result also correlates with the work of Mann *et al*, (2011) who reported that the methanolic and hexane extracts had antimicrobial activity against *Aspergillus ninger, Staphylococcus aureus*, and *Escherichia coli.*

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