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Antimicrobial activities of some medicinal plants used in folklore remedies in Enugu State, Nigeria

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Abstract

Background: The adoption of traditional medicine as an alternative or complementary therapy is increasingly recognized in primary healthcare across the globe due to its effectiveness in treating mild, chronic, and often difficult-to-cure ailments such as wound infections and diseases. The aim of the study was to evaluate the antibacterial activities of traditional wound healing agents.

Materials and Methods: A total of 5 typed strains, including Providencia vermicola strain 6G (KC775772), Proteus terrae strain TYFW6 (MZ960174), Proteus vulgaris strain LC-693 (CP063314), Klebsiella aerogenes strain CX-70 (MH368390) and Pseudomonas aeruginosa strain PHB3 (JQ327806), were purchased from Prof. Tasie, a F.0. of the Department of Applied Microbiology and Brewing at Enugu State University of Science and Technology, Agbani. Aloe barbadensis miller (Aloe vera) gel was obtained from Ogbete main market in Enugu Metrpolis, honey was gotten from Nsukka, and Persea americana (avocado) leaves were obtained from Nkanu, they are all traditional wound healing agents that were procured from local vendors in Enugu State. Honey was examined for physicochemical parameters and subsequently extracted using acetone and ethanol solvents. The avocado leaves were macerated for twenty minutes and extracted using acetone and ethanol solvents, while the Aloe vera gel were lyophilized and extracted using acetone and ethanol solvents. Standard methodologies were implemented to conduct preliminary qualitative phytochemical analyses in order to identify concentrations of fundamental phytocomponents. The extracts of the avocado and Aloe vera gel were reconfigured to concentrations of 200, 100, 50, 25mg/ml using 5ml of dimethyl Sulfoxide (DMSO) (w/v). The honey extracts were reconstituted to concentrations of 200, 100, 50, 25mg/ml using 5ml of hot water. Using the diffusion technique with agar wells, the isolates were examined for their susceptibility to the extracts. Based on the results of the agar well diffusion method, the lowest concentration of inhibition was determined. Using the Kirby-Bauer disc diffusion method, the drug resistance pattern of isolates was examined.

Results: The presence of phytochemicals (phenol, terpenoids, flavonoids, alkaloid compounds, saponins, steroids, glycosides, and tannins) was identified in the phytochemical analysis of acetone and ethanol extracts of honey, Aloe vera gel, and avocado leaves. Greater groups of inhibition on all the isolates were observed in the ethanol solutions of honey, aloe vera gel, and avocado leaves at 200mg/ml. Proteus vulgaris exhibited the maximum zone of inhibition in ethanol extracts of honey, measuring 26mm. Providencia vermicola, Proteus terrae, Klebsiella aerogenes, and Pseudomonas aeruginosa exhibited zones of inhibition of 28mm respectively. Honey, aloe vera gel, and avocado leaves demonstrated favorable results in comparison to the control antibiotic, Ciprofloxacin. The extracts of avocado leaves, honey, and aloe vera gel had a minimum inhibitory concentration of 25mg/ml on all isolates. Septrin exhibited 100% resistance in all isolates, while Providencia vermicola, Proteus vulgaris, and Pseudomonas aeruginosa exhibited 100% resistance to augmentin. The majority of the isolates were sensitive to the antibiotics tested. The acetone extraction of avocado leaves exhibited the lowest efficacy in blocking the growth of wound isolates, while the ethanol extract of honey exhibited the highest efficacy at P >0.5 as per the study.

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Conclusion: Honey, Aloe vera gel and Avocado leaves are easily accessible, potent, economical and safe to man, therefore, it is recommended as a topical agent for the treatment of wounds in developing countries.

Keywords: Folk Medicine; Aloe vera gel; Honey; Avocado Leaves

1. Introduction

Traditional medicine can often be viewed as separate from folk medicine. According to this definition, folk medicine refers to age-old remedies and practices that are shared and utilized by non-professionals. Folk medicine includes healing methods, concepts of bodily functions, and ways to maintain health that are familiar to some individuals in a culture, shared informally as common knowledge, and practiced or implemented by anyone in the culture with previous experience1. A lesion is an absence of or a disruption of the genetic and histological or physiological integrity of tissue that is alive2. The body's own regrowth of skin as well as epidermal tissue is referred to as healing from wounds3. To restore the structural regularity and operate of the affected part, the fundamental concepts of ideal wound repair involve preventing further harm to tissues, removing insufficient organs, utilizing blood supply and oxygenation, providing proper nutrition, and creating a humid wrapped healing environment4. Debridement, irrigation, antibacterial agents, cellular grafts, corticosteroids, and antibiotics are among the current methods employed to treat wounds. The majority of these are linked to undesirable side effects, including the possibility for resistance from bacteria, hemorrhage, tissue injury, contact dermatitis, and a delay in wound recovery. The discovery and development of novel tissue-healing agents is necessary to address the challenging task of achieving superior wound healing. These compounds, alkaloids, and flavonoids are active components that are present in numerous plants and are crucial to the method of wound reconstruction5. Traditional medical systems rely on medicinal flora as their most abundant bio-resource for drug production. Plant extracts have been employed by humans to enhance their health and livelihood since the beginning of time. A diverse array of free radical scavenging molecules, including compounds that are phenolic (phenolic ones chemicals, flavonoids, or catechins, the substance known as pro quinones, the coumarins and tannins, for example etc.), nitrogen-containing substances (alkaloid molecules, amines, and betalains etc.), vitamins, terpenoids, carotenoids, and other secondary metabolites, are present in medicinal plants. For centuries, natural products, such as medicinal plants and herbs, have been employed to treat a variety of maladies on a global scale6. More than 80% of the global population continues to rely on these traditional remedies to treat their ailments. Honey and Aloe vera (aloe leaf barbadensis miller) are among the products that are significant in the recovery of wounds7. Aloe vera, avocado, and honey are utilized as a prevalent folk remedy globally. Aloe vera is frequently used in herbal remedies to address a variety of ailments, including illnesses such as cancer, diabetes, irritation, gastric dysfunction, ulcers, infections, wounds, and scars8. Aloe vera is frequently referred to as a burned plant or first aid plant as a result of its potential therapeutic properties in the treatment of burns and wounds. Avocado (Persea americana) is a polymorphic tree crop that has its origins in a wide geographical region, spanning from the eastern and central highlands of Mexico, through Guatemala, to the western coast of Central America9.10,11,12,13. It is a prominent tropical fruit that is abundant in protein-soluble lipids, vitamins A and B, and middling levels of vitamins D and E. It is an active fruit with high vitamin and mineral content. The leaves' juice, in turn, can be employed to treat general skin diseases such as scabies, ringworm, dermatitis, and ulcers. Honey is one of the natural marvels. It is a viscous, sweet, and aromatic liquid that honeybees (Apis mellifera) produce from the nectar of blossoms. It is renowned for its nutritive and medicinal properties, which are a direct consequence of the healthful constituents that are closely linked to its floral origin. The composition as well as the quality are contingent upon the nectar, the environmental variables of the region, the production method, and the conditions of handling and storage14. Antimicrobial properties. antibacterial, and antioxidant abilities, as well as its ability to enhance the immune response and treat lesions, have been attributed to its therapeutic potential15. Honey acts as an antibacterial agent and serves as a protective barrier against wound infection. These medicinal plant-based goods are wound purifiers that possess antimicrobial properties that facilitate natural wound treatment without increasing the likelihood of infections caused by bacteria that could impede the healing process in open wounds.

Wound infections are common in developing countries which can lead to wound sepsis. Bacteria directly invade wounds producing inflammation and fluid exudation which interferes with the healing process. Topical antimicrobials are one of the most important agents in wound care. It was hence thought worthwhile to evaluate the antimicrobial activity of aloe vera gel, avocado leaves and honey and correlate them to their wound healing potential.

2. Material and methods

2.1. Collection of Plant Samples

Aloe barbadensis miller (Aloe vera) gel was obtained from Ogbete main market in Enugu Metropolis, honey was obtained from Nsukka, and Persea americana (avocado) leaves were obtained from Nkanu, they are all traditional wound healing agents that were procured from local vendors in Enugu State. They were all collected with sterile bags and taken to the Applied Microbiology Laboratory at Enugu State University of Science and Technology for analysis.

2.2. Extraction of Avocado Leaves

A modified method of Irobi et al. (2014)16 was used. Avocado leaves were washed with distilled water. The avocado leaves were allowed to dry for 7 days at room temperature. Thereafter, they were ground to powder with a sterile blender and were stored in a sterile air-tight container until needed. A total of 165g each of the powdered avocado leaves was weighed into 3 conical flasks. Then, one conical flask was mixed with 5 drops of water and then 500 ml of each solvent (acetone and ethanol) was added to the other 2 conical flasks. The conical flasks were covered tightly, stirred for 2 hrs, and left for 48 hrs at room temperature with intermittent shaking. The extracts were filtered aseptically into a 1000 ml beaker with sterile muslin cloth and further filtered with sterile Whatman's filter paper size No. The acetone and ethanol extracts were evaporated at 50 ^{o}C using a rotary evaporator, and the crude extracts were stored at 4 ^{o}C in a refrigerator for further use.

2.3. Extraction of Aloe vera Gel17

The Aloe vera plant leaves were washed with distilled water. The leaves were incised and the gel was separated with the help of a sterile knife and divided. One part was used directly on the isolates while the other part was sent for lyophilization. For lyophilization, the gel obtained was concentrated under vacuum at 125 mm Mercury Vacuum at 50 ^{o}C for 2 mins. The gel obtained was lyophilized at 30 ^{o}C to get the dry powder18. A total of 250g of the powder was dissolved in 300 ml each of acetone and ethanol for extraction. 1 filter paper. The solvents were evaporated at 50 ^{o}C using a rotary evaporator to ensure proper concentration. The acetone and ethanol extract of the gel was labeled properly; the crude extracts were stored at 4 ^{o}C in a refrigerator for further use in sensitivity testing.

2.4. Determination of the Biochemical Properties of Honey

2.4.1. Determination of Colour Intensity

Using a Spectrophotometer, the photocell detector detects how much light and wavelength has passed through the sample. It then passes through a monochromater that divides and diffracts the light. Honey intensity is assessed by measuring with colour intensity ABS450, the absorbance of a 50% honey solution at 450 and 720nm19.

2.4.2. Determination of Water Content20

The prism of the handheld refractometer was cleaned and dried before use, and the prism of the refractometer was covered with a honey sample evenly. The refractometer was plugged, adjusted to 25 ^{O}C, and allowed to stabilize for 30 min. The reading of the refractive index was recorded. Each sample was measured twice and the average value was taken.

2.4.3. Determination of pH Level21

Honey was diluted into a 10% solution (w/v). The pH meter was calibrated at pH 3.7 (4.0), 7.0, and 9.0 before use and the mean of their results was recorded as the result. Thereafter, 10g of honey sample was dissolved in 75 ml of carbon dioxide-free distilled water. This solution was mixed by using a magnetic stirrer. The pH electrodes were immersed into the solution. Then, the pH value of the honey solution was recorded in percentage.

2.4.4. Extraction of Honey22

An aliquot of 500 ml honey samples was placed in firmly closed and sterile vials. A total of 250mls each of Honey was weighed into conical flasks. One part was used in its natural form while the others were added 500mls of solvent (Acetone and Ethanol) respectively. The conical flasks were covered tightly, incubated using a water bath at 50 ^{o}C for 30 min, and then cooled at room temperature to extract. The extracts were filtered aseptically into 100 ml reagent bottles with sterile muslin cloth and further filtered with Whatman's filter paper size No. The acetone and ethanol extracts were evaporated at 50 ^{o}c using a rotary evaporator, and the crude extracts were stored at 4 ^{o}C in a refrigerator for further use in sensitivity testing.

2.5. Phytochemical Screening

The extracts were subjected to qualitative phytochemical screening using standard phytochemical methods 23,24,25,26 to test on Alkaloids, Saponins, Glycosides, Phenols, Flavonoids and Tannins.

2.6. Collection of Organism

Pseudomonas aeruginosa (PHB3), Proteus vulgaris (LC-693), Providencia vermicola (KC775772), Proteus terrae (TYFW6), Klebsiella aerogenes (CX-70), and Klebsiella aerogenes (MH368390) were the kinds of bacteria that were identified from wound samples. Enugu State University of Science and Technology's Applied Microbiology Laboratory procured the bacterium from Prof. Tasie's stock culture.

2.7. Re-Isolation of Isolates

After typing the isolates, a sterile wire loop was used to distribute them on nutrient agar slants. The loops were removed after 36hrs of aerobic incubation at 37 °C. After each usage, the colonies were stored on nutrient agar slants at 4 ^{o}C.

Inoculum Preparation: A total of one loopful of each isolate in a 24hr incubation chamber at 37 °C using 5ml of sterile nutritional broth was cultured. Adjustment of each culture to 50% of MacFarland's standard was done.

2.8. Reconstitution of Extracts

In separate sterile test tubes, we separated the acetone and ethanol extracts of the avocado leaves and the aloe vera gel into two equal amounts, 5ml of Dimethyl Sulfoxide (DMSO) was added to each tube to get the concentration down to 200mg/ml. A 2ml of the initial sample was divided among four test tubes containing two milliliters of dimethyl sulfoxide (DMSO), yielding a concentration of 200, 100, 50 and 25mg/ml by weight; this was done to provide two-fold dilutions. Two clean test tubes were prepared by adding 5ml of hot water to 1000mg (1g) of acetone honey extract and ethanol honey extract, respectively to get an initial concentration of 200mg/ml, 2mls was transferred from one tube to four additional test tubes, each containing 2ml of hot water, in order to produce two-fold dilutions. A concentration of 200, 100, 50 and 25mg/ml was the end outcome of this process.

2.9. Antibacterial Assay

Antibacterial assay on the isolates were determined by agar well diffusion method16. The four isolates were streaked on Muller Hinton agar plates with 0.1 ml each, after having been matched with 0.5 McFarland standard. Sterile cork borers, measuring 6mm in diameter each, were used to penetrate the plates. Pipette sterile extracts ranging in concentration from 200 to 25mg/ml were introduced to the holes in increments of 0.1ml. Ciprofloxacin was used as a control. The culture plates were incubated at 37 ^{o}C for one day after being pre-diffused on the work surface for 30min. The next stage included taking millimeter-scale readings of the inhibitory zones.

2.10. Antimicrobial susceptibility

Isolates were examined for antibiotic susceptibility using disc diffusion and Mueller Hinton agar plates. The following ten(10) typical antimicrobial medications were used, all sourced from Maxicare Medical Laboratories: septrin (SXT) (30µg), chloramphenicol (CH) (10µg), sparfloxacin (SP) (10µg), ciprofloxacin (CPX) (10µg), amoxicillin (AM) (30µg), augmentin (AU) (30µg), gentamicin (CN) (10µg), perfloxacin (PEF) (30µg), tarivid (OFX) (10µg), and streptomycin (S) (30µg). Muller-Hinton agar was prepared, then put to sterilized Petri dishes and allowed to gel. Isolates were selected based on their compatibility with one another using the MacFarland criteria. Separate Muller Hinton agar plates were streaked with a loopful of each isolate before incubation at 37 °C for 24hr. Antibiotic discs were then placed on top of the plates. After 24hr of incubation at 37 ^{o}C, the size of the area of decreased growth (zone of inhibition) was measured according to the guidelines of the Clinical and Laboratory Standard Institute (CLSI) (2012)27.

2.11. Determination of Minimum Inhibition Concentration (M.I.C.)28

The agar well diffusion method was used to determine the minimum inhibitory concentration of the extracts. Multiple concentrations were achieved by 2-fold dilution for the antimicrobial susceptibility test: 200, 100, 50 and 25mg/ml. Before being put onto the Muller Hinton plates, 0.1ml of each isolate was matched to 0.5 McFarland's turbidity standard. Holes were then created using a 6mm cork borer. A total of 0.1ml of extract was added to each well. The plates of cells were incubated for the formation of the zone of inhibition after 24hr of incubation at 28 °C. The concentrations the extracts became inhibited is called the Minimum Inhibitory Concentration (MIC).

2.12. Statistical Analysis

A T-test was used to evaluate the data in the statistical analysis. Statistical significance was assessed by calculating the average of the replicates. By comparing the standard errors, we were able to determine a p-value at $P > 0.5$.

3. Results

3.1. Physiochemical Parameters of Honey Samples

From the study, the colour of the honey sample was Brown, colour intensity was 49nm, pH was 3.8, and water content was 22.5% which met with the conformity of a good honey (Table 1).

3.2. Results on Qualitative Phytochemical Screening of Acetone and Ethanol Extracts of samples

The phytochemical analysis of acetone and Ethanol extracts of Honey, Aloe vera Gel, and Avocado leaves showed the presence of some important phytochemicals such as phenol, terpenoids, alkaloids, flavonoids, saponins, steroids, glycosides, and tannins. The phytochemicals are of pharmacological importance as antioxidants, anti-bacterial, etc. (Table 2 and 3).

3.3. Antibacterial Activity of Extracts on Isolates

The extracts of avocado leaves, Aloe vera gel and honey exhibited various antibacterial activities against Providencia vermicola strain 6G (KC775772), Proteus terrae strain TYFW6 (MZ960174), Proteus vulgaris strain LC-693 (CP063314), Klebsiella aerogenes strain CX-70 (MH368390) and Pseudomonas aeruginosa strain PHB3 (JQ327806). At the highest concentration of 200 mg/ml, the zones of inhibition ranged from 5-28mm respectively for all samples and extracts (Table 4, 5, 6, 7 and 8).

3.4. Minimum Inhibitory Concentration (MIC) of Avocado Leaves, Honey and Aloe vera Gel Extracts

The least concentration that inhibited the growth of Providencia vermicola strain 6G (KC775772), Proteus terrae strain TYFW6 (MZ960174), Proteus vulgaris strain LC-693 (CP063314), Klebsiella aerogenes strain CX-70 (MH368390) and Pseudomonas aeruginosa strain PHB3 (JQ327806) was at 25mg/ml respectively (Table 9).

3.5. Antibiotics susceptibility of isolates

Providencia vermicola strain 6G (KC775772) was highly sensitive to ciprofloxacin and gentamicin at (12)20% and (6)10% respectively, Proteus terrae strain TYFW6 (MZ960174) was highly sensitive to ciprofloxacin and gentamicin at (17)21.8% and (2)2.6% respectively, Proteus vulgaris strain LC-693 (CP063314) was highly sensitive to ciprofloxacin and gentamicin at (16)17.4% and (18)19.6% respectively, Klebsiella aerogenes strain CX-70 (MH368390) was highly sensitive to ciprofloxacin and gentamicin at (18)38.3% and (3)6.4% respectively and Pseudomonas aeruginosa strain PHB3 (JQ327806) was highly sensitive to ciprofloxacin and gentamicin at (13) 26% and (20)40% respectively. All isolates were completely resistant to septrin (Table 10).

3.6. Efficacy of Extracts on Isolates

At $p > 0.5$, Ethanol extracts of honey showed the highest efficacy in the treatment of all the wound isolates (Figure 1-5).

Table 1 Physiochemical Parameters of Honey Samples

Sample	Physiochemical Parameters	Criteria Range	Test Value	Inference
Honey	Colour	Brown-Amber	Brown	within the standard limit
	Colour Intensity(nm)	36-73	49	within the standard limit
	Water Content (%)	Not less than 18%	22.5	within the standard limit
	pH	3.2-4.5	3.8	within the standard limit

KEY: nm: nanometer %: Percentage

Table 2 Results on Phytochemical Screening of Acetone Extracts of samples

S/NO	Samples	Phytochemical Properties							
		phenol	Terpenoid	Alkaloid	Flavonoid	Saponin	Steroid	Glycosides	Tannin
1	Honey	+	+	+	+	+	+	+	+
2	Aloe Vera Gel	+	+	+	+	+	+	+	+
3	Avocado Leaves	+	+	+	+	+	-	+	+

KEY: +: Present -: Not Present

Table 3 Results on Phytochemical Screening of Ethanol Extracts of samples

S/NO	Samples	Phytochemical Properties							
		phenol	Terpenoid	Alkaloid	Flavonoid	Saponin	Steroid	Glycosides	Tannin
1	Honey	+	+	+	+	+	+	+	+
2	Aloe Vera Gel	+	+	+	+	+	+	+	+
3	Avocado Leaves	+	+	+	+	+	-	+	+

KEY: +: Present -: Not Present

Table 4 Zones of Inhibition (mm) on Antibacterial Assay of extracts on Providencia vermicola

Extracts	Concentrations(mg/ml)					
		200	100	50	25	Control CIP
AO	Ethanol	20	19	17	16	20
	Acetone	15	12	9	5	20
H	Ethanol	28	28	27	18	20
	Acetone	20	18	17	6	20
AV	Ethanol	26	25	18	7	20
	Acetone	21	19	15	5	20

Where: AO: Avocado Leaves; mg/ml: milligrams per milliliter; H: Honey; CIP: Ciprofloxacin; AV: Aloe vera Gel; mm: millimeter

Table 5 Zones of Inhibition (mm) on Antibacterial Assay of extracts on Proteus terrae

Extracts	Concentrations(mg/ml)					
		200	100	50	25	Control CIP
AO	Ethanol	22	19	8	4	20
	Acetone	18	9	7	3	20
H	**Ethanol**	28	17	15	5	20
	Acetone	26	15	12	4	20
AV	**Ethanol**	27	25	16	3	20
	Acetone	26	20	14	5	20

Where: AO: Avocado Leaves; mg/ml: milligrams per milliliter; H: Honey; CIP: Ciprofloxacin; AV: Aloe vera Gel; mm: millimeter

Table 6 Zones of Inhibition (mm) on Antibacterial Assay of Extracts on Proteus vulgaris

Extracts	**Concentrations(mg/ml)**					
		200	**100**	**50**	**25**	**Control CIP**
AO	Ethanol	20	18	7	5	20
	Acetone	18	13	4	2	20
H	**Ethanol**	28	27	15	9	20
	Acetone	26	23	11	5	20
AV	**Ethanol**	28	21	14	6	20
	Acetone	24	20	9	4	20

Where: AO: Avocado Leaves; g/ml: milligrams per milliliter; H: Honey; CIP: Ciprofloxacin; AV: Aloe vera Gel; mm: millimeter

Table 7 Zones of Inhibition (mm) on Antibacterial Assay of extracts on Klebsiella aerogenes

Extracts	**Concentrations(mg/ml)**					
		200	**100**	**50**	**25**	**Control CIP**
AO	Ethanol	21	19	14	2	20
	Acetone	20	18	13	3	20
H	**Ethanol**	28	25	17	7	20
	Acetone	26	22	14	5	20
AV	**Ethanol**	28	26	20	8	20
	Acetone	26	23	15	3	20

Where; O: Avocado Leaves; mg/ml: milligrams per milliliter; H: Honey; CIP: Ciprofloxacin; AV: Aloe vera Gel; mm: millimeter

Table 8 Zones of Inhibition (mm) on Antibacterial Assay of extracts on Pseudomonas aeruginosa

Extracts	**Concentrations(mg/ml)**					
		200	**100**	**50**	**25**	**Control CIP**
AO	Ethanol	20	17	10	4	20
	Acetone	17	14	9	3	20
H	**Ethanol**	28	27	16	5	20
	Acetone	26	24	18	7	20
AV	**Ethanol**	28	27	13	3	20
	Acetone	25	22	11	4	20

Where: AO: Avocado Leaves; mg/ml: milligrams per milliliter; H: Honey; CIP: Ciprofloxacin; AV: Aloe vera Gel; mm: millimeter

Table 9 Minimum Inhibitory Concentration (MIC) of Avocado Leaves, Honey and Aloe vera Gel Extracts

Extracts	**Isolates**	**Sample**	**Concentrations(mg/ml)**		**MIC**
			50	**25**	
	Provindencia vermicola	A	-	+	25
		H	-	+	25
		AV	-	+	25

Ethanol	Proteus vulgaris	A	-	+	25
		H	-	+	25
		AV	-	+	25
	Proteus terrae	A	-	+	25
		H	-	+	25
		AV	-	+	25
	Klebsiella aerogenes	A	-	+	25
		H	-	+	25
		AV	-	+	25
	Pseudomonas aeruginosa	A	-	+	25
		H	-	+	25
		AV	-	+	25
Acetone	Provindencia vermicola	A	-	+	25
		H	-	+	25
		AV	-	+	25
	Proteus vulgaris	A	-	+	25
		H	-	+	25
		AV	-	+	25
	Proteus terrae	A	-	+	25
		H	-	+	25
		AV	-	+	25
	Klebsiella aerogenes	A	-	+	25
		H	-	+	25
		AV	-	+	25
	Pseudomonas aeruginosa	A	-	+	25
		H	-	+	25

Where: A : Avocado leaves; H: Honey; AV: Aloe vera Gel-: Growth; +: No growth; mg/ml: milligrams per milliliter

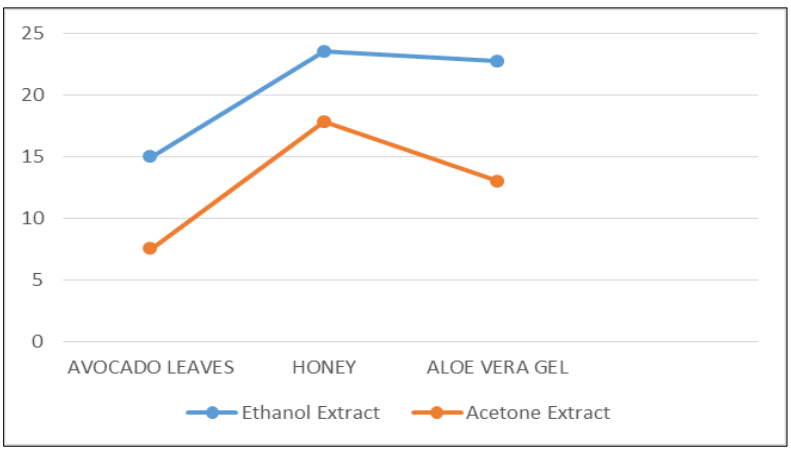

Figure 1 Efficacy of extracts on Providencia vermicola.

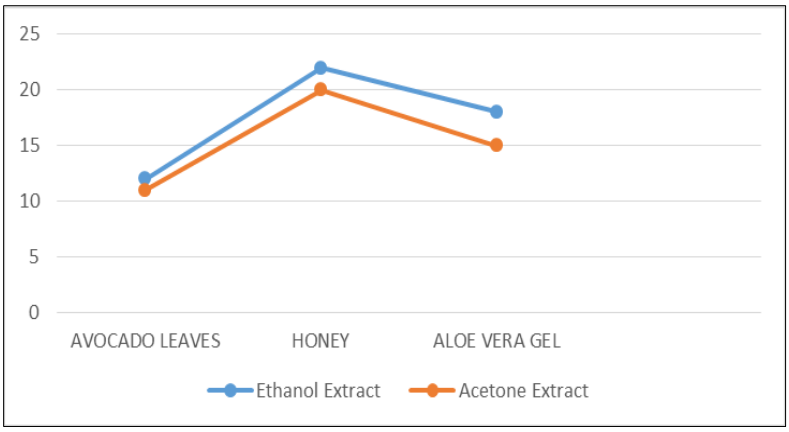

Figure 2 Efficacy of extracts on Proteus terrae

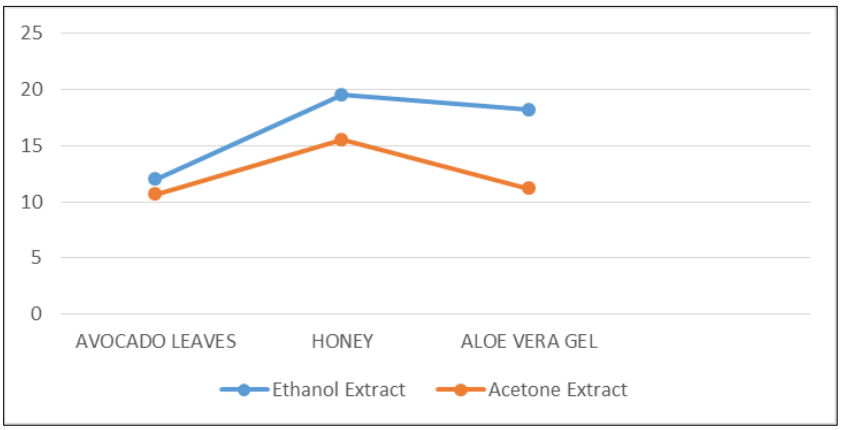

Figure 3 Efficacy of Extracts on Proteus vulgaris

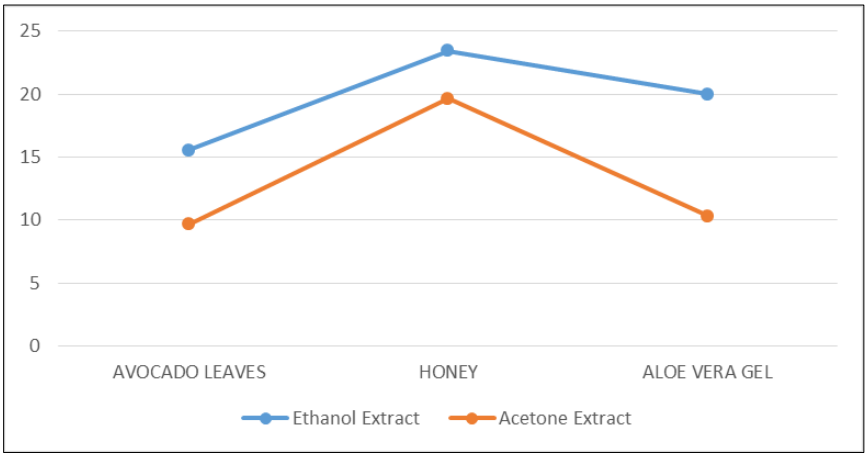

Figure 4 Efficacy of Extracts on Klebsiella aerogenes

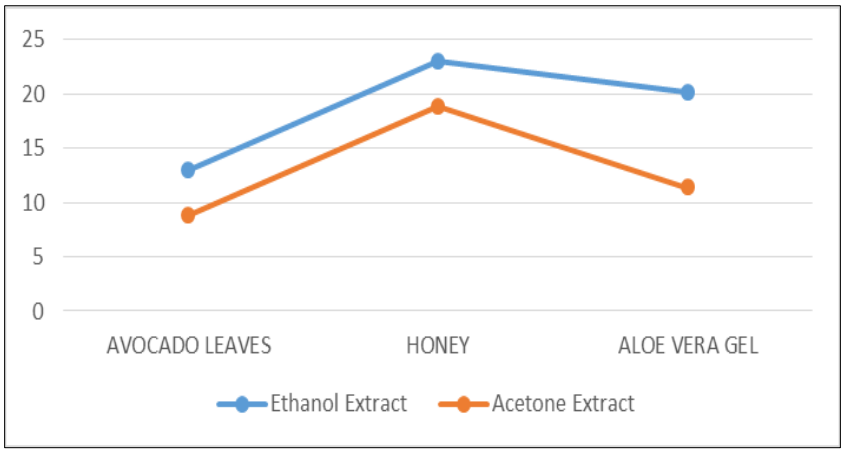

Figure 5 Efficacy of Extract on Pseudomonas aeruginosa

Table 10 Antibiotics susceptibility pattern of isolates

	Inhibitory Zone Diameter to Nearest millimeter(mm) (n=5)														
	Providencia vermicola			**Proteus vulgaris**			**Proteus terrae**			**Klebsiella aerogenes**			**Pseudomonas aeruginosa**		
Names of Antibiotics	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**
Chloramphenicol	3(60 %)	1(20 %)	1(20 %)	3(60 %)	-	2(40 %)	2(40 %)	1(20 %)	2(40 %)	3(60 %)	-	2(40 %)	5(100 %)	-	-
Ciprofloxacin	5(100 %)	-	-	4(80 %)	1(20 %)	-	5(100 %)	-	-	3(60 %)	2(40 %)	-	5(100 %)	-	-
Sparfolxacin	4(80 %)	1(20 %)	-	3(60 %)	2(40 %)	-	3(60 %)	1(20 %)	1(20 %)	3(60 %)	-	2(40 %)	3(60 %)	-	2(40 %)
Amoxicillin	3(60 %)	1(20 %)	1(20 %)	2(40 %)	3(60 %)	1(20 %)	3(60 %)	1(20 %)	1(2%)	4(80 %)	1(20 %)	-	3(60 %)	1(20 %)	1(20 %)
Augmentin	-	-	5(100 %)	-	-	5(100 %)	4(80 %)	-	1(80 %)	2(40 %)	2(40 %)	1(20 %)	-	-	5(100 %)
Gentamycin	4(80 %)	-	1(20 %)	4(80 %)	-	1(20 %)	3(60 %)	1(20 %)	1(20 %)	3(60 %)	-	2(40 %)	4(80 %)	-	1(20 %)
Pefloxacin	3(60 %)	1(20 %)	1(20 %)	4(80 %)	-	1(20 %)	3(60 %)	1(20 %)	1(20 %)	3(60 %)	-	2(40 %)	4(80 %)	-	1(20 %)
Tarvid	3(60 %)	1(20 %)	1(20 %)	-	-	-	4(80 %)	-	1(20 %)	3(60 %)	-	2(40 %)	5(100 %)	-	-
Streptomycin	-	-	-	-	-	-	-	2(40 %)	1(20 %)	2(40 %)	2(40 %)	1(20 %)	5(100 %)	-	-
Septrin	-	-	5(100 %)	-	-	5(100 %)	-	-	5(100 %)	-	-	5(100 %)	-	-	5(100 %)

Key: % : Percentage S: Sensitive I: Intermediate R: Resistant

4. Discussion

Folklore/Herbal medicine has gained global recognition recently, as medicinal plants continue to play an essential role in the healthcare systems of urban, semi-urban, and rural areas, because of the physiologically active components found in medicinal plants, they have historically been used to treat a wide range of illnesses and disorders29. Azaizeh et al. (2003)30 reported that approximately 80% of the global population relies on traditional medicine for their healthcare needs. This supports the findings of researchers who state that medicinal plants have been beneficial in managing mild, chronic, or seemingly incurable conditions and diseases. Numerous ethnobotanical studies have been carried out to record traditional healing practices, the plants utilized, their preparation methods, and the ways they are administered31,32,33,34,35. In Nigeria, scholars of ethnomedicine have historically and currently contributed to the growth of the traditional healthcare system36. This has resulted in a recent recognition and demand to incorporate this alternative form of therapy alongside the conventional treatments, which have been widely documented to have significant negative side effects, even though they do offer positive benefits for human health. The study's objective was to determine if honey, avocado leaves, and aloe vera gel's antibacterial characteristics were associated with their capacity to expedite the healing of single wounds. Typed isolates of the following bacteria were used from wound samples: Pseudomonas aeruginosa (JQ327806), Proteus vulgaris (CP063314), Providencia vermicola (KC775772), Proteus terrae (TYFW6), Klebsiella aerogenes (MH368390), and Proteus terrae (MZ960174). According to the study, the honey samples met all the necessary criteria for physicochemical qualities. Consistent with the findings of Almasaudi (2021)37, who also found honey samples with low pH, Table 1 reveals that the sample had a color intensity of 49 nanometers, a pH of 3.8, and a water content of 22.5%. Honey inhibits the growth of microorganisms and is compatible with a wide variety of foods due to its acidity and low pH. The presence of higher phenolic acids was found in darker varieties of honey, according to studies conducted by Gomes et al. (2011)38 and Eteraf-Oskouei and Najafi (2013)39. Furthermore, the renal system is particularly vulnerable to the harmful effects of honey adulteration due to the dramatic fluctuations in honey composition40. This characteristic greatly affects the stability, texture, and shelf life of honey, making it an essential consideration during extraction and storage. Examining the water activity of the honey samples which ranged from 0.650 to 0.720 is crucial for determining the stability of certain processed foods. Using phytochemical analysis, the researchers discovered that avocado leaves, aloe vera gel, and honey all included phenols, terpenoids, flavonoids, saponins, and glycosides. The results are in line with those of Eshun and He (2004)41 and Boudreu and Beland (2006)42, who also found lectins, terpenoids, and flavonoids in aloe. Antioxidants like phenolic compounds, organic acids, vitamins, and flavonoids boost the antibacterial capabilities of honey, aloe vera gel, and avocado and also neutralize free radicals produced by hydrogen peroxide43,44. Additionally, honey's antioxidant and anti-inflammatory properties are a result of its complex chemical makeup, and its immunomodulatory actions hasten the recovery time after an injury45. Akinpela and Onakoya (2006)46 noted that many phytoconstituents, such as glycosides, anthraquinones, alkaloids, flavonoids, and saponins, have shown antibiotic action. In terms of phytochemical components, ethanol proved to be the most efficient solvent. The level of activity of saponins is dictated by their inherent structure, as stated by Navarro et al. (2006)47. It is crucial to include saponin in traditional healing remedies since it is known to increase the expression of factors associated to proliferation, which means it stimulates the multiplication of skin cells48. Applying saponin to UV-exposed skin tissue increased fibroblast collagen synthesis and decreased matrix metalloproteinases formation, according to recent study49. Also, research by Lee et al. (2007)50 shown that saponin phosphorylated the Smad 2 protein, which in turn spurred skin fibroblasts to increase collagen production. It follows that saponin may promote matrix re-synthesis in skin wounds. Antibacterial activity against several strains of bacteria was demonstrated by extracts of ethanol and acetone of avocado leaf, aloe vera gel, honey, including Providencia vermicola 6G (KC775772), Proteus terrae TYFW6 (MZ960174), Proteus vulgaris LC-693 (CP063314), Klebsiella aerogenes CX-70 (MH368390), and Pseudomonas aeruginosa PHB3 (JQ327806). At the highest dosage of 200 mg/ml, all of the isolates had inhibitory zones that varied in size from 3 to 28 mm (Tables 4, 5, 6, 7 and 8). At the lowest dose of 25 mg/ml, all of the extracts exhibited antibacterial activity (Table 9). Our results are in line with those of Nwankwo et al. (2014)51, who also found that apiary honey is effective against clinical strains of S. aureus, E. coli, and Candida albicans. Honey has been used to treat chronic wounds because of its effectiveness in vitro against many multi-resistant bacteria, including methicillin-resistant Staphylococcus aureus (MRSA) and multi-resistant Pseudomonas aeruginosa52,53. The inner-leaf gel of Aloe vera reduced the growth of Streptococcus and Shigella species in vitro, according to Ferro et al. (2003)54.

Furthermore, it was discovered by Agarry et al. (2005)17 that aloe vera gel inhibited the growth of Trichophyton mentagrophytes (20.0mm), whereas the leaf inhibited the growth of Pseudomonas aeruginosa and Candida albicans. In addition to its antiviral, analgesic, and antibacterial properties, aloe vera has been shown to assist in the healing of both first- and second-degree burns55 and to eliminate a broad range of skin issues56,57. Atherton (2002) states that aloe vera's anthraquinones aid in bringing blood to the region, breaking up pus and dead cells, and clearing ulcers and wounds of debris. It has the potential to relieve a wide variety of skin conditions, including minor wounds, stings, bruises, poison ivy, and eczema. Joseph and Justin (2010)58 found that aloe vera may help the body repair wounds and

make more collagen, a protein that controls the aging process and the appearance of wrinkles. The results showed that according to Table 10, all of the tested isolates were very sensitive to ciprofloxacin (sensitivity rate of 100%) and totally resistant to septrin (resistance rate of 0%). No matter the reason, the fact that these bacterial isolates show signs of antibiotic resistance makes them a potential threat to public health as they might spread zoonotic illnesses and agents resistant to many medication classes. The results showed that each of the extracts significantly affected the isolates used in the experiment. Nevertheless, as compared to the control (ciprofloxacin), the honey, avocado leaf, and aloe vera gel extracts of ethanol and acetone performed better. Though all of the antibiotics tested were susceptible to the isolates, this conclusion is in line with that of Arbab et al., 202159, who also discovered that ethanol extracts of Aloe vera gel were more efficient than the antibiotics. Figures 1–5 show that, at a level higher than 0.5, all of the wound isolates were inhibited by the ethanol extract of honey. Aloe vera gel and avocado leaves followed, in that order. This is supported by the results of Nisbet et al. (2010)60, who discovered that groups given honey had a much faster recovery time than the control group. Research has shown that a combination of aloe vera gel, avocado leaves, and honey may suppress the growth of all wound isolates.

5. Conclusion

The study indicated that various concentrations of avocado leaf, honey, and aloe vera gel extracts all suppressed bacterial growth on wound isolates. The most potent inhibitory impact was shown with honey.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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