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Antibacterial activity of Honey, Bitter leaf and Garlic extracts on some *Escherichia coli, Salmonella typhi* and *Shigella* species

***A.D. Aondoackaa, C. Babayo, F.B. Auta, A.I. Yange, and K.B. Uta**
Department of Microbiology, Federal University of Agriculture, Makurdi, Nigeria.
*Correspondence E-mail: k.davidtime@gmail.com

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Abstract

Honey and some selected plants extracts: garlic (*Allium sativum*) and bitter leaf (*Vernonia amygdalina*) were studied for their antimicrobial properties on selected human pathogenic bacteria of the family enterobacteriaceae namely *Escherichia coli, Shigella* and *Salmonella*. All the extracts exhibited various degrees of inhibition against the organisms at different concentrations. The minimum inhibitory concentration (MIC) measured in mg/mL on *Escherichia coli*, *Salmonella typhi* and *Shigella* species for the treatments were as follows: bitter leaf extract: 6.25±0.00, 12.50±0.00 and 6.25±0.00, garlic: 9.38±9.77, 12.50±0.00, and 6.25±0.00, and honey: 6.25±0.00, 9.375±9.77, and 6.25±0.00. The zones of inhibition measured in millimetres (mm) were as follows: bitter leaf extract: 19.0±1.00, 22.50±0.25, and 13.0±1.00, garlic extract: 21.0±0.00, 15.50±0.25 and 15.50±0.25 and honey: 34.0±1.00, 36.0±1.00 and 33.50±0.25. Garlic extract had a minimum bactericidal concentration of 12.50 mg/mL against *Salmonella typhi*, and 25.00 mg/mL on *Shigella* spp. Similarly, the minimum bactericidal concentration for honey was observed to be 25.00 mg/mL and 50.00 mg/mL against *Salmonella typhi* and *Shigella* spp. respectively while no minimum bactericidal concentration was observed for bitter leaf extract. While garlic extract had the least bactericidal concentration against *Salmonella typhi*, none of the extracts showed any bactericidal concentration against *Escherichia coli*. This means that among the three treatments garlic was the most effective antimicrobial agent against *Salmonella typhi* at a lower concentration.

Keywords: Inhibition, Bactericidal, Antimicrobial, Minimum, Medicinal, Concentration, Extract

Introduction

Antimicrobial agents are very important in controlling the global impact of infectious diseases. The effect and value of antibiotics wanes as pathogens develop resistance to them and concomitantly, spread. The global surge in the emergence of superbugs against all kinds of antibiotics has posed a grave threat to public health [1, 2]. Therefore, there is an urgent need for the development of alternative antimicrobial agents. This has led to a re-evaluation of the therapeutic use of ancient remedies, such as plants and plant-based products, including honey [3,4].

Plants and herb extracts have been used in the treatment of human ailments for a very long time, and the practice has been passed on for generations. As in other developing countries, scientists in Africa are still researchinginto the local plants abundant in the continent for their possible use in traditional and orthodox medicine [5]. Research into traditional plants and herbs has received a boost due to the increasing resistance of pathogens to most of the commonly used antimicrobial agents [6].

According to a report [6], a medicinal plant is any plant in which one or more of its organ, contains substances that can be used for therapeutic purposes or which contains substances which can be used as precursors for the synthesis of useful drugs. The use of plant extracts and phyto-products is gaining attention due to their availability, cost effectiveness, proven nature of specificity, biodegradability, low toxicity, and minimum residual toxicity in the ecosystem [7]. Cold infusions of the leaves of bitter leaf have been used for the relief of stomach upset and haemorrhoids [8].

In this study, honey and some selected plant extracts; garlic (*Allium sativum*) and bitter leaf (*Vernonia amygdalina*) were studied for their medicinal properties on selected human pathogenic bacteria of the family enterobacteriaceae, namely *Escherichia coli, Shigella* spp. and *Salmonella typhi*.The study determined and compared the antimicrobial inhibition between honey, bitter leaf and garlic extracts on the enteric pathogens.

Materials and Methods

Sample collection and preparation

The enteric bacteria used were *Escherichia coli, Salmonella typhi*, and *Shigella* spp. They were bought from Institute of Medical and Veterinary Laboratory Science Vom, Jos, Nigeria and transported aseptically to the Microbiology Laboratory, Federal University of Agriculture Makurdi, Nigeria.

Bitter leaf samples were randomly collected from Logo I area of Makurdi town on 21st July 2018 and identified by an expert (Botanist) at the Department of Botany College of Biological Sciences, Federal University of Agriculture Makurdi, Nigeria. The leaves were washed under running

tap water and were spread to dry at room temperature in the Laboratory for two weeks. The dry leaves were pounded into powdered form using a mortar and pestle and stored in airtight container. The various extracts were prepared from the powdered leaves.

One hundred grams of the powdered material were weighed using digital weighing balance (Labtech BL20001) and were soaked in 500 mL of ethanol. The suspension was then filtered through Whatman No. 1 filter paper after 48 hours. The filtrate was air dried until used for the antimicrobial test. The type of extraction carried out was cold maceration extraction. The extracts were tested for sterility by plating on nutrient agar and incubated for 24 h at 37^0 C.

Fresh garlic (*Allium sativum*) bulbs were purchased from a retail food store. The garlic bulbs were peeled, and were homogenized using a sterile mortar and pestle. Two hundred grams of the homogenized garlic were weighed using a digital weighing balance (Labtech BL20001) and soaked in 500mL of ethanol.

The suspension was then filtered through Whatman No. 1 filter paper after 48 hours. The filtrate was air dried until used for the antimicrobial test. The type of extraction carried out was cold maceration extraction. The extracts were tested for sterility by plating on nutrient agar and incubating for 24h at 37^0 C.

The qualitative screening test for phytochemicals of honey and plant extracts were carried out following standard procedures of [9].

Saponins
Two millilitres of the ethanolic extracts in a test tube was shaken for two minutes. Frothing which persisted on shaking was taken as evidence for the presence of saponins.

Alkaloids
Three millilitres of ethanolic extract was stirred with 5ml of 1% HCL on a steam bath for twenty minutes. The solution obtained was cooled and filtered and to the filtrate was added few drops of Mayer's reagent/Picric acid. A cream precipitate indicated the presence of alkaloids.

Phenolics
Two drops of 5% ferric chloride were added to five millilitres of the ethanolic extracts in a test tube. A greenish precipitate was taken as an indication of phenolics.

Tannins
One millilitre of freshly prepared 10% potassium hydroxide was added to 1ml of the ethanolic extracts and aqueous extracts. The presence of a dirty white precipitate was taken as indication of tannins.

Flavonoids
To a volume of three millilitres of the ethanolic extracts, 1ml of 10% sodium hydroxide was added. A yellow colouration indicated the presence of flavonoids.

Sterile nutrient agar plates were prepared and allowed to solidify. Standardized organisms of 0.1mL were introduced into the plates and a sterile bent glass rod was used to spread the inoculum evenly on the surface of the agar and the excess drained off. The plates were left on the bench for 1 hour so that the inoculum will diffuse into agar.

Determination of the zones of inhibition
A sterile cork-borer of 5mm diameters was used to make 5 ditches on the plates. Varying concentrations of the extracts: 200 mg/mL, 100 mg/mL, 25 mg/mL were made and 0.5 mL of the extracts were dropped in each of the appropriately labelled plates and controls were set up for each plate by adding 0.5 mL of the appropriate solvent into the 5^{th} ditch. The plates were duplicated and left on the bench for few minutes for the extracts to diffuse into the agar and later incubated at 37^0 C for 24 hours. After incubation the zone of clearance around each ditch was measured using a metric ruler by taking measurement from the edge of the plate to the point where the growth of the organism started. The diameter of the zone of inhibition which represents antibacterial activity was measured. This procedure was separately carried out for each of the extract, i.e. honey, bitter leaf and garlic extract.

Determination of the minimum inhibitory concentration (MIC)
The broth dilution method was used to determine the MIC. Varying concentrations of the extracts were used ranging from 5 mg/mL – 200 mg/mL. Each concentration containing 0.1 mL was added to each 9 mL of nutrient broth containing 0.1 mL of standardized test organisms of bacterial cells. The tubes were incubated for 24 hours at 37^0 C. Controls were equally set up by using solvent and test organism without the extracts. The highest dilution of the tested extracts to inhibit growth (no turbidity in the tube) was considered as the MIC value of the extracts batch against the tested bacterial species.

Sample from the tubes used in MIC determination which did not show any visible growth after the period of incubation were streaked on nutrient agar (NA) plates. The lowest concentration of the extracts indicating a bacterial effect after 24 hours of incubation at 37^0 C was regarded as the Minimum Bactericidal Concentration (MBC) [10]. Data from this study were obtained in triplicates for each of the parameters determined. Results were presented as mean ± SD of triplicate analyses and statistically tested for significance, using analysis of variance (ANOVA)

Results and Discussion

Table 1 shows the cultural, morphological and biochemical characteristics of bacterial isolates used for this study. All the isolates are Gram negative rod shaped bacteria. They are all catalase positive and urease and citrate negative. Only *Escherichia coli* is indole positive while *Salmonella* differ from *Shigella* by being motile and hydrogen sulphide producing.
The qualitative phytochemical screening of the extracts is shown in Table 2. The various phytochemicals seen in this extracts were said to be the reason for their antimicrobial activity. All the extracts were found to contain saponin,

tannin and phenol, positive, while garlic was flavonoid negative. Bitter leaf and honey extract were shown to be alkaloid negative.

The mean zones of inhibition of the bacteria using the three extracts are presented in table 3. Bitter leaf exerts its highest inhibition against *Salmonella* with 22.50 mm while *Shigella* is least with zone of 13.0 mm. For garlic extract, the highest zone was against *Escherichia coli* (21.00 mm) while *Salmonella* and *Shigella* have zone diameter of 15.50±0.25 mm each. Honey exert an inhibition zone of 34, 36 and 33.5mm for *Escherichia coli, Salmonella* and *Shigella* respectively.

Table 1.Cultural, Morphological and Biochemical Characteristics of Isolates

Variable	Identified Bacteria		
	Escherichia coli	*Salmonella typhi*	*Shigella spp*
Colony colour	Pink	Pale	Pale
Colony shape	Circular	Circular	Circular
Morphology	Rod	Rod	Rod
Gram stain reaction	**-**	**-**	**-**
Catalase	**+**	**+**	**+**
Citrate	**-**	**-**	**-**
Urease	**-**	**-**	**-**
Indole	**+**	**-**	**-**
Motility	**+**	**+**	**-**
H_2S	**-**	**+**	**-**
MR	**+**	**+**	**+**

Key: - = means negative,**+ =** means positive, **H_2S =** hydrogen sulphide.

Table 2. Qualitative Phytochemical Screening of the Extracts.

Extracts	Saponin	Phenol	Tannin	Flavonoid	Alkaloid
Bitter Leaf	+	+	+	+	_
Garlic	+	+	+	_	+
Honey	+	+	+	+	_

Key: - means negative, + means positive.

Table. 3: Zone of Inhibition (mm) of the extracts on the test bacteria.

Extracts/Controls	*Escherichia coli*	*Salmonella*	*Shigella*
Bitter leaf	19.0±1.00	22.50±0.25	13.0±1.00
Garlic	21.0±0.00	15.50±0.25	15.50±0.25
Honey	34.0±1.00	36.0±1.00	33.50±0.25
Ciprofloxacin (positive control)	26.0±0.00	34.0±0.00	45.0±1.00
Ethanol (negative control)	0.00	0.00	0.00

The minimum inhibitory MIC of the extracts against each bacterial isolate is shown in table 4. Bitter leaf was inhibitory at 6.25 mg/mL against *Escherichia coli* and *Shigella*, while for *Salmonella* it was inhibitory at 12.50 mg/mL. Garlic was inhibitory at 9.375 mg/mL, 12.50 mg/mL and 6.25mg/ml respectively for the three isolates (*Escherichia coli, Salmonella* and *Shigella*). Honey's MIC was 6.25 mg/mL, 9.375 mg/mL and 6.25 mg/mL respectively.

The minimum bactericidal concentration (MBC) of the extracts against the test isolates is shown in Table 5. Bitter leaf extract was not bactericidal at any of the tested concentration. Garlic and honey were bactericidal against *Salmonella typhi* and *Shigella* at 12.50 and 25 mg/mL for garlic and 25 and 50 mg/mL for honey.

Table. 4.Minimum Inhibitory Concentration (mg/mL) of the extracts on the test bacteria.

Extracts	*Escherichia coli*	*Salmonella*	*Shigella*
Bitter Leaf	6.25±0.00	12.50±0.00	6.25±0.00
Garlic	9.375±9.766	12.50±0.00	6.25±0.00
Honey	6.25±0.00	9.375±9.766	6.25±0.00

Table 5. Minimum Bactericidal Concentration (mg/mL) of the extracts on the test bacteria.

Extracts	*Escherichia coli*	*Salmonella*	*Shigella*
Bitter Leaf	_	_	_
Garlic	_	12.50	25.00
Honey	_	25.00	50.0

Key: - = No MBC.

The zone of inhibition of bitter leaf extract against the three isolates (*Escherichia coli, Salmonella typhi, and Shigella*) is in consonance with the findings of a similar study [11], who reported a reasonable zone of inhibition against *Escherichia coli,Salmonella*, and *Shigella* species. Though, [12] reported a lower zone of inhibition against *Escherichia coli* which oppose this result. The antimicrobial activity of this plant extract on bacteria may be attributed to its phytochemicals like alkaloids, saponin, tannin and glycoside. Also garlic extracts showed a higher zone of inhibition against *Escherichia coli,* but lower against *Salmonella* and *Shigella* species. This finding also agree with previous research of [13] who reported a lower zone of inhibition for *Salmonella typhi* and higher for *Escherichia coli.* While the result disagree with that of [14] who reported a lower zone of inhibition against *Escherichia coli.*

Similarly, the zones of inhibition of honey were higher for all the three isolates.This also agrees with the work of [15] who reported a higher zone of inhibition for *Escherichia coli* and *Salmonella typhi.* Likewise, [16] also reported a higher zone of inhibition for several Gram positive and Gram negative organisms including the test isolates.

The minimum inhibitory concentration of the various bacterial isolates against the antimicrobial agents used showed that, bitter leaf and garlic were inhibitory to *Escherichia coli* and *Shigella* at a lower concentration as compared to *Salmonella.* This is in line with [17] who also reported a lower MIC value for garlic as low as 3.2 mg/mL. Finally, the minimum bactericidal concentration of the extracts on the isolates showed that, bitter leaf was not bactericidal at any of the tested concentrations. While garlic showed a lower MBC against *Salmonella* and *Shigella,* and honey was observed to have a higher MBC against *Salmonella* and *Shigella* respectively. In addition, the extracts showed no MBC against *Escherichia coli.* This means that *Escherichia coli* may not be totally eliminated by honey, garlic or bitter leaf, but its growth can be inhibited by them.

Conclusion

All the extracts exhibited inhibitory activity to the growth of the test bacteria at various concentrations. Honey showed the highest inhibition while the least concentration of the extracts inhibited *Shigella*, followed by *Escherichia coli* which were inhibited by honey and bitter leaf at the same concentration, and *Salmonella typhi* was inhibited by the extracts at higher concentrations. The lowest concentration of all the extracts inhibited the growth of *Shigella* species as compared to other test bacteria. Among the three extracts, garlic was the most effective antimicrobial agent capable of totally eliminating one of the tested bacteria with the least concentration. Therefore the

result obtained from this study can serve as incursion into the use of garlic for the production of antimicrobial agents.

Declaration of conflicting interests

The author declared no potential conflicts of interest

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