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Antibacterial potency of fortified and unfortified honey on some clinical bacterial isolates

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ABSTRACT: *The use of herbal medicine to treat infection has been practiced since the origin of mankind, and honey produced by Apis mellifera is one of the oldest traditional medicines considered important in the treatment of various human ailments. However, differently processed honeys exhibit different antibacterial properties, thus this study was aimed at investigating the antibacterial potency of fortified honey, and compared with the unfortified and natural honey tested on Escherichia coli and Salmonella typhi. The antibacterial susceptibility of Escherichia coli and Salmonella typhi on the four honey samples (honey fortified with ginger, honey fortified with lemon, unfortified honey and natural honey) was determined using the agar ditch method. Honey fortified with lemon shows higher antibacterial activity especially on Salmonella typhi with the zones of inhibition of 25, 28, 29.5, 30 and 31 mm at the concentrations of 20, 40, 60, 80 and 100 % (v/v) respectively, than unfortified honey and natural honey. This study revealed that fortified honey mostly honey fortified with lemon has increased antibacterial activity due to the presence of fortifying agents than the unfortified honey. The antibacterial activity of fortified honey may depend on the quantity of the fortifying agents present in the honey samples as honey fortified with ginger produced a contrary result; this means that the fortifying agents may not necessarily be applied in honey to add to its antibacterial potency rather used as a flavoring agent.*

KEYWORDS: *Antibacterial activity, Clinical bacterial isolates, Fortified and unfortified honey, Fortifying agents, Zones of inhibition.*

I. INTRODUCTION

Honey produced by *Apis mellifera* is one of the oldest traditional medicines considered to be important in the treatment of several human ailments [1]; as herbal medicine has been widely employed in the treatment of diseases since the origin of mankind. Honey is the sweet substance; produced when the nectar and deposit from plant are gathered, modified and stored in the honey comb by honey bees [2]. According to [3], honey has been extensively used as healing agent throughout the human history in addition to its widespread usage as popular food. Different cultures have extensively used honey as a medicine for many health issues and disorders.

Honey is an excellent source of flavonoids, a natural antioxidant which have the ability to protect against allergens, viruses and carcinogens [4]. Also as related by [5], honey includes hydrogen-peroxide, flavonoids and phenolic acids plus many other unidentified properties. Also the chemical composition of honey is said to comprise of seven tetracycline, fatty acids, lipids, amylase, ascorbic acid, peroxidase and fructose all of which are attributed to its antimicrobial activity together with high osmolarity, low pH (3.6-3.7), content of phenol (inhibine), peroxidase, glucose and fructose in honey and the presence of tetracycline derivatives of fatty acids [6]. The antibacterial activity of honey is highly complex due to the involvement of multiple compounds and due to the large variation in the concentrations of these compounds among honeys [7]. Acidity is also said to be one of the factors that contributes to the antibacterial property of honey [8].

In some bee products, the antibacterial activity of honey is attributed to the presence of "inhibin", which acts as an antibacterial factor other than hydrogen peroxide [9], more recently, methylglyoxal and the antimicrobial peptide bee defensin-1 were identified as important antibacterial compounds in honey [7].

Honey has been used as a medicine throughout the human history [10]. Also antibacterial properties of honey were recognized more than a century ago and have subsequently been extensively studied [11]. In modern medicine, particularly in the last three decades, interest in the application of honey for the treatment of infections has increased. Recently, the use of honey as a therapeutic agent has been re-discovered especially in the field of medicine thus gaining acceptance as an antibacterial agent. Many studies demonstrated the use of honey when antibiotic treatments had failed to clear the infection [10]. The control of infection by honey is said to be attributed to the high osmolarity while its hydrogen peroxide contents, lysozyme and other unidentified substances from certain flora sources are responsible for its antibacterial properties [12].

Recent advancement in industrial production and processing of honey especially in food and drug industries introduced fortification of honey with different fortifying agents known to possess certain individual medicinal value. These fortified honeys usually are enriched with fortifying ingredients such as ginger and lemon thus serves its purpose and may have an additive antimicrobial effects. The fortifying agents in honey (ginger and lemon) may act in synergy with honey and thus enhance the property of honey's antibacterial effect.

The need to identify a common and cheap herbal remedy for the prevention and treatment of sore-throat, mouth sore and dental caries, especially in a developing nation, prompted the investigation of the therapeutic potentials of ginger and honey [13].

Ginger (Zingiber officinale Roscoe, Zingiberacae) is a medicinal plant that has been widely used in herbal medicines all over the world, the plant is reported to have antibacterial, anti-oxidant, antiprotozoa, anti-fungal, anti-emetic, anti-rhinoviral, antiinflammatory, anti-insecticidal activity [14]. The antimicrobial activity of ginger has been described and studied [15]. Lemon (Citrus fruit), an acidic fruits is another important fortifying ingredient which contain fabulous source of vitamin and a wide range of essential nutrients required by the body.

Honey can be fortified to add to the flavor of the honey (as a flavoring agent) and to add to the antibacterial activity of the honey. Herbal preparation of honey and ginger are used as an essential ingredient in the preparation of most herbal concoctions [13].

The antibacterial effect of honey varies due to their composition, thus varieties of honey may have different degrees of antibacterial potency. The antibacterial/antimicrobial effects of honey are clearly obvious as proved by several scientific works. However there exist limited literatures demonstrating the antibacterial effects of fortified honey regardless of the fortifying agents.

The aims of this study were to investigate the antibacterial activity of fortified honey on clinical bacterial isolates hence compare its potency with unfortified and natural honey samples.

II. MATERIALS AND METHODS

2.1 Source of Honey

Varieties of honey used for this study include: fortified (honey fortified with lemon and honey fortified with ginger), unfortified and natural honey samples. Both fortified and unfortified honey were EL-LYON's honey, produced and packaged by EL-LYON Honey Granulation Technology, Technology Incubation Centre (TIC) 49, Barde way, Jalingo, Taraba State, Nigeria. The EL-LYON honey samples were purchased from a retail outlet in Gombe metropolis while natural honey was obtained from a local honey store in Gombe Metropolis. Various honey samples collected in their container were preserved at 4 ^{0}C in the refrigerator to protect the honey from oxidation and temperature degradation.

2.2 Clinical bacterial isolates

The clinical bacterial isolates used (*Escherichia coli and Salmonella typhi*) in this work were obtained from the Medical Microbiology Laboratory of Specialist Hospital, Gombe State. These clinical bacteria isolates were subjected to the following:

2.2.1 Sub-culturing and purification of test organisms

Stock cultures of *Escherichia coli and Salmonella typhi* used for this study were sub-cultured on nutrient agar by picking a single colony and streaking on nutrient agar plate hence incubated at 37 ^{0}C for 24 hours. Colonies of fresh cultures of the different test organisms (*Escherichia coli and Salmonella typhi*) from the overnight sub-cultured plates were picked with sterile inoculating loop and pure cultures of the test organisms were obtained by streak plating method. Distinct colony were picked from the pure culture plates and sub-cultured on agar slants to obtain stock of the pure culture for each of these test organisms.

2.2.2 Identification and Confirmation of clinical bacteria isolates

The tests organisms including *Escherichia coli and Salmonella typhi* were identified and confirmed using standard microbiological procedures such as gram staining and microscopy, and confirmatory biochemical test. The biochemical tests carried out to identify and confirm these clinical bacterial isolates include catalase test, coagulase test, urease test, indole test, oxidase, methyl red, H_2S test, Simmon citrate, motility test and Kligler iron agar test as described by Cheesebrough, (2006).

2.2.3 Standardization of Inocula

Fresh cultures for this study were prepared by transferring a loop full of colony from the pure culture plates of *Escherichia coli and Salmonella typhi* into different test tubes containing nutrient broth then incubated for 24 hours (overnight) at 37 ^{0}C.

9 mL of distilled water was dispensed in empty test tubes and 1 mL from the overnight cultures was dispensed into test tube containing 9 mL distilled water, further direct dilution was done for the three bacterial isolates until the turbidity matches 0.5 McFarland's standards.

2.3 Preparation of Stock and Standard Solutions of Honey

For all the four (4) honey samples, the undiluted form of the honey was used as stock concentration (assumed 100 % (v/v)). Various standard solution concentrations such as 20, 40, 60, 80 and 100 % (v/v) were prepared from the stock by diluting with required quantities of distilled water. The negative controls [0 % (v/v)] were set up with equivalent quantities of distilled water.

2.4 Antibacterial Susceptibility/Sensitivity Test

Antibacterial susceptibility test was carried out using the agar ditch method as described by [16] to investigate the antibacterial activity of the various honey samples (honey fortified with ginger, honey fortified with lemon, unfortified honey, and natural honey) on the two clinical bacterial isolates *Escherichia coli and Salmonella typhi*.

Different honey concentrations used for antibacterial susceptibility test were 20, 40, 60, 80 and 100 % (v/v). 1 mL of each standard inocula was pour plated into sterile, empty petri plates together with 15-20 mL of molten nutrient agar media. The plates were allowed to set after which six equidistant wells of 5 mm in diameter were drilled using a sterile cork borer at different sites on the plates. 20 µL of different concentrations (20, 40, 60, 80, 100 % (v/v)) of the honey samples were separately and carefully placed in the different wells using sterile syringe. The plates were allowed to stay for 15 minutes for proper diffusion of honey then incubated for 18 to 24 hours at 37 ^{0}C.Various zones of inhibitions were observed, measured (in diameter) and recorded.

2.5 Data analysis

The experiment was carried out in duplicates, the diameter zones of inhibition were measure in millimeter (mm) and data generated were used to calculate average diameter zones of inhibitions hence results presented in tables and suitable line graph designated as figures.

III. RESULTS AND DISCUSSION

Various honey samples (honey fortified with ginger, honey fortified with lemon, unfortified honey and natural honey) collected were tested for their antibacterial activity using the two clinical bacterial isolates (*Escherichia coli and Salmonella typhi*).

TABLE I Antibacterial susceptibility of the two clinical bacterial isolates on various honey samples

Organisms different	Honey samples	Diameter zone of inhibition (mm) produced by Concentrations of honey samples				
		20	40	60	80	100 % (v/v)
Salmonella typhi	Honey + ginger	18	20.5	23	26	26.5
	Honey + lemon	25	28	29.5	30	31
	Unfortified honey	18	20.5	22.5	24.5	25.5
	Natural honey	13	14.5	18.5	20.5	21
Escherichia coli	Honey + ginger	10	16.5	18	22	29.5
	Honey + lemon	22.5	24.5	26	27	28
	Unfortified honey	15.5	16.5	18.5	19.5	28.5
	Natural honey	19.5	24.5	26	28.5	32.5

mm: millimeter

Table I represents the results of the antibacterial susceptibility of *Escherichia coli and Salmonella typhi* on various honey samples tested using agar ditch method. From table I, the highest and the lowest zones of inhibition produced by the different honey samples tested on *Salmonella typhi* were 31 mm at 100 % (v/v) concentration for honey fortified with lemon and 13 mm at 20 % (v/v) concentration for natural honey. Also the highest and lowest zones of inhibition produced by various honey samples tested against *Escherichia coli* were 32.5 mm for 100 % (v/v) concentration for natural honey and 10 mm at 20 % (v/v) concentration of honey fortified with ginger.

The figures below describe the antibacterial activities of the different honey samples tested separately on *Escherichia coli and Salmonella typhi*.

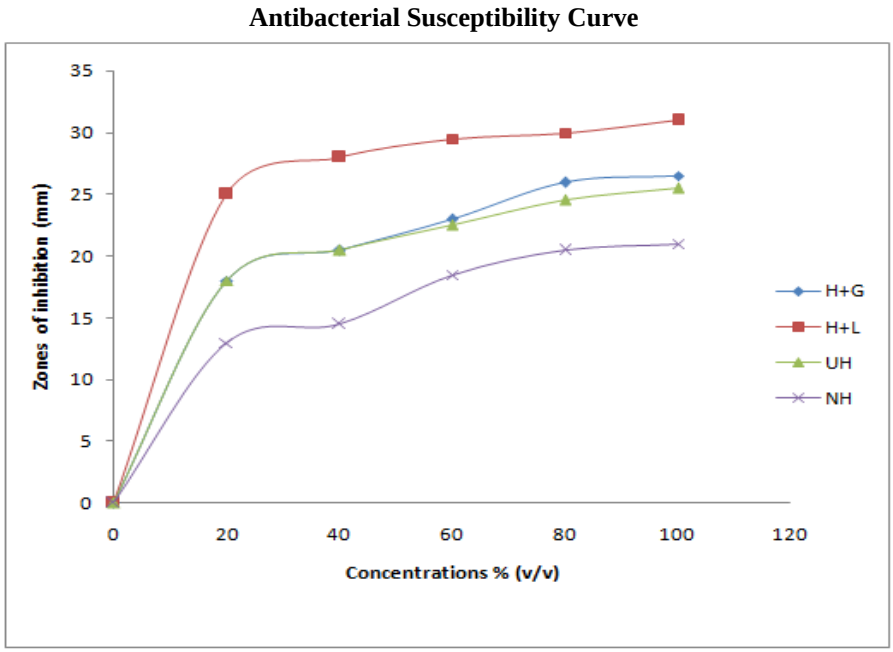

Fig. 1 shows the average zones of inhibition produced by various concentrations of honey tested on *Salmonella typhi*, H+G: Honey fortified with Ginger, H+L: Honey fortified with lemon, UH: Unfortified honey, NH: Natural honey.

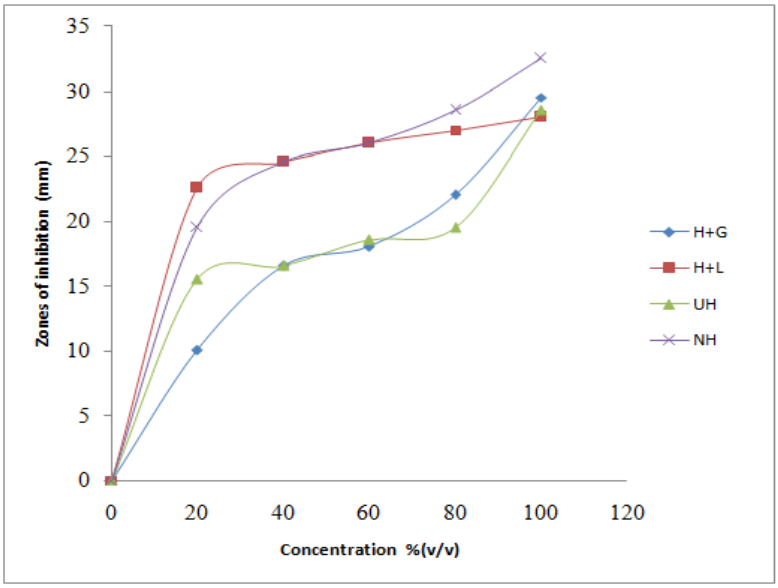

Fig. 2 shows the average zones of inhibition produced by various concentrations of honey samples tested on *Escherichia coli*. H+G: Honey fortified with Ginger, H+L: Honey fortified with lemon, UH: Unfortified honey, NH: Natural honey

From this study (table I), all the honey samples (honey fortified with ginger, honey fortified with lemon, unfortified honey and natural honey) showed reasonable antibacterial activities on *Escherichia coli and salmonella typhi*. According to [17] and [18], there are three explanations for the antibacterial property of honey, which includes osmotic effect, acidity and hydrogen peroxide. The control of infection by honey is said to be attributed to the high osmolarity while its hydrogen peroxide contents, lysozyme and other unidentified substances from certain flora sources are responsible for its antibacterial properties [12]. Also [19] related that antibacterial activities of honey can be due to the presence of inhibines including flavonoids, phenolic acids and some unidentified components in honey.

Research conducted by [20], indicated that diluted honey can activate glucose oxidase and then produce hydrogen peroxide which contributes to the antibacterial property of honey. In addition, honey is an excellent source of flavonoids, a natural antioxidant which have the ability to protect against allergens, viruses and carcinogens [4]. These studies (12, 17, 18, and 19) indicated that the antibacterial activity of honey can be attributed to its antibacterial properties or components. It is therefore worthy to state that these active components of honey are importantly responsible for the antimicrobial effects observed in all the honey samples used in this work.

In fig. 1-2, the highest zones of inhibition were observed at 100 % (v/v) concentrations of the various honey samples whereas the lowest were observed at 20 % (v/v) concentrations of the different honey samples tested on *Escherichia coli and salmonella typhi*. Also, it is evident that the zones of inhibition produced by the various honey samples is proportional to the concentration of honey sample used, this implies the inhibitions produced increases as the concentration of these honey samples increases. This phenomenon is expected as this is same with the study carried out by [21].

In comparing the antibacterial activities of fortified and unfortified honey tested on the two clinical bacterial isolates; fig. 1 described the antibacterial susceptibility of *Salmonella typhi* to different honey sample, honey fortified with lemon shows higher antibacterial activity with the zones of inhibition of 25, 28, 29.5, 30 and 31 mm at the concentrations of 20, 40, 60, 80 and 100 % (v/v) respectively, than unfortified honey with 18, 20.5, 22.5, 24.5, and 25.5 mm as zones of inhibition at 20, 40, 60, 80 and 100 % (v/v) concentration, this correlates with the findings of [22], also higher than honey fortified with ginger and natural honey.

Also fig. 2 shows the antibacterial activities of the various honey samples tested on *Escherichia coli*, here, the antibacterial activity of honey fortified with lemon was still higher than unfortified honey with the zones of inhibition of 22.5, 24.5, 26, 27 mm at 20, 40, 60, 80 % (v/v) concentrations respectively except for 100 % (v/v) but same with the natural honey. Reference [23] recently reported the presence of limonoids in lemon, which can be considered responsible for activity against many clinically, isolated bacterial strains. As clearly shown in figure 1, the higher antibacterial activity of honey fortified with lemon may be attributed to the antibacterial components of lemon combined with that of honey. The work of [22] studied the susceptibility of bacteria to honey and lemon and concluded that the combine effect of honey and lemon is higher than the individual effect. Lemon possesses significant antibacterial properties and investigations showed that lemon was active against bacteria [23]. Lemon has been reported to exhibit antimicrobial activity against *Vibrio cholera* [25]**.**

There was inconsistency in the antibacterial susceptibility of *Escherichia coli and Salmonella typhi* on various honey samples as seen in figures 1 and 2; As Reference [9] stated that the inhibitory activity caused by the osmotic effect of honey dilutions obviously depends on the species of bacteria. Although, [26] reported that the antibacterial activity of honey does not depend completely on its high osmolarity but on the release of the hydrogen peroxide.

Based on the findings of this study, it can be noted that honey fortified with lemon shows higher antibacterial activity on *Salmonella typhi* and *E. coli* than unfortified honey, but honey fortified with ginger showed indifference. Unfortunately, the concentration of the fortifying agents used in enriching these honey samples was not indicated on the containers by the manufacturer. The natural honey also shows higher antibacterial activity, this may be due to the higher antibacterial components of the natural honey sample [27].

It is imperative to note that fortified honey samples (honey fortified with lemon and honey fortified with ginger) showed relatively dissimilar antibacterial activity on the two clinical bacterial isolates when compared; this implies that the concentration of various fortifying agent in the honey differs, hence not stated or unknown. Therefore, these fortifying agents might have been used in the honey as flavoring agents.

3.1 CONCLUSION

Several studies have demonstrated the antibacterial potency of honey samples. In this study, the antibacterial activity of fortified honey was tested on clinical bacterial isolates then compared with unfortified and natural honey.

From this study, it can be inferred that fortified honey has increased antibacterial activity than the unfortified honey due to the presence of fortifying ingredients especially in the case of honey fortified with lemon. It is of the essence to further state that the antibacterial property of fortified honey may be dependent on the quantity of the fortifying agents present in the honey, as honey fortified with ginger produced indifference result.

Also the concentration of fortifying or enriching agents present in the fortified honey sample is unstated; this is because that the fortifying agents may not necessarily be applied to add to the antibacterial potency of honey rather used as a flavoring agent.

3.2 RECOMMENDATIONS

- In fortifying honey, the concentration/quantity of the fortifying agents should be increased to add to its antibacterial potency, not only serve as flavoring agents.
- Also the quantity of the fortifying agents should be clearly indicated to enable evaluation of the extent of increase in the antibacterial potency of the honey.
- Other fortifying agents such as garlic, *Moringa oleifera* should also be introduced to increase the antibacterial potency of honey samples.
- Research should be conducted to compare the antibacterial activities of fortified honey with commercial antibiotics sensitivity disc; its findings might be important to the field of medicine.
- Research should be extended to other microorganisms of medical importance other than bacteria to ascertain the extent of antimicrobial intensity of the honey samples.

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