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Biochemical screening of pure honey and its antibacterial activity on some bacterial isolates compared with a common antibiotics

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ABSTRACT: *The biochemical components and antibacterial efficacy of pure honey produced by honeybee(Apis mellifera) was investigated. Biochemical analysis revealed the presence of reducing sugar, saponins, glycosides, alkaloids and flavonoids and absence of phenols and tannins. Antibacterial activity of pure honey on some medically important bacteria including Staphylococcusaureus, Salmonella typhi, Escherichia coli, Klebsiella pneumonia and Pseudomonas aeruginosa was determined using the agar well diffusion method. The result shows that pure honey exhibit strong antibacterial activity producing zones of inhibition against the tested bacteria.Also, honey sample used in this study showed antibacterial activity than the commercially available antibiotics. Our findings shows that honey,apart from their roles as food and supplements, could be suitable for the treatment of various infection caused by bacteria hence should be used as a preventive and curative measure to common diseases related to the test organisms.*

KEYWORDS: *Antibacterial efficacy, antibiotics, bacteria, biochemical analysis, pure honey.*

I. INTRODUCTION

Microorganisms have developed resistance to many antibiotics and this has created serious clinical problems in the treatment of infectious diseases.Plethora of studies has emerged towards using natural products as an alternative therapy. One natural food product that has gained great momentum is honey. Researchers round the globe have worked both *in vitro* and *in vivo* to spark the unknown benefits of the inestimable values of honey as well as its application [1].The use of traditional medicine to treat infections has been practiced since the origin of mankind, and honey produced by *Apis mellifera* is one of the oldest traditional medicine considered to be important in the treatment of several human ailments. The belief that honey is a nutrient, a drug and an ointment has been carried into our days, and thus, an alternative medicine branch, called apitherapy, has been developed in recent years, offering treatments based on honey and other bee products against many diseases including bacteria infections [2].

Honey is a thick, sweet liquid made by bees from the nectar of flowers. It contains water, glucose, fructose proteins, vitamins and minerals [3]. It is also defined as the natural sweet substance produced by honeybees from the nectar of blossoms or from the secretions of living parts of plants or excretions of plants sucking insects on the living parts of plants, which honeybees collect, transform and combine with specific substances of their own, store and leave in the honeycomb to ripe and mature [4].

There are basically two types of honey, apiary and forest honeys. Honey produced by the honeybees, *Apis ceranaindica* and *Apis mellifera*, in apiaries and collected by modern extraction method are called apiary honey. They are transparent and free from foreign materials. In contrast, those produced by rock bee, *Apis dorsata*, or by wild nest of *Apis ceranaindica* in forests and collected by the crude method of squeezing the comb are known as forest honeys. They are turbid owing to the abundance of pollen, wax, brood (bee larvae), parts of bees and plat materials. It is therefore necessary to filter the honey to separate the suspended particles [5].Honey primarily contains sugar and water. Sugar accounts for 95-99% of honey dry matter, majority of these are simple sugars, fructose (38.2%) and glucose (31.3%), which represents 85-95% of total sugars. These are "simple" sugars, 6-carbon sugars that are readily absorbed by the body [6]. Other sugars include disaccharide such as maltose, sucrose and isomaltose, few oligosaccharides are also present. Water is the second most important component of honey. Its content is critical, since its affects the storage of honey. The final water content depends on numerous environmental factors during production such as weather and humidity inside the hives, but also on nectar conditions and treatment of honey during extraction and storage [6].

The colour of honey can vary from nearly colourless to dark brown and its consistency can be fluid, viscous or partly to entirely crystallized. The nectar source visited by the honey bees leads to variation in colours, flavors and aroma[7]. Honey is characteristically acidic with pH of between 3.2 and 4.5, which is low enough to be inhibitory to many animal pathogens [8]. The multi facet properties of honey anchored in the scientific world is regarded as a sweetener, functional food, antioxidant, antimicrobial, antiseptic, pre-biotic probiotic, immunomodulatory, anti-inflammatory, anti-tumor and anti-cancer effect amongst others [9, 10, 11, 12]. Above and beyond its therapeutic effect or medicinal attributes [13,14], it is also of potential use as bio indicators for environmental contamination [15]. It is commonly used as a base for ointment and has successfully been applied in surgical dressing to avoid septic infections [16]. Honey is gaining acceptance as an agent for the treatment of ulcers, bed sores and other skin infections resulting from burns and wounds [17, 18]. Moreover, it can be used on skin grafts and infected skin graft donor sites successfully [19]. Honey may promote the repair of damaged intestinal mucosa, stimulate the growth of new tissues and work as an anti-inflammatory agent [20, 21]. The healing properties of honey can be ascribed to the fact that it offers antimicrobial activity, maintain a moist wound environment that promotes healing and has a high viscosity which helps to provide a protective barrier to prevent infection [22]. Furthermore, honey has been employed to shorten theduration of diarrhea in patients with bactericidal gastro-enteritis due to bacterial infection [23].

The antibacterial activity of honey was first recognized in 1892, by Van Ketel [24]. Numerous studies demonstrated that honey possesses antimicrobial activity[25]. It destroys and/or inhibits the growth of some pathogenic vegetative microorganisms [26]. An antifungal action has also beenobserved for some yeasts and species of Aspergillus and Penicillium [27], as well as the common dermatophytes [28].Two millennia before bacteria were identified as the cause of disease, physicians at that time were aware that certain types of honey are the best therapy for particular ailment and infections [29]. Honey was used to treat infected wounds as long ago as 2000 years before bacteria were discovered to be the cause of infection [30]. When applied topically to wounds, osmosis would be expected to draw water from the wound into the honey helping to dry the infected tissue and reduce bacteria growth [31]. Laboratory studies and clinical trials have shown that honey is an effective broad-spectrum antimicrobial agent. Honey has been reported to have inhibitory effect on several bacteria including aerobes and anaerobes, Gram-positive and Gram-negative and is effective against methicillin resistant *Staphylococcusaureus* (MRSA), β-hemolytic *Streptococci* and vancomycin-resistant enterococci (VRE) [32, 33].

Pure honey has been shown to be bactericidal to many pathogenic microorganisms including *Salmonella spp*, *Shigellaspp*, Other enteropathogenic like *Escherichia coli*, *Vibrocholerae* and other gram-negative and gram-positive organisms [34, 35]. The ability of honey to kill microorganisms has been attributed to its high osmotic effect, high acidic nature, hydrogen peroxide concentration and its chemical nature, which include its content of tetracycline derivatives, peroxides, amylose, fatty acids, phenols, ascorbic acid, terpenes, benzyl alcohol and benzoic acid [36, 25]. Most types of honey generate hydrogen peroxide when diluted because of the activation of the enzyme glucose oxidase, which oxidize glucose to gluconic acid and hydrogen peroxide [37]. Hydrogen peroxide is the major contributor to the antimicrobial activity of honey, and the different concentrations of this compound in different honeys result in their varying antimicrobial effects [25]. It has further been reported that physical property along with geographical distribution and floral sources may play an important role in the antimicrobial activity of honey [38].The antibacterial activity screening and phytochemical analysis of essential plants has been of great interest in the discovery of drugs effective in the treatment of several diseases [39].

The present study was therefore to investigate the chemical constituents and antibacterial efficacy of pure honey on some pathogenic organisms involved in causing infections in humans compared with that of the conventional antibiotic that is commonly used in the treatment of infections.

II. MATERIALS AND METHODS

2.1 Sample Collection and Mode of Identification of Pure/Original Honey

The honey samples used in this study were purchased from Gashaka Local Government Area, in Taraba State, Northeastern Nigeria. Several experiments were conducted to ascertain that the honey samples were pure and original. These include:

I. Dropping some of the sample onto sand: if it is a pure honey, it will not sink immediately.
II. Pouring a small quantity into a cup of water: if pure, it will go down to the bottom of the cup without mixingup with the water except when stirred.
III. Dipping a finger into the honey sample, dropping one or two drops on the ground: if it is pure, it will go down like a thread without breaking.

The samples were then collected in sterile screw-caped container and were kept in a dark, cool and dry place (at room temperature) overnight before they were finally transported to the Department of Science Laboratory Technology, Federal Polytechnic Bali for processing.

2.2 Processing of Honey Samples

Each sample was first filtered with a sterile mesh to remove debris, viscosity was reduced by heating honey at 30°C for 30 minutes.The samples were checked for purity by inoculating on blood agar plates and incubated overnight. Uncontaminated samples were stored at refrigeration temperature of about 4℃ until used.

2.3 Microorganisms

The microorganisms used in this study which are also known to be potentially pathogenic to humans were obtained as clinical isolates from optimum laboratory, Jimeta-yola, Adamawa State, Nigeria. They include *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella* spp. and *Salmonella* spp. Biochemical tests such as Catalase test, Lactophenol test, indole test, Oxidase test and Gram stain were carried out to confirm the identity of the organisms.These organisms were maintained in agar slants at 4℃ until used.

2.4 Biochemical Analysis

The sample was screened for the following compounds: reducing sugar, saponins, glycosides, alkaloids, phenols, flavonoids and tannins using standard laboratory techniques [40, 41].

2.5 Antimicrobial Susceptibility Testing

The antibacterial activity of pure honey in comparison with standard antibiotic ciprofloxacin (100mg/ml) in vitro on the isolates was determined by the ager well diffusion method as described by [42 and 43]. This was done using pour plate method in which small colonies from each clinical isolates of the test organisms were made into suspension with 1ml of sterile distilled water in test tubes. 0.1ml of each suspension was dispensed into sterile petridishes after which melted and sterilized nutrient agar maintained at 45℃ was poured (15 aliquot) into the respective plates. The plates were allowed to set, four equidistant wells of 6mm in diameter were punched in each plate using a sterile cork borer. To each of the wells, 0.2ml of pure undiluted honey was introduced. A well filled with sterile water served as control and the plates were allowed to stay for15 minutes for pre-diffusion to take place followed by incubation for 24-48 hrs at 37℃. The zones of inhibition were measured with the use of a metric rule.

IV. RESULTS AND DISCUSSION

Biochemical analysis of pure honey is shown in table 1. The result shows that honey contains reducing sugar, Saponins, glycosides, alkaloids and flavonoids. The efficacy of pure honey against different bacteria is shown in table 2. The sample showed effective antibacterial activity against both gram-positive and gram-negative bacteria as indicated by their zones of inhibition. *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* showed greater level of susceptibility (30mm, 24mm and 22mm respectively) than other tested bacteria. *Salmonella typhi*was the most resistant isolate tested in this study as it showed the least zone of inhibition of 18mm. Table 3 shows the antibacterial activity of a standard drug on the bacteria isolates. Ciprofloxacin showed maximum zone of inhibition (21mm) against *S. typhi* and the least zone of inhibition (17mm) was against *Pseudomonas aeruginosa*.

Table 1: Biochemical analysis of pure honey

Chemical constituent	Confirmation
Reducing sugar	+
Saponins	+
Glycosides	+
Alkaloids	+
Phenols	-
Flavonoids	+
Tannins	-

Keys: + = Presence of constituent, - = Absence of constituent

Table 2: Antibacterial activity of pure honey against some bacteria isolates.

Organisms (Bacteria)	Dose (ml)	Zone of inhibition (mm)
Staphylococcus aureus	0.2	24
Salmonella typhi	0.2	18
Escherichia coli	0.2	30
Klebsiella pneumonia	0.2	20
Pseudomonas aeruginosa	0.2	22

Table 3: Antibacterial activity of a standard drug (ciprofloxacin 100mg/ml) against some bacterial isolates.

Organisms (Bacteria)	Dose (ml)	Zone of inhibition (mm)
Staphylococcus aureus	0.2	20
Salmonella typhi	0.2	21
Escherichia coli	0.2	18
Klebsiella pneumonia	0.2	19
Pseudomonas aeruginosa	0.2	17

The result of the biochemical screening conducted on pure honey as shown in table 1 revealed the presence of some bioactive components such as reducing sugar, Saponins, glycosides, alkaloids andflavonoids while phenols and tannins are absent. These compounds are known to have some therapeutic properties against several pathogens which supports its traditional use for curing disease. The presence of these active agents confirms similar research conducted by [44] who also observed similar constituents in honey.Flavonoidshave been documented to posses potent antioxidant and free radical scavenging effect [45]and have strong anticancer activity[46, 47]. They are also known to have anti-inflammatory, anti-allergic and anti-viral properties and help in the healing of wounds and treatment of skin diseases due to their ability to neutralize the acidity of wounds, and inflammation [48, 44].The honey samples were also revealed to contain saponins. Saponin has the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness [49, 48]. More so, saponins have been found to be an antibacterial substance on the cell wall of many organisms [50]. Saponins cause a reduction of blood cholesterol by preventing its re-absorption. The presence of alkaloid indicates that honey can be used as basic medicinal agents for their analgesic, antispasmodic and bactericidal effects [51, 52]. Glycosides are known to lower blood pressure according to many reports [53]. Plants containing reducing sugar are of great value to living organisms since they are primary metabolites and are directly involved in their growth, development and reproduction.Absence of other phytoconstituents (Phenols and tannins) may be due to certain factors such as climatic conditions, temperature and soil fertility. Furthermore, this study also showed that some bacteria were more sensitive to honey than others. This phenomenon was observed in table 2 were *E. coli, S. aureus* and *P. aeruginosa* were more sensitive while *K. pneumonia* and *S. typhi* were less sensitive.

The result obtained from this study, indicate that the honey sample used in this study showed antibacterial activity than the commercially available antibiotics as shown in Table 2 and 3.

V. CONCLUSION

Pure honey used in this present study revealed the presence of bioactive components that has both bactericidal and bacteriostatic activity. This therefore justifies its usefulness in clinical and folklore practices against diseases caused by bacteria. However, dosage remains a challenge.

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