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Phytochemical screening and antimicrobial activity of apiary honey produced by honey bee (*Apis mellifera*) on clinical strains of *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*

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Honey produced by honeybee (*Apis mellifera*) which is used in herbal medicine was examined for its chemical constituents and antimicrobial activity. The phytochemical analysis of honey showed the presence of alkaloids, flavonoids, saponins, steroids, reducing sugar and glycosides. Antimicrobial activity of honey on fresh hospital isolates: *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* obtained from Glanson Medical Laboratory Awka were determined using well diffusion method. The result shows that the honey produced by *mellifera* has strong antimicrobial activity against *E. coli* and *S. aureus* but not against *C. albicans*. The result obtained shows that the honey produced a zone of clearance of 45 and 34 mm on *S. aureus* and *E. coli*, respectively. The result of minimum inhibitory concentration (MIC) determined on liquid culture was 20%v/v for both *S. aureus* and *E. coli* while the minimum bactericidal concentration (MBC) determination of the sample showed 20% and 30%v/v for *S. aureus* and *E. coli*, respectively. Our result shows that honey, apart from their role as food additives and supplements, may also be utilized as effective and cheap sources of antibacterial agents for the treatments of bacterial infections.

Key words: Apiary honey, *Apis mellifera*, antibacterial activity, minimum inhibitory concentration, minimum bactericidal concentration, clinical isolates.

INTRODUCTION

The use of plant for healing is as ancient and universal as medicine itself. Plants act generally to stimulate and supplement the body's healing forces. They are the natural food for human beings. Many infectious diseases are known to be treated with herbal remedies throughout the history of mankind. Today, plant materials continue to

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play a major role in primary health care as therapeutic remedies in many developing countries. Plants still continue to be almost the exclusive source of drugs for the majority of the World's population. The antimicrobial 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 (Ainslie, 1999). Herbs have played an important part in our developments. Approximately 25% of all prescription drugs are derived from trees, for example, shrubs of foxglove, morphine and codeine are derived from the opium, and quinine from cinchoma bark etc. The essential differences between herbal and conventional medicine is that; in conventional medicine, the most active constituent is extracted from the plant, synthesized in the laboratory to make drugs while in herbal medicine, the extract from the whole plant are used (W.H.O, 2000).

The scope of herbal medicine is extended to include fungal such as mushroom and bee products like honey as well as animals, shells and certain animal parts. Man has been so blessed in nature. Honey is a natural gift to man from Mother Nature which is made available to us from the mysterious kingdom of the bees, but it is quite unfortunate that we have cared less to know what is contained in this wonderful nature's kits (Akinpelu, 2000).

The resistance of antibiotics against pathogens has triggered research scientists to venture for substitute curatives. It is indeed of paramount importance to unveil new therapies directed at novel targets as budding to alternatives to antibiotics as well as validation of traditional remedies (Jenkins et al., 2011). Plethora of studies has emerged towards natural products in addressing the dearth and limitations of current therapies. One natural food product which has gained great momentum is honey. Honey, a natural product of very high nutritive value is made when the nectar (floral) and sweet deposits from plants (non floral) are gathered, modified and stored in the honeycombs by honeybees of the genera *Apis* and *Meliponini* (Namias, 2003; Al-jabri, 2005). Its composition and quality vary greatly with the botanical source of nectar as well as environmental and climatic conditions. Depending on its quality, honey can contribute to the health and nutritional status of humans. These beneficial actions have been ascribed to its antimicrobial, anti-inflammatory and anti-oxidant potential. Interestingly, honey is gradually receiving attention as a complementary and an alternative source of treatment in modern medicines. It is active against antibiotic-sensitive and antibiotic-resistant strains of micro-organisms and has the potential not to select for further resistant strains (Manyi-Loh et al., 2011).

There are basically two main types of honey, apiary and forest honeys. Honeys produced by the honeybees, *Apis cerana indica* and *Apis mellifera*, in apiaries and collected by the 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 from wild nests of *A. cerana indica* 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 plant materials. It is therefore necessary to filter the honey to separate the suspended particles (Subrahmanyam, 2007).

Researchers round the globe have worked both *in vitro* and *in vivo* to spark the unknown benefits of the inestimable attributes of honey as well as its applications (Cursons, 2010; Irish et al., 2008; Kumari et al., 2010; Zaid et al., 2010). In the modern era, the different biological, chemical and physical properties of honey have revealed several beneficial claims through different techniques. The multi facet properties of honey anchored in the scientific world is regarded as a sweetener, functional food, antioxidant, antimicrobial, antiseptic, prebiotics, probiotics, immunomodulatory, anti-inflammatory, anti-tumor and anti-cancer effect amongst others (Jenkins et al., 2011; Conway et al., 2010; Fauzi et al., 2011). Above and beyond its therapeutic effects or medicinal attributes (Mohapatra et al., 2011; Conti et al., 2007), it is also of potential use as bio-indicators for environmental contamination (Celechovska and Vorlova, 2001). 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 botanical spectrum or the nectar source visited by the honey bees leads to variation in colours, flavours and aroma (C. A. C, 1996).

Honey is well known for its antibacterial activity, which was first reported in 1892. Since ancient times, honey has been used for treatment and prevention of wound infections. With the advent of antibiotics, the clinical application of honey was abandoned in modern Western medicine, though in many cultures it is still used. For all antibiotic classes, including the major last resort drugs, resistance is increasing worldwide (Walsh, 2003; Levy and Marshal, 2004); and even more alarming, very few new antibiotics are being developed. The potent activity of honey against antibiotic-resistant bacteria resulted in renewed interest for its application (Cooper et al., 2002a; Cooper et al., 2002b; Efem et al., 1988). Several honeys have been approved for clinical application. The incomplete knowledge of the antibacterial compounds involved and the variability of antibacterial activity are however major obstacles for applicability of honey in medicine. In recent years, the knowledge on the antibacterial compounds in honey has expanded.

According to the United States National Honey Board (1994) and various international food regulations, honey is a sweet aliment produced by honey bees (*A. mellifera*) and derived from the nectar of flowers. It also stipulates a pure product that does not allow the addition of any other sweetener, but is not limited to water or other substance. Honey gets its sweetness from monosaccharide such as fructose, glucose and has approximately the same

sweetness as that of granulated sugar. Honey is one of the oldest traditional medicines considered to be important in the treatment of respiratory ailment, gastrointestinal infection and various other diseases (W.H.O, 1996). It is being used effectively in dressing of wounds, burns and skin ulcers to reduce pain and odour quickly. The ability of honey to kill microorganisms has been attributed to its high osmotic effect, high acidic nature (pH 3.2 to 4.5), hydrogen peroxide concentration and its phytochemical nature; that is, its content of tetracycline derivatives, peroxide, fatty acids, phenols etc.

The aim of this study was therefore to evaluate the chemical constituents and antimicrobial potential (bacteriostatic and bactericidal effect) of honey produced by honey bees (*A. mellifera*) on *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* strains isolated from wound, feces and vaginal swab of patients.

MATERIALS AND METHODS

Sample collection and mode of identification of pure/original honey

Honey samples were collected from harvesters at Nsukka, Enugu state Nigeria. The samples were confirmed to be honey by conducting several experiments, which included:

i) Dipping a match stick into the honey and striking it: the matches will burn if it is a pure honey and the honey will even act as a fuel while the match is burning.
ii) Dropping some of the sample onto sand: if it is a pure honey, it will not sink immediately.
iii) Dipping a finger into the honey and trying to drop one or two drops on the ground: if it is pure, it will go down like a thread without breaking.
iv) Pouring a small quantity of honey into a cup of water: if pure it will go down to the bottom of the cup without mixing up with the water except if stirred.

These entire tests were done to ascertain that the sample was pure and original honey was used. It was then filtered with a sterile mesh to remove debris and then stored in a sterile bottle before use.

Phytochemical analysis of honey produced by *A. mellifera*

The sample was screened for the following compounds: alkaloids, flavonoids, glycosides, phenols, saponins, tannins and reducing sugar using standard laboratory techniques (Harbonrne, 1992; Sofowara, 1993).

Confirmatory identification of test organisms

The test organisms used in this work were obtained from Glanson Medical Laboratory Awka, Anambra State. The organisms are: *E. coli, S. aureus, C. albicans.* The following biochemical tests were carried out to confirm the identity of the organisms: Gram stain, Catalase test, Oxidase test, Indole test and lactophenol test.

Antimicrobial screening of honey

The antimicrobial activity was tested using the agar well diffusion method (Chung et al., 1990). Nutrient agar was used to study antibacterial susceptibility while Sabouraud dextrose agar was used for antifungal susceptibility test. Twenty - four to 48 h broth cultures of the test organisms was diluted to 10^{-2}. One millilitre of the diluted culture was added to 100 ml of sterile molten Nutrient agar (40 to 45°C) and Sabouraud dextrose agar for the yeast in a 250 ml flask. The content was mixed very well and 20 ml of it poured into each Petri dish and allowed to solidify. A sterile cork borer (a metallic hollow cylinder) was used to create wells in the agar. The wells were aseptically filled with the honey sample using a dropping pipette and the plates incubated at 37°C for 24 h or at 25°C for 72 h for bacteria and fungi, respectively. Zones of inhibition were measured after incubation.

Determination of minimum inhibitory concentration (MIC)

The honey produced by honey bees (*A. mellifera*) was used to determine the MIC on the bacterial organisms in liquid culture. The MIC is the lowest concentration that is able to inhibit any visible bacterial growth on the culture tube (Prescott et al., 2008). The following concentrations of the honey sample; 1, 2.5, 7.5, 10, 15, 20 and 30%v/v corresponding to the following volumes; 0.1, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0 and 3.0 ml were made in test tube each containing 10 ml of sterile nutrient broth. In each test tube 0.5 ml was added at 24 h culture diluted to 10^{-5}. The tubes were examined for visible growth after 24 h incubation.

Determination of minimum bactericidal concentration (MBC)

MBC is the lowest concentration of the sample that prevents bacteria growth after incubation or required to kill the organism (Prescott et al., 2008). This was obtained by streaking out the samples from the MIC tubes that showed no visible growth on nutrient agar plates. MBC were indicated by failure of the organism to grow on the media plates after 24 h incubation.

RESULTS

Phytochemical analysis of honey produced by honeybees (*A. mellifera*)

The results of the phytochemical analysis show that the honey contains alkaloids, flavonoids, saponins, glycosides, and reducing sugar (Table 1). The intensity of colour change is a semi - quantitative measure of the amount of each chemical present in the sample and is represented in Table 1 by the number of plus signs (+). The results of antimicrobial screening are shown in Table 2.

The sample showed antibacterial activity against *S. aureus* and *E. coli* with zone of inhibition/clearing of 45 and 34 mm in diameter, respectively while there was no inhibition on *C. albicans*.

Minimal inhibitory concentration (MIC)

The minimal inhibitory concentration observed to be 20% (2 ml of the sample in 10 ml of broth) for both *S. aureus* and *E. coli*.

Table 1. Phytochemical analysis of apiary honey produced by *Apis mellifera.*

Parameter	Value (Inference)
Alkaloids	++
Flavonoids	+++
Saponins	+
Tannins	-
Phenols	-
Glycosides	+
Reducing sugar	++

+++, Highly present; ++, moderately present; +, slightly present; -, Nil. Antimicrobial screening of honey produced by honeybees (*Apis mellifera*).

Table 2. Zone of inhibition of the honey produced by *Apis mellifera* on the test organisms.

Test organism	Zone of inhibition in diameter (mm)
S. aureus	45
E. coli	34
C. albicans	nil

Table 3. Minimal bacterial concentration (MBC) of the honey (*Apis mellifera*) on the test organisms.

Concentration (%)	Test organism	
	S. aureus	***E. coli***
10	+	+
15	+	+
20	-	+
30	-	-

+, Growth; -, No growth

Minimal bacterial concentration (MBC)

The MBC for *S*. *aureus* was observed to be 20% while that of *E*. *coli* was 30%v/v of the sample (Table 3).

DISCUSSION

The result of the phytochemical analysis of honey as shown in Table 1 indicates that alkaloids, flavonoids, glycosides, saponins and reducing sugar are present in the honey sampled here. These classes of compounds are known to possess therapeutic properties against several pathogens and are therefore supporting its traditional use in curing diseases. Saponins detected in honey have been found to be an antibacterial substance on cell wall of many organisms (Harborne, 1992).

Flavonoids help in the healing of wounds and treatment of skin diseases due to their ability to neutralize the acidity of wounds, and inflammation. Plants containing alkaloids are used in the treatment of malaria, cold, and cough (Thomson, 1987). Treatment of heart diseases could be because of flavonoid, saponins and glycosides which stimulate heart, especially saponin that remain within gastrointestinal tract. Some interact directly with dietary cholesterol producing an insoluble complex which prevents the cholesterol from being absorbed. Dietary saponins reduce plasma cholesterol level in primate thus having the potential to lower the risk of coronary heart diseases in humans (Macrae et al*.,* 1993).

Results of the well diffusion test, reported in Table 2 showed that the honey sample has antibacterial activity against both Gram positive and Gram negative organisms but not against yeast cells. This is an indication that honey can be a potential treatment for diseases caused by *S*. *aureus* and *E*. *coli.* Similarly, previous work has shown that honey has been used to heal recalcitrant wounds whereby it was found to be effective *in vitro* against a wide range of multi-resistant organisms including methicillin resistant *S. aureus* (MRSA), vancomycin-resistant *Enterococci* (VRE) and multiresistant *Pseudomonas aeruginosa* (Cooper et al., 2002; George and Cutting, 2007). Our findings are also in agreement with the work of Nzeako and Hamdi (2000). Their study has shown that honey (*A. mellifera*) has an antimicrobial activity against *S. aureus* and *E*. *coli*. Another study by Kingsley (2000) also reported that honey completely inhibited major wound infectious pathogens such as *Staphylococcus pyogenes* and *S*. *aureus*. Similarly, a study by Mogessie Ashenafi (1994) reported that tazma mar honey produced by sting-less bees (*Apis mellipodae*) was found to be effective against some food-borne pathogens of humans, including *Staphylococcus typhimurum*, *Staphylococcus enteriticlis* and *E*. *coli*. The result of our study is consistent with the above studies. Furthermore, Rendel et al. (2001) demonstrated that acidification of wounds speeds healing; this being attributed to low pH increasing the amount of oxygen off load from hemoglobin in the capillaries. Actually, acidification prevents ammonia produced by bacteria metabolism from harming body tissues (Williams et al., 2009).

The honey used in this research did not inhibit the growth of *C. albicans*. This result does not agree with the work of Koc et al. (2009), who in their study demonstrated *in vitro* that honeys from different floral sources in Turkey had antifungal activity at high concentration of 80% v/v against 40 yeast species, including *C. albicans, Candida krusei, Candida. glabrata* and *Trichosoporon* spp. Cutaneous and superficial mycoses like ringworm and athletes foot are found to be responsive to honey (Bansal et al., 2005). This disagreement may be as a result of differences in the experimental conditions.

In this study, the minimum inhibitory concentration (MIC) was observed to be 20% for both *S*. *aureus* and *E*.

coli. In contrast to this report, Molan (1999) observed that honey produced by honeybees (*A. mellifera*) could inhibit most of the test organisms including *S*. *aureus* and *E*. *coli* at a very low concentration (2.5 to 7.5%v/v). Another study by Molan (2000) reported that the minimum inhibitory concentration (MIC) and the minimum bacteria concentration (MBC) for *E*. *coli* were found to be 7 and 10%, respectively. The variation in the antimicrobial potential of honey used in this present study as compared to the previous studies highlights that the source of the nectar may have contributed to the difference in the antimicrobial activities of honey; that is, the flowers from which bees gathered nectar to produce the honey, since flora source determines many of the attributes of honey; for example flavour, aroma, colour and composition of honey is highly variable as demonstrated by Mogessie (1994). The variation may also be attributed to differences in growth rate of pathogens, nutritional requirement, temperature, inoculums size and the test methods itself (Gaill and Jon, 1995).

The presence of antimicrobial substances as demonstrated by zone of inhibition showed distinctly the efficacy of apiary honey as a medicine for the treatment of ailments caused by *S*. *aureus* and *E*. *coli*.

Conclusion

This work has shown that apiary honey produced by *A. mellifera* has both bacteriostatic and bactericidal activity when tested. Moreover, the pharmacological, standardization and clinical evaluation on the effect of honey are essential before using it as a preventive and curative measure to common diseases related to the test organisms. Therefore, the antibacterial activity of honey produced by *A. mellifera* against clinical bacterial isolates indicates the usefulness of the honey in clinical practice against bacterial but not fungal (*C. albicans*) infections.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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