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Antimicrobial and Proximate Properties of Some Processed Honey in Ado-Ekiti

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Abstract: The antibacterial activity and proximate composition of five processed honey samples collected from different location in Ado-Ekiti were evaluated. The honey samples were diluted to concentration of 50%, 75% and 100% (w/v) for sensitivity test against *Staphylococcus aureus, Proteus* spp., *Escherichia coli, Pseudomonas* spp., *Salmonella* spp., *Shigella* spp., *Klebsiella* spp.and *Bacillus* spp. using agar well diffusion method. The proximate composition analysis of the samples was also determined using titrimetricmethod. Generally, the honey samples showed inhibitory potency at different concentrations,showing a range of 6-24mm at concentration of 50% (w/v), 7-28mm at concentration of 75% (w/v) and 0-30mm at concentration of 100% (w/v) on the agar well diffusion plates. The proximate values of honey samples showed that the ash content ranged from 0.05% to 0.79%, moisture content; 16.8% to 21.5%, nitrogen content; 0.21% to 0.54%, total acidity; 23.88 to 33.42meq/kg, hydrated lactose; 0.38 to 0.54% and pH; 4.31 to 4.43. The present study has however proven honey as a very promising topical antimicrobial agent against the infection caused by antibiotic resistant bacteria; as a further assurance of the potency of the honey processed and sold in Ado-Ekiti, Nigeria.

Key Words: Antibacterial, honey, proximate composition, Ado-Ekiti

Introduction

The widespread use of antibiotics both inside and outside of medicine is playing a significant role in the emergence of resistant bacteria, serving a growing threat to public health throughout the world (Goossens *et al*., 2005). Evolutionary pressure from the use of antibiotics has played a role in the existence of muilti-drug resistance varieties and the spread

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of resistance between bacterial species (Hawkey and Jones, 2009). The essentials of antimicrobial agents in reducing the global burden of infectious diseases however call for urgent need of alternative antimicrobial strategies. This situation therefore has led to re-evaluation of the therapeutic use of ancient remedy including extracts of different plants and biologically active compounds isolated from natural species used in herbal medicine "like Honey" (Basualdo *et al.,* 2007).

Honey is the natural sweet substance produced by honeybees from the nectar of blossoms or from the secretion of living parts or excretions of plant sucking insect on the living parts of plant, which honeybees collect, transform and combine with specific substance of their own, store and leave in the honey comb to ripen and mature (Codex, 2011). Although the use of honey as a traditional remedy for microbial infections dates back to ancient time (Dustman, 1989; Molan, 1992), but then had a limited use in medicine due to lack of scientific support (Ali *et al*., 1991). It has been rediscovered and it is gaining acceptance as an antibacterial treatment of topical infections relating to burns and skin ulcer (Abuharfeil, *et al*., 1999; Fakoor and Pipelzadez, 2007). It is also employed in wound therapy (Alvrez-suarez *et al*., 2010) and gastritis caused by enteropathogenic bacteria (Halawani, 2006). It is well established that honey inhibits a broad-spectrum of bacterial species as investigations have been conducted on some commercial honey in Nigeria. Internationally, honey (e.g. Manuka) has been demonstrated to be effective against several human pathogens including *Escherichia coli, Enterobacter aerogenes*, *Salmonella* t*yphimurium* and *Staphylococcus aureus*, (Lusby *et al.*, 2005; Visavadoa *et al*., 2006).

The antimicrobial activity of honey could be attributed to several factors like acidity and osmotic effect of honey, presence hydrogen peroxide in honey (Mundo *et al.*, 2004), phytochemical component and also the *in-vitro* antibacterial activity of honey in the induction of increase lymphocytes and phagocytes' activities (Halawani, 2006; Kwakman*et al*., 2010). Other investigators have identified flavonoid in honey, particularly caffeic acid and ferulic acid as the mostly likely contributors (Wahdan, 1998).

Several authors reported that different honey varies substantially in the potency of their antibacterial activity depending on their plant source (Wilkinson *et al*., 2005). It has further been reported that physical property along with geographical distribution and different floral source may play important role in the antimicrobial activities of honey (Taormina *et al*., 2001). Hence this study aims at examining the antimicrobial potency of processed honey sold in Ado-Ekiti, Ekiti-State, Nigeria.

Materials and methods

Study Area/ Collection of Samples

Honey samples were obtained from five different locations in Ado-Ekiti namely; Oasis supermarket (processed honey), Basiri (processed honey), Ilokun market, Adehun (processed honey) and State Hospital (comercial honey). Honey samples were collected aseptically using sterilized sampling bottles, labeled and preserved at 37^{o}C before used.

Preparation of Microbiological Media

All the media used for the antibacterial assay were prepared and sterilized according to manufacturer's instructions, using moist heating with the aid of autoclave which operates at a temperature of 121^{o}C for 15 min. The media used include Nutrient agar, Mueller Hilton agar and Peptone water.

Preparation of Inocula

The organisms used in this experimental study were obtained from the stock cultures in the Department Microbiology, Ekiti State University, Ado-Ekiti. The test organisms were S*taphylococcus aureus*, *Proteus* spp., *E.coli*, *Pseudomonas* spp.*, Salmonella* spp., *Shigella* spp., *Klebsiella* spp. and *Bacillus* spp. The organisms were grown and maintained from an original freeze-dried culture as described in the British standard and preserved on nutrient agar slants in a refrigerator at 4^{o}C after growth at 37^{o}C for 24 h.

The determination of the bactericidal and bacteriostatic activity of these honeys were carried out using suspension of each organism, in peptone water under aseptic condition and incubated at room temperature 37^{o}C for 3 hours.

Assay of Antibacterial Activity

Honeys were screened for total antibacterial activity against *S.aureus, Proteus* spp., *E.coli, Pseudomonas* spp. *Salmonella* spp., *Shigella* spp., *Klebsiella* spp. and *Bacillus* spp. each on different sterile plate containing molten nutrient agar using the agar well diffusion method according to Allen, (1999). Two loopfuls of each test organism were suspended in 5ml of peptone water and incubated at room temperature for 3 hours. The culutre was adjusted to equal the density of a Barium Sulphate ($BaSO_4$) (0.5 McFarland turbidity standards. After the turbidity was obtained, the suspension was introduced on to a prepared/set Mueller Hilton agar using the pour plate method (Cheesbrough, 2004). A sterile cork borer (6mm diameter) was used to make four wells in the agar. A 0.2ml amount of each of solution containing 50%, 75% and 100% (w/v) of honey sample was filled into each well using micro pipette and forth well was filled with distilled water serving as control and incubated at 37^{o}C for 24 hours. Antibacterial activity was assessed by measuring the diameter of the zone of clearance to the nearest whole millimeter.

Proximate Composition Analysis

The free lactose and total acidity of honey samples were determined by the titrimetric method using 0.05MNaOH and 0.05M HCl (AOAC, 1990). Ten grams of honey sample was dissolved in 75ml CO_2-free distilled water and the pH of the resulting solution was measured using a KENT EIL 7020 (Kent industrial measurement Limited, Surrey, England) pH-meter (Odeyemi *et al*., 2011). Moisture content was determined by drying 2.0g of honey sample at 70^{o}C to constant weight in hot air oven (AOAC, 1990).

Ash content was determined by drying 5.0g sample in porcelain crucible at 105^{o}C for 3 hours in hot air oven to prevent loss by foaming. The dried sample was then ashed in furnace at 600^{o}C to constant weight, cooled and weighed.

Results and discussions

The result of the evaluation of antimicrobial activity carried out on honey samples obtained from five different locations in Ado-Ekiti revealed that different concentration of the honey studied, exhibited various degree of activity against each of the test bacteria ranged from 6.00 – 24.0mm at concentration of 50% (w/v), 7.00 – 28.0mm at concentration of 75% (w/v) and 0.00 – 30.0mm at concentration of 100% (w/v) (Table1).

The agar well diffusion assay revealed that development of inhibition zones against microbial growth depends on the concentration of honey i.e. as honey concentration increases, the antibacterial effect of the honey samples on the organisms also increases (Badawy *et al.*, 2004). The inhibition zones observed against *Pseudomonas* spp., *Klebsiella* spp., *Bacillus* spp., *Salmonella* spp. and *Shigella* spp. showed proportionality with the increase in concentration of the honey samples from 50% to 100% (w/v). This is evident with a similar observation reported by Agbagwa *et al.*, (2010).

The present study also established the fact that, test type of organism determines the potency of honey as an antimicrobial agent (Table 1). The zone of inhibition observed against *S.aureus* at 100% concentration of honey 4 (Adehun honey) was significantly larger compare to other test honey samples at the same concentration. *Staphylococcus aureus* showed total resistance to honey 3 (Ilokun honey) at a concentration of 100% (w/v); indicating no antimicrobial effect of honey obtained from Ilokun on *S. aureus*. Whereas the zone of inhibition at 100% (w/v) of honey 3 and honey 5 (State Hospital honey) against *Proteus* spp. was significantly wide compared to other test honey samples. Likewise, a great susceptibility was observed in *Klebsiella* spp. each of the honey sample at 100% (w/v) showing highest inhibition zone for honey 3 (32mm), followed by honey 1 (30mm) and the least of 18mm for honey 4; in comparison to other test pathogens. This is in accordance with

previous reports of Adeleke *et al*. (2006); Basualdo *et al*., (2007) who emphasized on the bactericidal effect of honey to be dependent on the concentration and the nature of the bacteria to be tested.

The proximate properties of different samples of honey used for evaluation are depicted in Table 2 showing the ash content of the honey samples varied between 0.05 and 0.79%, which falls within the range reported for Nigerian honey samples from other locations by Agbagwa *et al.* (2010); Adeleke *et al*. (2006) and other countries by Jeffery and Echazarreta, (1996); Malika *et al*. (2005). The flora origin of

Tab. 1: In-vitro Susceptibility of the Extracts of Processed Honey.

Samples	Sampling location and brand	Concentration of honey (w/v)	Strains of bacterial for assay/zone of inhibition (mm)							
			Staphylococcus aureus	*Proteus* spp.	*Escherichia coli*	*Pseudomonas* spp.	*Salmonealla* spp.	*Shigella* spp.	*Klebsiella* spp.	*Bacillus* spp.
1	Oasis Honey	50%	9	13	7	10	15	14	24	18
2	Basiri Honey		8	15	6	6	10	18	18	24
3	Ilokun Honey		15	18	21	9	6	10	20	16
4	Adehun Honey		14	15	17	16	10	8	8	10
5	State Hospital Honey		6	20	21	7	14	6	16	14
1	Oasis Honey	75%	11	15	10	12	19	16	28	24
2	Basiri Honey		7	18	7	10	15	21	24	28
3	Ilokun Honey		13	16	22	12	10	12	26	18
4	Adehun Honey		18	20	19	15	16	13	12	16
5	State Hospital Honey		12	26	21	16	17	8	22	17
1	Oasis Honey	100%	13	19	14	14	21	18	30	28
2	Basiri Honey		9	15	10	16	18	25	28	32
3	Ilokun Honey		0	29	28	12	14	16	32	26
4	Adehun Honey		20	14	24	18	17	16	18	18
5	State Hospital Honey		10	28	26	19	22	14	26	20

Tab. 2: Proximate composition of processed honey.

Samples	Sampling location and brand	pH	Ash (%)	Nitrogen (%)	Acidity (meq/100g)	Moisture Content (%)	Lactose (%)
1	Oasis honey	4.40	0.79	0.54	33.42	21.52	0.38
2	Basiri honey	4.43	0.62	0.26	27.54	16.81	0.54
3	Ilokun honey	4.32	0.05	0.37	23.88	17.60	0.44
4	Adehun honey	4.35	0.31	0.33	25.64	17.25	0.42
5	State Hospital honey	4.31	0.22	0.48	31.56	18.63	0.46

honey has been reported responsible for the variability in ash content by Molan, (1992). The moisture content of the honey samples varied between 16.8 and 21.5% (Table 2), which conform tothe range reported for floral honeys by Badawy *et al.*, (2004).

Moisture content is practically the most important parameter that determines quality of honey, since it affects storage life and processing characteristic. The variations in the moisture content of honey have been attributed to the composition and floral origin of honey (Malika *et al*., 2005).The strong interaction of sugar in honey with water molecules may decrease the water available for microorg-anisms. The low moisture content of honey also forms an important part of the system which protects honey from being degraded by microorganisms.

The nitrogen content of the honey sample also varied between 0.26 and 0.54%. The nitrogen content of the samples fall within the range reported for Nigerian honeys (Jefrey and Echazarreta, 1996). The total acidity values were below the maximum limits (40meq/kg) set internationally for honey. The values obtained for total acidity falls within the range reported for Moroccan honey by Malika *et al*. (2005). The acidity of honey contributes to its activity against microorganisms. The values for free lactose are summarized in Table 2 showing a range between 0.38 and 0.54%. The pH values of the honey samples ranged from 4.31 and 4.43 correlate with the pH range of 3.2 and 4.5 of honey reported by White (1975) and have lower pH than the Nigerian honey that range from 4.31 – 6.0 as reported by Adeleke *et al*. (2006). Since acidic pH of honey is desirable; acidification has been shown to promote healing by causing oxygen release from hemoglobin (O'Grady *et al*., 2009). Meanwhile pH value of honey samples obtained in this study is low enough to prevent the growth of many species of bacteria.

Conclusion

The present study has also proven honey as a very promising topical antimicrobial agent

against infections caused by antibiotic resistant - bacteria and in the treatment of chronic wound infections that do not respond to antibiotic therapy, since microbial resistance to honey has never been reported. It also established the antibacterial activity of honey sold in Ado-Ekiti with different location against the bacterial strains studied. Furthermore, the low pH value exhibited by these honey samples is an advantage in preventing the growth of many species of bacteria. It is worthy to note that only Ilokun honey sample at full strength (100% w/v) showed no inhibitory activity against *S.aureus*. The activity of honey against the tested organism is however of great importance as they remain major threat to human health.

We can infer from this that, several processed honeys sold in some locations in Ado-Ekiti possessed antibacterial property, therefore, could be recommended for use in treating some bacterial infections. Moreover the exploration of antimicrobial activity of honey has gained the interest of several research workers; it would therefore be interesting to investigate the antibacterial activity of honey from other parts of Ekiti State and works are on-going in our laboratories on this aspect.

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