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The Influence of Starch of Ginger on the Antibacterial Activity of Honey of Different Types from Algeria against *Escherichia coli and Staphylococcus aureus*

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Abstract: The purpose of this study was to establish an additive starch action of ginger on the potential antibacterial activity of honey with respect to *Escherichia coli* and *Staphylococcus aureus* in vitro. Five varieties of honey of different botanical origin with addition of starch prepared from rhizomes of ginger with various concentrations were used. For each variety of honey the minimum inhibitory concentration (MIC) and minimum additive inhibitory concentration (MAIC) were determined by the agar diffusion method. The starch of the ginger which was the subject of the study showed a remarkable additive potential on the antibacterial effect of honey against the bacteria tested, where as no activity was noted when the starch is employed only. The MAIC for the five varieties of honeys tested ranged between 3 and 15% (vol/vol) and 2 and 14% (vol/vol) against *E. coli* and *S. aureus*, respectively. The MIC range for honey alone was 5-70% (vol/vol) and 5-40 % (vol/vol) against *E. coli* and *S. aureus*, respectively, Within sight of these results, it seems that the starch of ginger strongly decreases the MIC of honey, thus letting hope for a honey benefit and would constitute an alternative way against the resistance to bacteria.

Key words: Honey · Starch of Ginger · Potential antibacterial · Additive effect

INTRODUCTION

Staphylococcus aureus is an important pathogen both in community acquired and healthcare associated infections. The organism has successfully evolved numerous strategies for resisting the action of practically all antibiotics [1]. *Escherichia coli* is a widespread intestinal commensal organism found in human and animal, resulting from fecal contamination or contamination during animal slaughter. It is often found in soil, water and foods [2].

Since ancient times, honey has been known to possess antimicrobial properties, as well as wound-healing activity [3, 4]. Microbial resistance to honey has never been reported, which makes it a very promising topical antimicrobial agent. Indeed, the in *vitro* activity of honey against antibiotic-resistant bacteria [5, 6] and the reported successful application of honey in the treatment of chronic wound infections that were not responding to antibiotic therapy [7] have attracted considerable attention [8, 9]. Honey is a complex material but is primarily a saturated or super-saturated solution of sugars which largely consists of glucose and fructose (84%) and the high percentage of sugars makes it of high osmolarity. Although present in much lesser quantities than glucose and fructose, honey also contains other carbohydrates including disaccharides (sucrose and maltose) and oligosaccharides which seem to vary depending on the floral source of the honey [10]. Honey contains small amounts of different enzymes and the most important ones are diastase (α- and β-amylase), invertase (α-glucosidase), glucose-oxidase, catalase and acid phosphatase [11].

IT is suggested that amylases present in honey originating from bees and pollen are responsible for hydrolysis of starch chains to randomly produce dextrin

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and maltose that increase the osmotic effect of honey and consequently increase the antibacterial activity [12, 13]. Ginger (*Zingiber officinale Roscoe*) is widely used in cooking and phytotherapy because of its volatile oil and oleoresin. Nonetheless, ginger has a considerable amount of starch (up to 40%, dry basis) with potential applications [14]. The importance of starches is long recognized as they are an important source of energy and contribute to the structure and texture of foods [15]. This study was carried out to evaluate the antibacterial properties of honey and starch when used jointly to manage the bacterial diseases of origin.

MATERIALS AND METHODS

Honey and Plant Samples: From the 2010 harvest, five varieties of honeys of different botanical origin, namely: citrus (A), jujube (B), orange (C), multi floral (D) and *Eucalyptus* (E) were collected from hives located in western Algeria. All honeys were kept in glass vials, protected from light at temperature of 4°C.

Rhizomes of ginger purchased from a local market in Tiaret (Algeria) were washed with water, peeled, weighed, reduced to smaller pieces and properly ground using an electric grinder. Enough quantity of water was added to soak the material for 5 h and it was sieved with a clean muslin cloth. The ground mass was thoroughly washed with water onto the muslin cloth into a collecting vessel to release the starch granules embedded in the parenchyma cells. The content of the collecting vessel was then allowed to settle for 2 h and the yellowish supernatant was decanted. The whitish starch mixture was stirred with addition of water and allowed to stay for 2 h and the supernatant decanted. Series of redispersions and decanting were done to remove impurities. The settled starch was scrapped off and placed into white paper to dry in open air. The starch was then milled and weighed [16].

Determination of Antibacterial Activity: *Bacterial Strains*: Clinical isolates of *Escherichia coli* and *Staphylococcus aureus* were obtained from the stock culture of the Department of Microbiology, Institute of Science, Ibn-Khaldoun University, Algeria.

Preparation of Bacterial Inoculums: Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loop full of cells from each stock culture of *E. coli* and *S. aureus* to test tubes of nutrient agar medium and incubating without agitation for 24h at 37°C. The cultures were diluted with fresh nutrient agar broth to achieve optical densities corresponding to 2.0×10^6 colony forming units (CFU/ml) [17].

Minimum Inhibitory Concentration Measurement: Increased concentrations of honey (10-50 % vol/vol) were incorporated into nutritive agar media to test their efficiency against *Escherichia coli* and *Staphylococcus aureus*. Each plate with final volume of honey and media of 5 ml was inoculated and incubated at 37ºC for 24 h. The MIC was determined by finding the plates with the lowest concentration of honey on which the strain didnt grow [18]. All MIC values were expressed in % (vol/vol).

Minimum Additive Inhibitory Concentration Measurement: To evaluate the effect of starch on the antibacterial action of honey, a 1 % starch solution was prepared using sterile water. Different volumes from the stock solution were added to a range of honey concentrations lower than the MIC. The same volume of starch solution that has given inhibition with honey was added alone to media as control to check whether or not starch alone has an inhibition effect against *Escherichia coli* and *Staphylococcus aureus* [18]. An equivalent volume of water was added to honey instead of starch solution as a control to confirm that inhibition was not due to the dilution of honey. The final volume in each plate was 5 mL. Starch content in media ranged between 1 and 8% (w/vol). Honey and starch as well as honey and water were incubated for 24 h at 37ºC before being incorporated into media. Plates were inoculated and incubated at 37ºC for 24 h [18]. All inoculations were carried out in duplicates.

RESULTS

The results of our work indicated that the five varieties of honeys tested have a antibacterial property. The intensity of effect on the growth of germs, varied according to the botanical honey origin and the type of germ tested. As a whole, the five varieties presented antibacterial activities against *E. coli* at concentrations from 5 to 70% (vol/vol) and 5 to 40% (vo/vol) for *S. aureus* (Table 1) highest MIC was obtained with the honey of *Eucalyptus* 5% (vol/vol) while weakest MIC was obtained with the honey of citrus 70% (vol/vol). For the starch no effect was observed *in vitro* (Table 1).

Table 2: Minimum inhibitory concentration (MIC) and Minimum additive inhibitory concentration (MAIC) of tested honeys

Isolats	*E. coli*		*S. aureus*		Control	
	MIC % (v/v)	MAIC % (v/v)	MIC % (v/v)	MIAC % (v/v)	MIC %(w/v)	MIC %(w/v)
Sample	Honey only	Honey and Starch	Honey only	Honey and Starch	Starch only	Water and starch
Honey A	70	10: 4	40	10: 4	0	0
Honey B	30	15: 8	20	14: 8	0	0
Honey C	20	10: 4	10	5: 4	0	0
Honey D	10	5: 3	10	4: 2	0	0
Honey E	5	3: 2	5	2: 1	0	0

MIC: minimum inhibitory concentration

MAIC: minimum Additive inhibitory concentration

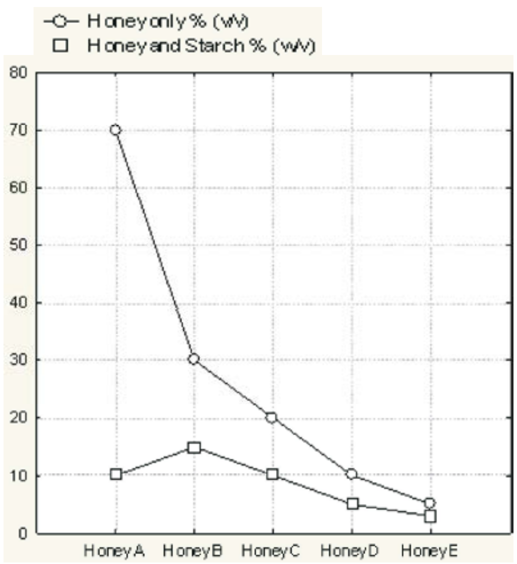

Fig. 1: Additive effect of starch of ginger against *E. coli*

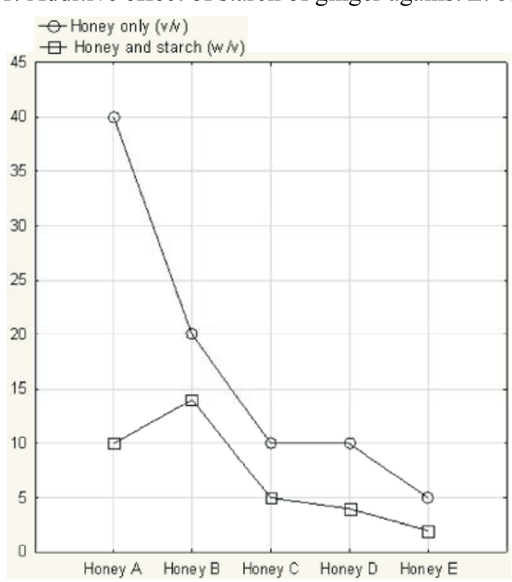

Fig. 2: Additive effect of starch of ginger against *S. aureus*

The additive effect between honey and starch of ginger as regards our five varieties of honeys studied against *S.aureus* showed that the MIC for the five varieties of honeys were by decreasing order of effect; 2% (vol/vol) &1% (w/v), 5% (vol/vol) & 4% (w/v), 10% (vol/vol) & 4% (w/v) and 14% (vol/vol) & 8% (w/vol) (Table 2). For *E. coli*: 3% (vol/vol) & 2%(w/v), 5% (vol/vol) & 3%(w/v), 10 %(vol/vol) & 4%(w/vol) and 15%(vol/vol) & 8%(w/v) (Table 1 & Figure 1, Figure 2).

DISCUSSION

Many work was interested, during this last decade, with the products of the hive and in particular honey, efficient product against the germs secreted by the bees as a possible source of new pharmaceutical and medical agent. According to the standard method [19] the gélose Mueller Hinton should be employed. In our case there would be likely to be phenomena artefactuels because honey contains amylases and the gélose Mueller Hinton contains the amidon [20] and the effects of amylases of honey on the starch would be likely to truncate the results because there is an additive effect between honey and the starch [21]. Honeys have long been recognized for their antimicrobial activity against bacteria, moulds and yeasts with unique properties that render it bacteriostatic and bactericidal. The high osmotic pressure, low water activity, low pH, low redox potential, hydrogen peroxide and other phytochemical factors might contribute to the honey antimicrobial nature[22]. Their relative importances depend on the sensitivity of the species and the level of additional factors in any honey [23, 24]. Using corn starch and three varieties of Algerian honeys Boukraa and Amara [25] have shown an additive effect of starch and honey against *Escherichia coli* and *Staphylococcus aureus* with a MIC drop ranging between 8% and 17,39% and 13,78% and 63,63% respectively. By using ginger starch in our study instead of corn starch used by Boukraa and Amara [25], we obtained better results, with a MIC drop ranging between 85, 14 and 36% for *E. coli* and 58% and 26% for *S. aureus*. But it must be mentionned that the honey varieties used by Boukraa and Amara [25] was different from ours. It seems then that ginger starch for a reason or another is more effective than corn starch, perhaps in regard to its lesser resistance to hydrolysis by amylases. In other hand, Torley et *al*. [30] reported that, honeys from different sources show a varied effect on starch gelatinization with starch viscosity increasing with addition level for some honeys, but decreasing with increasing addition level for other samples. Neither honey nor starch have adverse effects on tissues, so they can be safety used in wounds and inserted in cavities and sinuses to clear infection.

CONCLUSION

Our results confirm the economic interest of the addition of the starch to honey in order to apply it at the therapeutic level.

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