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Combined antibacterial activity of stingless bee (*Apis mellipodae*) honey and garlic (*Allium sativum*) extracts against standard and clinical pathogenic bacteria

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PEER REVIEW

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Comments

This is a very good finding in which the author investigated the synergistic antimicrobial activity of mixture of garlic extract and *Apis mellipodae* honey against pathogenic bacteria. The results are interesting that garlic in combination with honey can serve as an alternative natural antimicrobial drug for the treatment of pathogenic bacterial infections.

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ABSTRACT

Objective: To investigate the synergic antibacterial activity of garlic and tazma honey against standard and clinical pathogenic bacteria.

Methods: Antimicrobial activity of tazma honey, garlic and mixture of them against pathogenic bacteria were determined. Chloramphenicol and water were used as positive and negative controls, respectively. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration of antimicrobial samples were determined using standard methods.

Results: Inhibition zone of mixture of garlic and tazma honey against all tested pathogens was significantly ($P \leq 0.05$) greater than garlic and tazma honey alone. The diameter zone of inhibition ranged from (18±1) to (35±1) mm for mixture of garlic and tazma honey, (12±1) to (20±1) mm for tazma honey and (14±1) to (22±1) mm for garlic as compared with (10±1) to (30±1) mm for chloramphenicol. The combination of garlic and tazma honey (30–35 mm) was more significantly ($P \leq 0.05$) effective against *Salmonella* (NCTC 8385), *Staphylococcus aureus* (ATCC 25923), *Lysesria monocytogenes* (ATCC 19116) and *Streptococcus pneumonia* (ATCC 63). Results also showed considerable antimicrobial activity of garlic and tazma honey. MIC of mixture of garlic and tazma honey at 6.25% against total test bacteria was 88.9%. MIC of mixture of garlic and tazma honey at 6.25% against Gram positive and negative were 100% and 83.33%, respectively. The bactericidal activities of garlic, tazma honey, and mixture of garlic and tazma honey against all pathogenic bacteria at 6.25% concentration were 66.6%, 55.6% and 55.6%, respectively.

Conclusions: This finding strongly supports the claim of the local community to use the combination of tazma honey and garlic for the treatment of different pathogenic bacterial infections. Therefore, garlic in combination with tazma honey can serve as an alternative natural antimicrobial drug for the treatment of pathogenic bacterial infections. Further *in vivo* study is recommended to come up with a comprehensive conclusion.

KEYWORDS

Garlic, Honey, Antimicrobial activity, Bactericidal activity, Inhibition zone, Minimum inhibitory concentration, Minimum bactericidal concentration

1. Introduction

The use of traditional medicine to treat infection has been practiced since the origin of mankind and honey produced by honeybees (*Apis mellifera*) is one of the oldest traditional medicines considered as traditional remedy for microbial infections[1,2]. Antimicrobial action of honey depends on the presence of hydrogen peroxide which is

produced enzymatically in honey[3]. The glucose oxidase is secreted from the hypopharyngeal gland of the bee into the nectar to assist in the formation of honey from the nectar. The hydrogen peroxide and acidity produced by two reactions (glucose and oxygen chemically react to produce gluconic acid) has antimicrobial potential[3]. Another antimicrobial activity of honey also depends on osmolarity effect which inhibits bacteria. The high sugar

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concentration of honey ties-up water molecules, so that bacteria would have insufficient water to support their growth^[4]. The antimicrobial activity of honey is also related to its geographical region and flower from which the final product is derived^[5,6]. As a result, most of antimicrobial studies against pathogenic bacteria were made on honey produced by the honey bee^[7,8]. However, the scientific work on the honey produced by *Apis mellipodae* (*A. mellipodae*, stingless bee) is limited^[9,10].

In Ethiopia, honey produced by *A. mellipodae*, is considered to be important in traditional treatment of respiratory ailments, surface infections, diarrhea and various other diseases in line with treatments using honey^[4]. Ashenafi has evaluated the antibacterial effect of *A. mellipodae* honey on *Salmonella typhimurium*, *Salmonella enteritidis*, *Escherichia coli* (*E. coli*), *Bacillus cereus* and *Staphylococcus aureus* (*S. aureus*) at concentration of 10%, 15%, and 20% in brain heart infusion broth^[4]. According to this study, retarded growth and inhibition were noted at concentration of 15%, and 20%. The *A. mellipodae* honey, according to Ahenafi, may be effective to treat food born infections at low concentration^[4].

Garlic (*Allium sativum*) has a long history as a treatment for cold, cough and asthma, and is reported to strengthen the immune system^[11]. Moreover, garlic is widely used to treat alzheimer's disease, cancer, cardiovascular disease including atherosclerosis, strokes, hypertension, thrombosis and hyperlipidemias, infections, enhance children's conditions and dermatologic applications, as well as reduce stress^[12–14]. Generally, garlic has long been known to have antibacterial, antifungal, anticancer and antiviral properties^[13–17]. The main antimicrobial constituent of garlic has been identified as the oxygenated sulfur compound, thio-2-propene-1-sulfinic acid S-allyl ester, which is referred to as allicin^[18].

According to Omoya and Akharaiyi^[19,20], the mixture of honey and ginger showed high antimicrobial activity against pathogenic Gram positive and negative bacteria. But still there is not any scientific report about the synergic effect of honey and garlic extract. In Ethiopia, people use mixture of *A. mellipodae* honey and garlic to treat different types of diseases such as cold, cough, asthma, diarrhoea and respiratory infections. People use *A. mellipodae* honey and garlic in various combinations, there is not any scientific report about the synergic effect of these substances. Therefore, it is necessary to investigate synergic antimicrobial effect of *A. mellipodae* honey and garlic mixture. The aim of the present investigation is to study the synergic antibacterial effect of *A. mellipodae* honey and garlic mixture against standard and clinical pathogenic bacteria. The outcome of this investigation may be used as baseline to formulate antimicrobial substance from the combination of honey and garlic extract.

2. Materials and methods

2.1. Preparation of garlic extract

Matured fresh garlic bulbs were purchased from Gondar town market. The cleaned bulbs of garlic were peeled, weighed (55 g) and homogenized aseptically using a sterile

mortar and pestle. The homogenized bulbs were squeezed using sterile cheese cloth. Out of 100 g garlic bulb, 34.5 g juice which was considered as 100% in concentration was recovered^[21]. From the garlic 50%, 25%, 12.5% and 6.25% (v/v) of extracts were prepared by diluting the concentrated juice with appropriate volumes of sterile distilled water for determination of antimicrobial activity, minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). *A. mellipodae* honey was also diluted using the same procedure.

2.2. Source and dilution of honey

The *A. mellipodae* honey used in this study was obtained from South Gondar, Ethiopia. The *A. mellipodae* honey sample was first filtered with a sterile mesh to remove debris. Pure (100.0%) honey was referred to as 'neat'. It was then diluted with sterile distilled water to different concentrations: 12.5%, 25.0% and 50.0% (v/v).

2.3. Test pathogenic bacteria

All standard bacterial cultures, namely, *E. coli* (ATCC 25922), *Salmonella typhi* (clinical isolate) (*S. typhi*), *Listeria monocytogenes* (ATCC 19116) (*L. monocytogenes*), *S. aureus* (ATCC 25923), *Shigella flexneri* (ATCC 12022) (*S. flexneri*), *P. vulgaris* (ATCC 881) (*P. vulgaris*), *Salmonella* NCTC 8385 and *S. pneumonia* (ATCC 63) were obtained from Ethiopia Health and Nutrition Research Institute, while *Shigella dysenteriae* (*S. dysenteriae*) (clinical isolate) was obtained from University of Gondar Medical and Health Sciences Hospital. Most of them are commonly involved in causing gastroenteritis, pneumonia, wound and urinary tract infections.

2.4. Determination of antibacterial activity of honey and garlic extracts

McFarland standard No. 0.5 was prepared according to Andrews^[22]. Standard and clinical isolates of pathogenic bacteria were inoculated and spread over on Muller-Hinton (MH) agar and incubated for 24 h. 2–3 colonies were peaked up by wire loop aseptically into sterile saline solution and the turbidity was adjusted to the 0.5 McFarland's standard solution (a concentration of 1.5×10^8 CFU/mL)^[22]. Each MH agar plate was uniformly seeded by means of sterile swab dipped into the suspension and streaked on the agar plate surface, and the plates was left on the bench for excess fluid being absorbed. Using a sterile cork borer (6 mm diameter, 4 mm deep and about 2 cm apart) wells were made in the agar medium. Using a micropipette 100 μ L of sample with the concentration of 50% was added into the wells in the plate. The plates were incubated at 37 °C for 24 h. The mean diameters of inhibition zones were measured in mm and the results were recorded. The inhibition zones with diameter less than 12 mm were considered as having no antibacterial activity. A positive control well was equally filled with chloramphenicol (32 μ g/mL) while sterile, distilled water served as negative control.

2.5. Antibiotic sensitivity testing

The test microorganisms were also tested for their

antimicrobial activity against the commonly prescribed antibiotics using similar methods for the determination of antimicrobial activity of honey and garlic extracts.

2.6. Determination of MIC

The MIC of the extracts was determined according to methods described by Shahidi and Akinyemi *et al*[23,24]. Extracts were diluted to concentrations ranging from 6.25% to 25% for garlic, honey and a mixture of garlic with honey (1:1, v/v). To each dilution of honey, garlic extract and a mixture of both, in nutrient broth tubes were seeded 100 μ L of the standard and clinical bacterial inoculum. Negative control tubes with no bacterial inoculation were simultaneously maintained. Tubes were incubated aerobically at 37 °C for 24 h. The MIC was defined as the lowest concentration of garlic extract that completely inhibits the growth of the organism.

2.7. Determination of MBC

Dilutions showing no visible growth for the MIC was subcultured onto a fresh MH agar plate and incubated at 37 °C for 24 h. The lowest concentration of the extracts yielding no growth on the MH plate was recorded as MBC[20].

2.8. Data analysis

All data were analyzed using SPSS version 16.0. Means

Table 1

Comparison of inhibition zone among mixture of garlic extract and *A. mellipodae* honey, garlic extract and *A. mellipodae* honey (50%) against standard and clinical isolated pathogenic bacteria.

Source of antimicrobial agents	Sum of squares	Df	Mean	F square	Sig.
Mixture of garlic extract and <i>A. mellipodae</i> honey (equal volume) (50%)	696.000	8	87.000	87.000	0.00
Garlic extract (50%)	154.741	8	19.343	20.890	0.00
Tazma honey (50)	204.000	8	25.500	25.500	0.00
Chloramphenicol (31 μ g/mL)	1082.741	8	135.343	146.170	0.00

Df: Degree of freedom; Sig.: Significance.

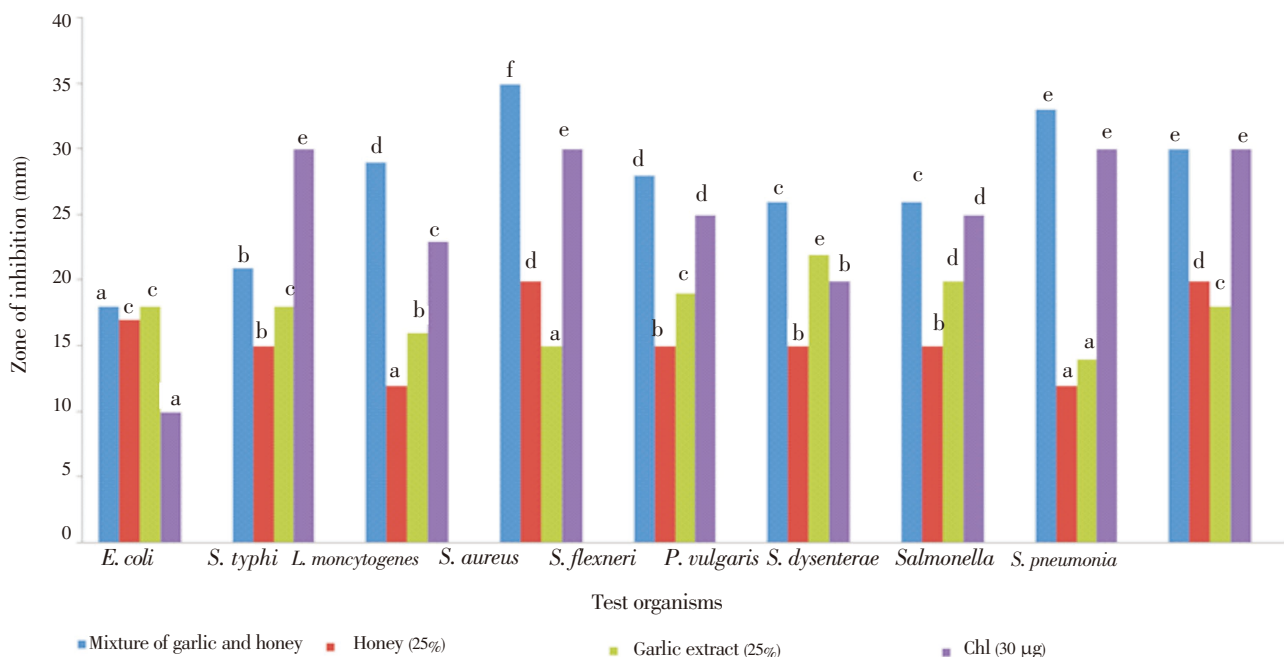


Figure 1. Antibacterial activity of different concentrations of garlic, hone and mixture of garlic and honey (v/v) by agar well diffusion method. Chl: Chloramphenicol. Values are means of triplicate determinations. Values of the same colour bars followed by different letters are significantly different ($P < 0.05$).

and standard deviations of the triplicate analysis were calculated using analysis of variance (ANOVA) to determine the significant differences in the means, followed by Duncan's Multiple range test ($P < 0.05$) when the F-test demonstrated significance. The statistically significant difference was defined as $P < 0.05$.

3. Results

Inhibition zone of mixture of garlic extract and *A. mellipodae* honey, garlic extract and *A. mellipodae* honey alone against standard and clinical pathogenic bacteria is presented in Table 1. Inhibition zone of mixture of garlic extract and *A. mellipodae* honey against all tested pathogens was significantly ($P < 0.05$) greater than that of garlic extract and *A. mellipodae* honey alone. Inhibition zone of *A. mellipodae* honey against all pathogenic bacteria was statistically ($P < 0.05$) greater than inhibition zone of garlic extract but less than that of garlic extract and tenegn honey mixture. Inhibition zone of chloramphenicol against all tested pathogenic bacteria was greater than that of garlic extract and tenegn honey mixture.

The inhibition zones of *A. mellipodae* honey and garlic extract mixture, garlic extract and *A. mellipodae* honey alone against standard and clinical pathogenic bacteria are presented in Figure 1. The inhibition zone of mixture of *A.*

mellipodae and garlic extract (30–35 mm) against *Salmonella* NCTC 8385, *S. aureus* (ATCC 25923) and *S. pneumonia* (ATCC 63) were significantly ($P \leq 0.05$) greater than the rest test pathogenic bacteria. The diameter of inhibition zone ranged from (18±1) mm to (35±1) mm for mixture of garlic extract and *A. mellipodae* honey, (12 ±1) mm to (20±1) mm for *A. mellipodae* honey and (14±1) mm to 22±1) mm for garlic extract as compared with (10±1) mm to (30±1) mm for chloramphenicol at the various concentration used. The inhibition zone of mixture of tazema honey and garlic extract against *E. coli* (ATCC 25922) was statistically ($P \leq 0.05$) less than the other tested pathogens. The inhibition zone of mixture of tazema honey and garlic extract against *S. flexneri* (ATCC 12022), *L. monocytogenes* (ATCC 19116), *S. pneumonia* (ATCC 63) were significantly ($P \leq 0.05$) greater than *S. dysenterae* (clinical isolate), *P. vulgaris* (ATCC 881), *E. coli* (ATCC 25922), *S. typhi* (clinical isolate) and less than the rest pathogenic bacteria.

The inhibition zone of *A. mellipodae* honey (17–20 mm) against *S. aureus* (ATCC 25923), *S. pneumonia* (ATCC 63) and *E. coli* (ATCC 25922) were significantly ($P \leq 0.05$) greater than the rest pathogenic bacteria. The inhibition zone of tazema honey to *S. typhi* (clinical isolate), *S. flexneri* (ATCC 12022) and *P. vulgaris* (ATCC 881) were greater ($P \leq 0.05$) than *L. monocytogenes* (ATCC 19116) and *Salmonella* NCTC 8385.

The inhibition zone of garlic extract (20–22 mm) against *P. vulgaris* (ATCC 881) and *S. dysenterae* (clinical isolate) were statistically ($P \leq 0.05$) greater than the rest tested pathogenic bacteria, while the inhibition zone of garlic extract against *Salmonella* NCTC 8385 and *S. aureus* (ATCC 25923) were significantly ($P \leq 0.05$) less than the rest pathogenic bacteria. The inhibition zone of chloramphenicol to *S. flexneri* (ATCC 12022), *S. dysenterae* (clinical isolate), *S. typhi* (clinical isolate), *Salmonella* NCTC 8385, *S. pneumonia* (ATCC 63) and *S. aureus* (ATCC 25923) were statistically greater than the rest pathogenic bacteria. Results of this study also showed a considerable antimicrobial activity of garlic and *A. mellipodae* honey.

The diameter of the inhibition zones against commonly prescribed antibiotics are shown in Table 2. The mean inhibition zone of mixture of garlic extract and *A. mellipodae* honey (27.33 mm) was greater than the mean inhibition zone of commercially used antibiotics (24.03 mm) against tested pathogens. Those commercial antibiotics are widely used to treat the infections caused by both gram negative and gram positive bacteria. The mixture of garlic extract and *A. mellipodae* honey may serve as a new choice of antibacterial agents against commonly encountered pathogens.

MIC of different concentration of garlic, *A. mellipodae* and mixture of garlic and *A. mellipodae* honey (v/v) are shown in Table 3. MIC of mixture of garlic extract and *A. mellipodae* honey at 6.25% against total test bacteria was 88.9%. MIC of mixture of garlic extract and *A. mellipodae* honey at 6.25% against Gram positive and negative were 100% and 83.33%, respectively. MIC of mixture of garlic extract and *A. mellipodae* honey against *L. monocytogenes* (ATCC 19116)

was 12.50%, but in the rest pathogenic bacteria, the MIC was reduced by half (6.25%). On the other hand, MIC of *A. mellipodae* honey against *E. coli* (ATCC 25922), *Salmonella* NCTC 8385, *L. monocytogenes* (ATCC 19116) and *S. dysenterae* (clinical isolate) were 12.50%, but in the rest tested pathogenic bacteria it was 6.25%. MIC of garlic extract against all pathogenic bacteria was 6.25%.

Table 2

Antibiogram of commonly used commercial antibiotics against the test bacteria.

Test bacteria	Antibiotics (µg)	Maximum zone of inhibition (mm)
<i>E. coli</i> (ATCC 25922)	C30	10
	SXT	16
	Na	22
<i>S. pneumonia</i> (ATCC 63)	C30	30
	SXT	30
	Na	24
<i>L. monocytogenes</i> (ATCC 19116)	C30	23
	SXT	24
	Na	28
<i>S. flexneri</i> (ATCC 12022)	C30	25
	SXT	27
	Na	28
<i>Salmonella</i> NCTC 8385	C30	30
	CN10	30
	E15	10
	SXT	18
	Na	26
<i>S. aureus</i> (ATCC 25923)	C30	32
	CN10	30
	E15	30
	SXT	20
	Na	22
<i>S. dysenterae</i> (clinical isolate)	C30	25
	CN10	30
	SXT	10
	Na	20
	E15	15
<i>P. vulgaris</i> (ATCC 881)	C30	20
	CN10	28
	E15	30
<i>S. dysenterae</i> (clinical isolate)	C30	24
	CN10	24
	E15	32
	SXT	25
	Na	23
The mean inhibition zone	Total antibiotics	24.03

C: Chloramphenicol, CN: Cephalixin, E: Erythromycin, SXT: Trimethoprim-sulfamethoxazole, Na: Nalidixic acid.

MBC of different concentrations of garlic, *A. mellipodae* and mixture of garlic and *A. mellipodae* honey (v/v) was presented in Table 4. The bactericidal activity of garlic extract, *A. mellipodae* honey, and mixture of garlic extract and *A. mellipodae* honey against all tested pathogenic bacteria at 6.25% concentration were 66.6%, 55.6% and 55.6%, respectively. MBC of mixture of garlic extract and *A.*

Table 3

MIC determination of different concentrations of garlic, hone and mixture of garlic and honey.

Test organisms	MIC (% of dilution)		
	Mixture of honey and garlic	Honey	Garlic extract
<i>E. coli</i> (ATCC 25922)	6.25	12.50	6.25
<i>Salmonella</i> NCTC 8385	6.25	12.50	6.25
<i>L. monocytogenes</i> (ATCC 19116)	12.50	12.50	12.50
<i>S. aureus</i> (ATCC 25923)	6.25	6.25	6.25
<i>S. flexneri</i> (ATCC 12022)	6.25	6.25	6.25
<i>P. vulgaris</i> (ATCC 881)	6.25	6.25	6.25
<i>S. dysenteriae</i> (clinical isolate)	6.25	12.50	6.25
<i>S. typhi</i> (clinical isolate)	6.25	6.25	6.25
<i>S. pneumonia</i> (ATCC 63)	6.25	6.25	6.25
Total test bacteria at 6.25% concentration	88.90	55.60	88.90
Total test bacteria at 12.5% concentration	11.10	44.40	11.10
Total Gram positive bacteria at 6.25% concentration	100.00	100.00	100.00
Total Gram negative bacteria at 6.25% concentration	83.33	50.00	83.33

mellipodae honey against *E. coli* (ATCC 25922), *Salmonella* NCTC 8385, *L. monocytogenes* (ATCC 19116) and *S. aureus* (ATCC 25923) was 12.50%, while against the rest pathogenic bacteria it was 6.25%. On the other hand, MBC of *A. mellipodae* honey against *E. coli* (ATCC 25922), *L. monocytogenes* (ATCC 19116), *P. vulgaris* (ATCC 881) and *S. dysenteriae* (clinical isolate) was 12.50%, while against the rest pathogenic tested bacteria, it was 6.25%. MBC of garlic extract against *Salmonella* NCTC 8385, *L. monocytogenes* (ATCC 19116) and *S. aureus* (ATCC 25923) was 12.5% but against the rest pathogenic bacteria was 6.25%. MBC of garlic extract, *A. mellipodae* honey and combination of garlic extract and *A. mellipodae* honey against *L. monocytogenes* (ATCC 19116) was 12.50%. Therefore, all those antimicrobial agents under this investigation were relatively not efficient to kill such tested pathogenic bacteria in low concentration.

Table 4

MBC determination of different concentrations of garlic, honey and mixture of garlic and honey.

Test organisms	MBC (% of dilution)		
	Mixture of honey and garlic	Honey	Garlic extract
<i>E. coli</i> (ATCC 25922)	12.50	12.50	6.25
<i>Salmonella</i> NCTC 8385	12.50	6.25	12.50
<i>L. monocytogenes</i> (ATCC 19116)	12.50	12.50	12.50
<i>S. aureus</i> (ATCC 25923)	12.50	6.25	12.50
<i>S. flexneri</i> (ATCC 12022)	6.25	6.25	6.25
<i>P. vulgaris</i> (ATCC 881)	6.25	12.50	6.25
<i>S. dysenteriae</i> (clinical isolate)	6.25	12.50	6.25
<i>S. typhi</i> (clinical isolate)	6.25	6.25	6.25
<i>S. pneumonia</i> (ATCC 63)	6.25	6.25	6.25
Total test bacteria at 6.25% concentration	55.60	55.60	66.60
Total test bacteria at 12.5% concentration	44.40	44.40	33.33

4. Discussion

The synergic antimicrobial effect of garlic extract and *A.*

mellipodae honey mixture against all pathogenic bacteria was found to be greater ($P \leq 0.05$) than other antimicrobial agents (garlic extract and *A. mellipodae* honey alone). That may be the reason why the local society or community widely use the mixture of garlic and *A. mellipodae* honey to treat different pathogenic bacterial infections. The traditional knowledge about the treatment of infectious disease using natural products or substances should be investigated using scientific methods. The mean inhibition zone of mixture of garlic extract and *A. mellipodae* honey (27.33 mm) was greater than the mean inhibition zone (24.03 mm) of commercially used antibiotics against tested pathogens. It may be the basic reason why the local society or community widely preferred to use the mixture of garlic and *A. mellipodae* honey to treat different pathogenic bacterial infections. Therefore, mixture of garlic extract and *A. mellipodae* honey may be effective to treat both gram positive and negative bacterial infections at low concentrations. According to this study, mixture of garlic extract and *A. mellipodae* honey has larger inhibition zone against tested pathogenic bacteria than garlic extract and *A. mellipodae* honey. Although mixture of garlic extract and *A. mellipodae* honey has high antimicrobial activity, garlic extract and *A. mellipodae* honey alone also have good potential of a broad spectrum of activity against both Gram-positive and Gram-negative bacteria.

In this study, aqueous extract of garlic inhibited the growth of enteric pathogens such as *E. coli*, *S. typhi*, *S. flexneri* and other pathogens at low concentration which is in line with the report of Shobana *et al.* and Sadeghian and Ghazvini^[25–27]. All tested pathogens were also sensitive to *A. mellipodae* honey. However, the mixture of garlic extract and *A. mellipodae* honey was more effective against test pathogens than garlic extract and *A. mellipodae* honey separately. Garlic in combination with *A. mellipodae* honey can serve as alternative natural antimicrobial drug for the treatment of pathogenic bacterial infections.

As it is clearly presented and shown in the data for determination of MIC and MBC, synergic antimicrobial action

of garlic and *A. mellipodae* honey mixture may attribute to allicin in garlic, and osmolarity, acidity, hydrogen peroxide generation and phytochemical components considered in honey, as reported by Molan^[28,29]. Allicin is identified as the oxygenated sulfur compound, thio-2-propene-1-sulfinic acid S-allyl ester, which is generally referred to as allicin^[18]. The main antimicrobial effect of allicin is due to its chemical reaction with thiol groups of various enzymes, *e.g.* alcohol dehydrogenase, thioredoxin reductase, and RNA polymerase, which can affect essential metabolism of cysteine proteinase activity involved in the virulence of *E. histolytica*^[30]. In brief, allicin interferes with RNA production and lipid synthesis. If RNA cannot be produced, or produced in less amount then protein synthesis will be severely affected. In short, no more protein synthesis takes place without the action of mRNA, rRNA and tRNA. Allicin also affects lipid synthesis and as a result phospholipid bilayer of the cell wall cannot be formed correctly in both gram positive and gram negative bacteria. All these contribute a lot to impact on the growth of bacteria with the presence of allicin or AGE^[21].

The antimicrobial role of honey is attributed to its high osmolarity, acidity (low pH) and content of hydrogen peroxide (H₂O₂) and non-peroxide components, *i.e.*, the presence of phytochemical components like methylglyoxal^[31–33]. The antimicrobial agents in honey are predominantly hydrogen peroxide, of which the concentration is determined by relative levels of glucose oxidase, synthesized by the bee and catalase originating from flower pollen^[32]. The hydrogen peroxide and acidity produced by the reaction of glucose and oxygen (glucose + O₂ → gluconic acid + H₂O₂) in honey may contribute a lot to inhibitory and bactericidal activity against pathogenic bacteria.

The cumulative effect of allicin in garlic and high osmolarity, acidity (low pH), content of hydrogen peroxide (H₂O₂) as well as the presence of phytochemical components like methylglyoxa in honey may play a great role in the inhibitory and bactericidal activity against pathogenic bacteria. This finding strongly supports the claim of the local community to use the combination of *A. mellipodae* honey and garlic for the treatment of different pathogenic bacterial infections. This paves a way to consider and acknowledge the traditional knowledge for the treatment of infectious diseases using natural resources like honey and garlic.

Conflict of interest statement

I declare that I have no conflict of interest.

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I am thankful to University of Gondar for financial assistant (UOG/Budget/no.6215) for the successful completion of research work. I also would like to express my appreciation

to the Department of Biotechnology for the facilities provided during this study. I would like to thank Muluken Dejen for her contribution to purchase *A. mellipodae* honey from South Gondar.

Comments

Background

In Ethiopia, people use *A. mellipodae* honey and garlic mixture to treat different types of diseases such as cold, cough, asthma, diarrhoea and respiratory infections. But still there is no any scientific report about the synergic effect of any type of honey and garlic extract. People use *A. mellipodae* honey and garlic in various combinations, there is no any scientific report about the synergic effect of these substances. Therefore, there is a need to investigate synergic antimicrobial effect of *A. mellipodae* honey and garlic mixture.

Research frontiers

This finding strongly supports the claim of the local community to use the combination of *A. mellipodae* honey and garlic for the treatment of different pathogenic bacterial infections. So, garlic in combination with *A. mellipodae* honey can serve as alternative natural antimicrobial drug for the treatment of pathogenic bacterial infections. Further *in vivo* study is recommended to come up with a comprehensive conclusion.

Related reports

There are different reports on the separate issues of antimicrobial effects on honey and garlic. However, a report on the synergistic effect of honey and garlic is scarce. This finding fills this research gap and may help base information for further *in vivo* research.

Innovations and breakthroughs

The finding of the study paves a way to consider and acknowledge the traditional knowledge for the treatment of infectious diseases using natural resources like honey and garlic.

Applications

Garlic in combination with *A. mellipodae* honey can be used as antimicrobial agent to different pathogenic bacteria. As recommended by the author it needs further validation and then it would be important for the community as it is routinely used as food.

Peer review

This is a very good finding in which the author investigated the synergistic antimicrobial activity of mixture of garlic extract and *A. mellipodae* honey against pathogenic bacteria. The results are interesting that garlic in combination

with *A. mellipodae* honey can serve as alternative natural antimicrobial drug for the treatment of pathogenic bacterial infections.

References

- [1] Mandal MD, Mandal S. Honey: its medicinal property and antibacterial activity. *Asian Pac J Trop Biomed* 2011; **1**(2): 154–160.
- [2] Namita P, Mukesh R. Medicinal plants used as antimicrobial agents: A review. *Int Res J Pharm* 2012; **3**(1): 31–40.
- [3] Patel RV, Thaker VT, Patel VK, Shukla P, Bhatnagar P, Patel A. *In vitro* study of changing antibiotic sensitivity and resistance by honey on gingival inflammation during orthodontic treatment—a preliminary report. *Orthodontic Cyber J* 2010. [Online] Available from: <http://orthocj.com/2010/06/in-vitro-study-antibiotic-sensitivity-resistance-honey/>. [Assessed on 2 April, 2013].
- [4] Efem SEE, Udoh KT, Iwara CI. The antimicrobial spectrum of honey and its clinical significance. *Infect* 1992; **20**(4): 227–229.
- [5] Taghizadeh M, Saffari M, Pourbabaei M, Mahboubi M. Antimicrobial activity of different honey samples against *Pseudomonas aeruginosa* *in vitro*. *Biharean Biol* 2011; **5**(2): 113–115.
- [6] Omoya FO, Akharaiyi FC. A pasture honey trial for antibacterial potency on some selected pathogenic bacteria. *J Nat Prod* 2010; **3**: 5–11.
- [7] Sherlock O, Dolan A, Athman R, Power A, Gethin G, Cowman S, et al. Comparison of the antimicrobial activity of Ulmo honey from Chile and Manuka honey against methicillin-resistant *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. *BMC Complement Altern Med* 2010; **10**: 47.
- [8] Chute RK, Deogade NG, Kawale M. Antimicrobial activity of Indian honey against clinical isolates. *Asiatic J Biotech Res* 2010; **1**: 35–38.
- [9] Ashenafi M. The *in vitro* antibacterial activity of “Tazma Mare” honey produced by stingless bee (*Apis mellipodae*). *Ethiop J Health Dev* 1994; **8**(2): 109–117.
- [10] Temaru E, Shimura S, Amano K, Karasama T. Antimicrobial activity of honey from stingless honeybees (Hymenoptera; Apidae; Meliponinae). *Pol J Microbiol* 2007; **56**(4): 281–285.
- [11] Borek C. Antioxidant health effects of aged garlic extract. *J Nutr* 2001; **131**(3): 1010–1015.
- [12] Bongiorno PB, Fratellone PM, LoGiudice P. Potential health benefits of garlic (*Allium Sativum*): A narrative review. *J Complem Integr Med* 2008; **5**(1): 1–24.
- [13] Ekwene UN, Elegalan NN. Antibacterial activity of ginger and garlic extracts on *Escherichia coli* and *Salmonella typhi*. *Int J Mol Med Adv Sci* 2005; **1**(4): 411–416.
- [14] Cellini LE, Campli Di, Masulli M, Di Bartolomeo S, Allocati N. Inhibition of *Helicobacter pylori* by garlic extract (*Allium sativum*). *FEMS Immunol Med Microbiol* 1996; **13**(4): 273–277.
- [15] Yoshida S, Kasuga S, Hayashi N, Ushiroguchi T, Matsuura H, Nakagawa S. Antifungal activity of ajoene derived from garlic. *Appl Environ Microbiol* 1987; **53**(3): 615–617.
- [16] Pan XY, Lif Q, Yu RJ, Wang H. [Comparison of the cytotoxic effects of fresh garlic diallyltrisulphide, 5-lurouracil, mitomycin and cis-DDP on two lines of gastric cancer cells]. *Zhonghua Zhong Liu Za Zhi* 1985; **7**(2): 103–105. Chinese.
- [17] Block E. The chemistry of garlic and onions. *Sci Am* 1985; **252**(3): 114–119.
- [18] Cavallito CJ, Bailey JH. Allicin, the antibacterial principle of *Allium sativum*. isolation, physical properties and antibacterial action. *J Am Chem Soc* 1944; **66**(11): 1950–1951.
- [19] Omoya FO, Akharaiyi FC. Mixture of honey and ginger extract for antibacterial assessment on some clinical isolates. *Int Res J Pharm* 2012; **2**(5): 127–132.
- [20] Patel RV, Thaker VT, Patel VK. Antimicrobial activity of ginger and honey on isolates of extracted carious teeth during orthodontic treatment. *Asian Pac J Trop Biomed* 2011; **1**: S58–S61.
- [21] Durairaj S, Srinivasan S, Lakshmanaperumalsamy P. *In vitro* antibacterial activity and stability of garlic extract at different pH and temperature. *Electr J Biol* 2009; **5**(1): 5–10.
- [22] Andrews JM. BSAC standard disc susceptibility testing method (version 5). *J Antimicrob Chemother* 2006; **58**(3): 511–529.
- [23] Shahidi GA. Evaluation of antimicrobial properties of Iranian medicinal plants against *Micrococcus luteus*, *Serratia marcescens*, *Klebsiella pneumonia* and *Bordetella bronchiseptica*. *Asian J Plant Sci* 2004; **3**(1): 82–86.
- [24] Akinyemi KO, Oladapo O, Okwara CE, Ibe CC, Fasura KA. Screening of crude extracts of six medicinal plants used in Southwest Nigerian orthodox medicine for anti-methicillin resistant *Staphylococcus aureus* activity. *BMC Complement Altern Med* 2005; **5**: 6.
- [25] Shobana S, Vidhya VG, Ramya M. Antibacterial activity of garlic varieties (ophioscordon and sativum) on enteric pathogens. *Curr Res J Biol Sci* 2009; **1**(3): 123–126.
- [26] Sadeghian A, Ghazvini K. Antimicrobial activity of garlic extract against *Shigella*. *Iran J Med Sci* 2002; **27**(3): 142–144.
- [27] Kadir Batcioglu K, Kargin FO, Satilmis B, Gul M, UyumLu AB, Buyuktuncel E, et al. Comparison of *in vivo* chemoprotective and *in vitro* antimicrobial activity of different garlic (*Allium sativum*) preparations. *J Med Plants Res* 2012; **6**(14): 2885–2894.
- [28] Molan PC. The antibacterial activity of honey: 1. The nature of the antibacterial activity. *Bee World* 1992; **73**: 5–28.
- [29] Goncagul G, Ayaz E. Antimicrobial effect of garlic (*Allium sativum*) and traditional medicine. *J Anim Vet Adv* 2010; **9**(1): 1–4.
- [30] Ancri S. Antimicrobial properties of allicin from garlic. *Microbes Infect* 1999; **2**: 125–129.
- [31] Weston RJ. The contribution of catalase and other natural products to the antibacterial activity of honey: a review. *Food Chem* 2000; **71**: 235–239.
- [32] Mavric E, Wittmann S, Barth G, Henle T. Identification and quantification of methylglyoxal as the dominant antibacterial constituent of manuka (*Leptospermum scoparium*) honeys from New Zealand. *Mol Nutr Food Res* 2008; **52**(4): 483–489.
- [33] Mukherjee S, Chaki S, Das S, Sen S, Dutta SK, Dastida SG. Distinct synergistic action of piperacillin and methylglyoxal against *Pseudomonas aeruginosa*. *Indian J Exp Biol* 2011; **49**(7): 547–551.