Journal of Pharmacovigilance
ISSN: 2329-6887

Alemseged et al., J Pharmacovigil 2018, 6:4
DOI: 10.4172/2329-6887.1000267

Research Article Open Access

Antibacterial Properties of Mixture Honey and Garlic (*Allium Sativum*) Extracts Against Respiratory Tract Infection Causing Bacteria

Muley Alemseged[1*], Samuel Adugna[1] and Ejigu Bayu[2]

[1]Department of Biology, College of Natural and Computational Science, Wolkite University PO Box 07, Wolkite, Ethiopia
[2]Department of Chemistry, College of Natural and Computational Sciences, Wolkite University, PO Box 07, Wolkite, Ethiopia

Abstract

In Ethiopia, different local communities use mixture of honey and garlic to treat coughing and other respiratory tract infections by traditional methods. Objective of the present study was to assess the antibacterial properties of mixture of honey and garlic extract against respiratory tract infection causing bacteria. The antibacterial activity of mixture of garlic extract and honey was evaluated by methods such as agar well diffusion method to observe zone of inhibition around the well as well as broth dilution method to evaluate the minimum inhibitory concentration of the extracts against the tested organisms. Five respiratory tract infection causing bacteria such as *Pseudomonas aeruginosa, Streptococcus pneumoniae, Klebsiella pneumoniae, Haemophilus influenza* and *Staphylococcus aureus* were included in the study. Chloramphenicol was used as standard antibiotics. Mean inhibition zones of mixture of garlic extract and honey was significantly (P 0.05) greater than honey alone against the tested pathogens. The range of zone of inhibitions of mixture of garlic and honey against the tested bacteria was between 25 to 31 mm whereas the range of inhibition zone of chloramphenicol was from 9 to 30 mm. The inhibition capacity of mixture of garlic extract and honey was greater than the commercial antibiotics such as Co-trimoxazole, Cefoxitin and Erythromycin. Finally it may be possible to conclude that, mixture garlic extract and honey has the capacity to treat respiratory tract infection causing bacteria.

Keywords: Garlic; Honey; Respiratory tract infection; Extracts; Antibacterials

Abbreviations: RIT: Respiratory Tract Infection; MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration

Introduction

In many developing countries, respiratory tract infection (RTI) is considered as one of the major public health problems leading to morbidity and mortality [1]. Over 50 million deaths recorded globally each year due to RIT which occurs in community as well as health care settings [2]. According to the WHO [3] Global Burden of Disease Study report , in Ethiopia, lower respiratory tract infections is one of the top three causes of disability-adjusted life years (DALYs).

The most common bacteria which cause respiratory tract infection are *Pseudomonas* spp., *Streptococcus* spp., *Proteus* spp., *Klebsiella* spp., *Staphylococcus* spp., *Enterobacter* spp., *Acinetobacter* spp., and *Haemophilus influenza* [4]. These above listed bacteria are frequently resistant to commonly used antibiotics like ampicillin, amoxicillin and kanamycin [5]. Due to this fact, recently scientists are giving more attention to the extraction active biological compounds from natural species used in herbal medicine [6].

Honey is a natural product which has been used by different cultures for its medicinal value [7]. It has been used as an antibacterial agent to treat ulcers, bed sores, skin infections, wounds [8] and recently it used to treat respiratory tract infections [9].

Garlic has been used to combat infectious disease by various societies throughout the world [10]. It has a great antibacterial capacity against both Gram-positive and Gram-negative bacteria [11,12]. It was also effective against antibiotics resistance as well as toxin producing bacterial strains [11]. Its extracts also show maximum inhibitory activities against different species that causes pneumonia [13].

In Ethiopia, honey and garlic are used extensively as a foods and traditional medicines. So, it is desirable to determine their antimicrobial activities. Different local communities use mixture of honey and garlic traditionally to treat coughing and other respiratory tract infection. So, based on the traditional knowledge and practice of communities, this study was design to assess the antimicrobial potentials of honeys and garlic's against respiratory tract infection causing pathogens.

Materials and Methods

This study was conducted at Wolkite University, Wolkite, SNNPR, Ethiopia.

Collection and extraction of honey

Raw wild honey samples (1 kg) were collected from the nests using sterile syringes. Samples of honey were filtered aseptically with a sterile mesh to remove debris and the filtered extracts were stored in dark bottles. This was considered as pure (100.0%) honey. The filtered extracts were then diluted with sterile distilled water with the following concentrations; 50%, 25%, 12.5% and 6.25% (v/v) [14].

Collection and preparation of garlic extract

Garlic (*Allium sativum*) bulbs were purchased from the market (Wolkite, Ethiopia). After that, the garlic bulbs was peeled, 100 g was weighed, and aseptically homogenized using a juicer. Then, using sterile

***Corresponding author:** Muley Alemseged, Department of Biology, College of Natural and Computational Sciences, Wolkite University, PO Box 07, Wolkite, Ethiopia, Tel: +251928675693; E-mail: mulen.alemseged@wku.edu.et

Received: July 14, 2018; **Accepted:** August 16, 2018; **Published:** August 23, 2018

Citation: Alemseged M, Adugna S, Bayu E (2018) Antibacterial Properties of Mixture Honey and Garlic (Allium Sativum) Extracts Against Respiratory Tract Infection Causing Bacteria. J Pharmacovigil 6: 267. doi:10.4172/2329-6887.1000267

cheesecloth the homogenized mixture was filtered. This was considered as the 100% concentration of the garlic extract. An appropriate volume of sterilized distilled water was used to dilute and prepare the following concentrations of the extracts 50%, 25%, 12.5% and 6.25% (v/v) [12-15].

Bacterial isolates

Five bacterial species which cause respiratory tract infection such as *Haemophilus influenza* (ATCC 49766), *Streptococcus pneumoniae* (ATCC 63*)* were obtained from Ethiopian biodiversity institution, Microbiology unit whereas the clinical insolates of *Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *staphylococcus aureus* were obtained from Ethiopian public health institution.

Determination of antibacterial activity honey and garlic mixture using agar well diffusion method

The tests were run in Mueller Hinton agar (Oxoid UK) for *Pseudomonas aeruginosa, Klebsiella pneumonia* and *staphylococcus aureus*. MHA supplemented with 5% sheep blood was used for *Streptococcus pneumoniae* (ATCC 63*)* and Haemophilus Test Medium (HTM) agar was used for *Haemophilus influenza* (ATCC 49766). The antibacterial activities of the honey and garlic samples were carried out using the Agar well diffusion method [16]. The test pathogens were aseptically inoculated sterile saline solution and the bacterial turbidity was calculated using Mc Farland 0.5 method (1.5 x 10^8 Cfu/ml) [17]. About 100 µL of bacteria isolates samples was poured into the Petri dishes. Five wells on the growth medium with diameters of 6 mm, 4 mm depth and 4 cm apart were made and filled up with honey, extract from garlic and mixture of honey and extract of garlic samples in equal proportions [16]. One well from each plates was filled with sterile distilled water as a negative control and another one was filled with chloramphenicol (31 µg/ml) as positive control. The plates were incubated at a temperature of 37°C for 24 hours.

Determination of MIC and MBC

The minimum inhibitory concentration and Minimum bactericidal concentration were done by broth dilution method as described by Akinyemi et al. [18]. Extracts of garlic, honey and mixture of both were diluted to prepare concentrations ranged from 6.25 up to 25%. Five hundred (500) µl of each concentration was added to 2 ml sterile liquid Trypticasein soy broth in test tubes arranging on a test tube rack. Then 1 ml (1.5 x 10^8 Cfu/ml) of an organism was added to the contentofthe test tubes and the test tubes was incubated at 37°C for 18 h. Five hundred (500) µl of the solutions of the water were added into the test tube stand used asnegative controls. The lowest concentration of extracts that did not show any visible growth bacteria in medium was taken as MIC. From the broth tubes that showed no growth, 100 µl of culture was taken and introduced into fresh appropriate agar plates. After incubation for 48 h, the plates were observed for growth. The concentration of the extracts that showed no visible growth after 24 h of incubation was recorded as the MBC.

Antibiotic susceptibility testing

Four antibacterial drugs were used to test the susceptibility of the test pathogenic bacteria. It was done using the Kirby-Bauer disk diffusion method [19]. The commercially available antibiotic discs such as Amikacin (AK^{30}), Co-trimoxazole (CoT^{25}), Cefoxitin (CX^{30}) and Erythromycin (ER^{15}) were used for this study. One hundred (100 µl) of the test pathogen was evenly spreading onto the entire surface of the agar plate. The plates were left to dry for some time before applying antibiotic disc. Then, antibiotic discs were placed carefully on the agar plates, which were then left an hour at a room temperature in order to diffuse the antibiotics into the agar medium. The plates were incubated for 24 h at 37°C. The antimicrobial activity of the commercial antibiotics was observed on the plates in the form of inhibition zone around the discs. After 24 h of incubation, the diameter of the inhibition zones was measured in millimeter using a scale.

Data analysis

Statistical analysis was done using SPSS 16.020 version window. The data was statistically analyzed using one way ANOVA. Statistical significance was accepted at $P \leq 0.05$.

Results and Discussion

Authors from different parts of the world have been demonstrated the antibacterial properties of honey and garlic extracts separately against different clinical isolates originated from wounds, enteric pathogens, urinary tract infections and nosocomial infections [7,9,16,20]. The present study was focused on the antibacterial properties of mixture of garlic extract and honey against the RIT causing pathogens.

In the present study, the means inhibition zones of mixture of garlic extract and honey, garlic extract alone and chloramphenicol against all tested pathogens were significantly ($P \leq 0.05$) greater than mean inhibition zones of honey alone (Tables 1-5). On the other, there was not any significance difference among the mean inhibition zone of garlic extract alone, the mean inhibition zone of mixture of garlic extract and honey and the mean inhibition zone of chloramphenicol. Local communities believed mixture of garlic and honey have great capacity of treating different infection including respiratory tract infections than using honey alone or garlic alone to treat different infections. Their claim were also supported by research from Gondar, Ethiopia [15] but in the present study, the inhibition capacity of mixture of honey and garlic extract was almost the as the inhibition of garlic extract alone. This may be due to the low inhibition capacity of honey used in this study.

Zones of inhibition of garlic extract alone, honey alone and mixture of honey and garlic extract against the test pathogenic bacteria were presented in Table 1. The zones of inhibition of mixture of garlic extract and honey as well as garlic extract alone against *S. pneumoniea* (ATCC 63) were 25 mm in diameter, whereas chloramphenicol showed 9 mm inhibition. The inhibition capacity of garlic extract alone as well as mixture of garlic extract and honey against *S. pneumoniea* (ATCC 63) were significantly greater ($P \leq 0.05$) than the inhibition zone of commercial Chloramphenicol. This shows, it might be possible to use garlic extract alone or mixture of garlic extract and honey as an alternative antibiotic treatment. The diameter inhibited area of garlic extract alone against *K. pneumoniae* was significantly ($P \leq 0.05$) greater than other tested pathogenic bacteria. Different researchers reported

Test organisms	Inhibition zone (mm)			
	Garlic	Honey	Mixture	Standard antibiotic
P. aeruginosa	25	10	30	30
*S. pneumoniae (*ATCC 63)	25	18	25	9
K.pneumoniae	40	20	25	30
*H .influenza (*ATCC 49766)	25	18	28	12
S. aureus	30	25	31	20

Table 1: Inhibitory zones of mixture of garlic extract and honey from honey bee, garlic extract and honey from honey bee against RTI causing bacteria.

Comparison of extracts	Mean	Std. Error	P-value	95% Confidence Interval	
				Lower Bound	Upper Bound
Garlic -Mixture	1.200	3.153	.708	-5.48	7.88
Garlic -Honey	10.800*	3.153	.003	4.12	17.48
Garlic -Chloramphenicol	2.000	3.153	.535	-4.68	8.68
Mixture -Honey	9.600*	3.153	.008	2.92	16.28
Mixture -Chloramphenicol	.800	3.153	.803	-5.88	7.48
Chloramphenicol -Honey	8.800*	3.153	.013	2.12	15.48
*. The mean difference is significant at ≤ 0.05.					

Table 2: One way ANOVA multiple comparisons of extracts inhibition.

Tested bacteria	Pure Honey		Mixture of extracts		Garlic extract	
	MIC	MBC	MIC	MBC	MIC	MBC
P.aeruginosa	12.5	25	6.25	12.5	6.25	6.25
S. pneumonia(ATCC 63)	12.5	25	12.5	12.5	6.25	12.5
K.pneumoniae	12.5	25	6.25	12.5	6.25	12.5
H.influenza(ATCC 49766)	12.5	25	12.5	25	6.25	12.5
S.aureus	12.5	12.5	6.25	6.25	6.25	6.25

Table 3: Minimum inhibitory concentration and minimum bactericidal concentration of different concentrations of the extracts (v/v) (%).

Comparison of MIC of Extracts	Mean Difference	Std. Error	P-value	95% Confidence Interval	
				Lower Bound	Upper Bound
Honey-Mixture	10.750*	2.574	.001	5.14	16.36
Honey-Garlic	11.250*	2.574	.001	5.64	16.86
Mixture-Garlic	500	2.574	.849	-5.11	6.11
*. The mean difference is significant at the 0.05 level.					

Table 4: One way ANOVA multiple comparisons of MIC of extracts.

Tested bacteria	Commercial antibiotics	Inhibition (mm)
P.aeruginosa	COT 25	13
	ER15	0
	AK 30	20
	CX30	9
S. pneumoniae(ATCC 63)	COT 25	28
	ER15	10
	AK 30	19
	CX30	11
K.pneumoniae	COT 25	10
	ER15	4
	AK 30	12
	CX30	5
H.influenza(ATCC 49766)	COT 25	10
	ER15	6
	AK 30	20
	CX30	11
S.aureus	COT 25	15
	ER15	5
	AK 30	15
	CX30	10
NB: Erythromycin (ER15), Amikacin(AK30), Co-trimoxazole (COT25), Cefoxitin(CX30)		

Table 5: Antibiotic sensitivity test of commonly used antibiotics against the test bacteria.

that various garlic extracts such as crude, aqueous and alcoholic extracts showed great antimicrobial properties against various microorganisms [12,13]. Nigerian researcher named, Abubakar reported that 100 mg/ml concentration of crude extract of garlic was effective against hospital acquired infection causing pathogens such *Staphylococcus aureus, Streptococcus pneumoniea* and *Pseudomonas aeruginosa* [21]. On his report he mentioned that *Staphylococcus aureus* was the most susceptible organism following by *Streptococcus pneumoniea*. In the present study *Klebsiella pneumonia* was the most susceptible microorganism.

According to this investigation, honey was effective against all tested pathogenic organisms except *P.aeruginosa,* which was the only resistance pathogen. Ahmed Mousa et al. [22] did research on different Algerian honey types, they reported that honey inhibited the growth of *S.aureus* at 50% concentration. Ahmed et al. [23] also studied the antibacterial activities of different Saudi Arabian honey which came from different floral sources and compared their inhibition capacity against *S.aureus* (ATCC 25923), *K.pneumoniae* (ATCC 27736) and *P.aeruginosa* (ATCC 27853), their findings indicates that the different honey samples were effective against the tested pathogens at concentration of 20.30%. Similarly, in this study honey inhibited all tested pathogens at concentration of 12.5% v/v (Table 4). On another case, one research from Nigeria reported that 100 mg/ml concentration of honey did not inhibit the growth of bacteria such as *S.aureus, P.aeruginosa* and *K.pneumoniae* [24].

Over all, in this study, the extracts were effective against the tested bacteria except *P. aeruginosa* was resistant to honey alone. The diameter ranges of inhibition of extracts were showed great variation like, mixture of garlic extract and honey was ranged from 25-31 mm, 25-40 mm was also for garlic extract alone, 10-25 mm for honey and also the diameter of zone inhibition of Chloramphenicol was ranged from 9-30 mm.

MIC and MBC of extracts against the tested pathogens were presented on Table 3. The MIC of mixture of honey and garlic against all tested pathogenic bacteria was 6.25%, except for *S. pneumonia* (ATCC 63) and *H.influenza* (ATCC 49766) which was 12.5%. MIC of garlic extract alone and honey alone against the tested bacteria were 6.25% and 12.5% respectively. Over all, the MIC of mixture of honey and garlic extract as well as garlic extract alone shows a significance difference to the MIC of honey alone (Table 4).

The MBC of mixture of honey and garlic extract against the tested pathogens was 12.5% except for *S.aureus* and *H.influenza* (ATCC 49766) which, their MBC were 6.25% and 25% respectively. Except for *P.aeruginosa* and *S.aureus,* the MBC of garlic extract against the tested pathogens was 12.5%. Similarly, MBC of honey alone against *S.aureus* was 12.5%. Commercial antibiotic Amikacin was effective against the tested pathogens except against *K.pneumoniae.* On other hand all the tested pathogens were resistance to Erythromycin and Cefoxitin and were not effective on the test pathogenic bacteria. Beside this antibiotic Co-trimoxazole was effective against *S. pneumonia* (ATCC 63) and *S.aureus* (Table 5).

As it was clearly stated in the data of well inhibition, MIC and MBC, honey and garlic juice were effective against the tested pathogens. So this indicates that, it might be possible to use honey and garlic as antibiotics against those tested pathogens, even if it needs further studies. The antimicrobial nature of garlic is due to the present of allicin [25,26]. It interferes the normal process of RNA production and lipid synthesis, which affects the synthesis of protein and cell wall of microorganisms [27] and also different compounds present in garlic like adjoene, enzymes (peroxidase and miracynase), different amino

acids such as cysteine, glutamine and methionine, vitamins B and C may also be responsible for its antimicrobial activity [28].

Honey's antimicrobial behavior is dependent on factors such as H_2O_2, PH, and low water activity, non-dissociated organic acid and Phenolic compounds [7,29]. Another report from Molan et al. [30] also said that the floral source of which, the honey is made up of contributes to the antimicrobial activity of honey. Lysozyme and another volatile compounds present in honey may have roles in the inhibition of microorganisms [31].

Conclusion

The traditional use of honey alone, garlic alone or mixture of both against respiratory tract infection causing bacteria was evident in this study. Honey and garlic are used as food additives and supplements in daily diet. Nutritionally both of them are very useful. As it was stated on the result, their antibacterial properties were also effective, so it may be possible to use them as an alternative treatment method to treat RTI or can be used together with the pharmaceutically prescribed antibiotics. It is also evident Pneumonia is a common disease in our country so including garlic and honey in our diet may help to minimize the epidemic.

Acknowledgements

We thank research and community service office of Wolkite University for funding our research project.

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