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(RESEARCH ARTICLE)

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To investigate the anti-bacterial activity of crude extracts of *Mintha pipertia* and *Allium sativum* against throat infection causing bacteria streptococci collected from various diagnostic laboratories and hospitals of Mingora swat

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Abstract

Streptococcus (S) pyogenes one of the major Gram-positive pathogens responsible for upper respiratory tract and nosocomial infections. This is a Gram Positive, aerobic, no motile, and non-spore-forming ubiquitous bacteria. The present study was conducted to evaluate the in vitro antibacterial activity of ethanol and aqueous extract of peppermint and garlic against 29 isolates of *S. pyogenes* recovered from the throat sample of patientsin Swat KPK-Pakistan. These isolates were purified to identify the target bacterial isolates by gram staining and biochemical tests. The Agar dilution and well method was applied for the antibacterial activity of garlic and peppermint extracts. All of the subject isolates were exposed to different concentrations of peppermint. MIC of aqua extract of peppermint was 250µL/mL and ethanol extract was 100µL/mL. The zone size was 29mm and 36mm respectively. So the ethanol extract of peppermint was found to be more effective as compared to the aqueous extract. On the other hand, Garlic has shown equal effectiveness of water and ethanol extraction with MIC value of all is 50µL/mL and zone of inhibition was 38mm. Ciprofloxacin antibiotic disk was used as reference standard.

Keywords: *Streptococcus pyogenes; Mentha piperita L; Allium sativum*; Antibacterial activity

1. Introduction

Group A streptococci are major bacterial pathogens responsible for sore throat. They are Gram Positive non motile, non spore forming cocci. They are part of human oral cavity normal flora [1]. The *Streptococcus pyogenes* is frequently reported pathogen in human of all age groups [2]. It is easily transferred from person to person through respiratory droplet while breathing, sneezing and talking. This infection is not so serious or complicated and resolves in five days with simple antibiotic treatment [3]. However, throat infections may cause complications particularly in children, old age and immune compromised persons. Major complications are pneumonia, kidney inflammation, rheumatic fever and meningitis [4]. In complicated bacterial infections, patients have complained of bleeding in salvia, Joint swelling and difficulty in breathing. In this situation patients are advised to take antibiotics [5]. However, home remedies likegargle with salt water, honey and lemon etc are also helpful to ease the condition and reduces the duration of infection [6]. In South Asia region home remedies have a rich tradition especially in remote areas. Lemon and ginger teas with honey are very popular and effective for treatment of upper respiratory infections [7-8]. Additionally, lemon and mint, apricot leaf, and anise teas, ginger balm, cumin water are in use for children and infant treatment [9]. The peppermint extract and oil is considered one of the very effective COVID 19 related upper respiratory tract infections and its complications

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[10]. Similarly, garlic is also effective against oral pathogens and considered an effective ingredient of mouth wash solutions [11].

Peppermint is a member of the mint (*Mentha*) family. Its botanical name is *Mentha piperita L.* It is regularly cultivated in Pakistan and considered as cash crop due to distinct test and aroma [12]. Since 1696 it is being used as a medicine in various forms for common cold, cough, inflammation of the mouth and throat, sinus infections, and other respiratory infections. Peppermint oil and its menthol components are in use as an inhalant for upper respiratory tract infections [13]. It is reported that peppermint oil and Plant extract are effective against Gram positive and Gram negative bacteria, viruses and fungi. The effective antibacterial ingredients are presents in whole leafs, leaf extracts and water of whole plants including roots. The main chemical compounds present in mint are limonene, cineole, menthone, menthofuran, isomenthone, menthyl acetate, isopulegol, menthol, pulegone and carvone [14]. Moreover, it is also suggested that oil from root and leaf have different chemical compositions [15]. Due to its uses the essential oil of peppermint are frequently traded around the world and it is considered as subject for food and pharmaceutical industries in developing and developed countries [16].

Like peppermint garlic (*Allium sativum*) is also one of the broad spectrum antimicrobial agents. Its organosulfur compounds are highly effective against viruses, fungi and bacteria [17]. The garlic is in use as a medicinal plant since ancient time (3000 B. C) [18]. The group (Allium) vegetables are consist upon 850 different species and commonly used all over the world [19]. The garlic is one of the highly important products ranks second to onion in Allium species . It increases the shelf life of food items by reducing microbial growth as well as protects the consumers from infectious agents [20]. Normally, garlic is considered good for high blood pressure, high levels of cholesterol or other fats in the blood, and hardening of the arteries. It is also helpful against common cold and osteoarthritis. Organosulfur (e. g. , allicin and alliin) and flavonoid (e. g. , quercetin) compounds are responsible for immunomodulatoryeffects of this healthy spice [20]. It is reported that peoples using it as daily,have less severe symptoms of cold and other respiratory tract infections [21]. It is in use for the treatment of upper respiratory tract infections particularly for infants [22]. Garlic is also an effective treatment option against multidrug resistant pathogens of *E. coli* and methicillin resistant *S. aureus* [23]. In a recent study it is reported that 10 mg/mL of garlic is effective against clinical and environment pathogens of *S. aureus* and *E. coli*. Additionally, it has also been reported that 2. 5% mouth wash solution of garlic is very effective against streptococci and other oral pathogens [24]. Similarly, garlic and peppermint extract also an effective treatment for oral cavity and its uses reduces the microbial load of saliva [25]. On the basis of these observations this study was designed to analyze the effect of garlic and peppermint as treatment option for upper respiratory tract infections. The focus of the study is Group A streptococci.

2. Material and methods

2.1. Sample collection and Isolation of Group A*streptococci*

This cross sectional study was carried out in Mingora, KPK-Pakistan. Samples were collected from tonsillar area and posterior pharyngeal wall with sterile cotton swabs from patients of pharyngitis/tonsillitis of all age groups. All samples were identified in two different laboratories (Ameerk Clinical Laboratory and Anwar Clinical Laboratory). The cotton swabs were processed for the isolation of group A streptococci within 30 minutes of collection and cultured on 5% sheep blood agar plate. The plates were incubated at 37°C for 24 hrs. The hemolysis on blood agar indicated the presumptive presence of group A streptococci. The colony morphology, gram staining, catalase test and bacitracin sensitivity (0. 04 units/disc) procedures was applied for further confirmation of bacteria. The confirmed pure cultures were preserved in tryptone soya broth with 20% glycerol at – 20 °C until further analysis.

2.2. Preparation of Ethanol Extract of Garlic and Peppermint

The fresh peppermint and garlic (*Allium sativum*) were purchased from local market and authenticated by department of Pharmacognosy. The fresh peppermint was washed with distilled water kept at room temperature in shade to avoid volatility of plant materials and maintain original color. The plants were air dried and crashed in sterile mortar-pestle for preparation of fine powder of peppermint. The powder was stored at cool dry place until next use. The 1 kg of dried material was transferred into a round bottom flask and refluxed with ethanol for 2 × 3 hrs [26].

The 250 gram garlic (*Allium sativum*) was peeled off and washed with de-ionized distilled water. The 100 g of cleaned garlic was re-washed in 95% ethanol and crashed in sterile mortar-pestle and sieved through double layer of sterile fine mesh cloth to make 100% extract. The filtrate was placed in a water bath at 40-50°C for 24 hrs to evaporate the ethanol. The thick paste of garlic was considered as 100% concentrated extract and stored at 4°C. The extract was further diluted in de-ionized distilled water. The ethanolic extract was prepared following same procedure with the exception of

solvent which was 95% ethanolic instead of sterilized distilled water. Antibacterial sensitivity testing using disc diffusion method Filter paper disc of 5mm diameter using whatman no. 1 filter paper was prepared and sterilized. The 10-µL of the Garlic and peppermint extract was aseptically transferred to each disc.

2.3. The Stock Solution

The 50 mg dried mass of garlic and peppermint extract was re-dissolved at room temperature in 100 mL of ethanol to prepare a stock solution at a concentration of 500 µg/mL. Further, 1 mL of stock solution of each extract was dissolved in 100 mL to prepare a solution of 5 µg/mL and all the experiments were conducted within this range (5–500 µg/mL).

2.4. Preparation of standard inoculums

The subject isolates of *S. pyogenes* were inoculated into Muller Hinton broth (MHB) supplemented with 5% de-fibrinated sheep blood and incubated at 35 ºC for 18 hrs. The growth was adjusted according to 0. 5 Macfarland standards (3. 0×10^8 CFU/mL) in fresh MHB.

2.5. Agar Diffusion Method

Antibacterial activity of ethanol extract of garlic and peppermint was conducted as described in Clinical and Laboratory Standard Guidelines (CLSI) [28-29]. Briefly, 500 µL (~10^8 CFU/mL) of subject culture of *S. pyogenes* was seeded on MH agar containing 5% defibrinated sheep blood. After 15 minutes 7 mm wells were made on agar plate with sterile tube. The wells were filled with 15 µL ethanol extract of garlic and peppermint. The ciprofloxacin antibiotic disk was also placed on agar surface as control. The plates were placed at 4ºC for 1 hr and incubated at 35 ºC for 24 hrs. The antibacterial activity was evaluated by measuring the width of the zone of inhibition (clear) of growth against. The zone around antibiotic disk was used as reference.

2.6. Minimum inhibitory concentration (MIC)

MIC was determined by the broth twofold macro dilution method in Muller Hinton brothsupplemented with 5% defibrinated sheep blood for bacterial strains according to a modification of the procedures reported earlier (Mazzanti et al. , 2000; NCCLS, 2000). MIC was defined as the lowest concentration of garlic and peppermint extractsthat allows no more than 20% growth of the bacteria, which is seen as the decreased number of colonies after removing the loop with 10 µL of each dilution on MHA and incubation at 37 ± 1 °C for 24hrs

2.7. Antibiotic Sensitivity Assay

Briefly, 500 µL (~10^8 CFU/mL) of subject culture of *S. pyogenes* was seeded on MH agar containing 5% defibrinated sheep blood. The antibiotic discs of Penicillin,Co-amoxiclav and Ciprofloxacin were used as standard for antibiotic sensitivity assay. The discs of antibiotics were placed on agar surface with help of sterile forceps and incubated at 35°C for 24 hrs. The antibacterial activity was evaluated by measuring the width of the zone of inhibition [27-28].

3. Results

A total of 53 swabs collected from different patients have shown the presence of bacterial colonies on nutrient agar, MacConkey's agar, and blood agar plates. These isolates were purified to identify the target bacterial strains. In the first step, Gram staining was applied to differentiate between Gram-positive and Gram-negative strains. The Gram-positive cocci in chains were then segregated and purified for confirmation. Out of the 76 isolates, 54 were identified as Gram-positive cocci. Further testing revealed that 42 were catalase-negative cocci in chains, while 22 were catalase-positive. All 42 catalase-negative isolates were found to be beta-hemolytic onbloodd agar and non-motile, and were subsequently classified as S. pyogenes. However, the sensitivity profile of these pathogens revealed that the majority were resistant to ciprofloxacin, with 25 out of 29 isolates (86%) showing resistance. Additionally, seven ciprofloxacin-resistant isolates of S. pyogenes were recovered from female patients, while 19 S. pyogenes strains recovered from male patients were resistant to ciprofloxacin. In contrast, penicillin and co-amoxiclav (amoxicillin with clavulanic acid) were highly effective against all S. pyogenes isolates used in the present study. All of these isolates were found to be sensitive to penicillin and co-amoxiclav, with no considerable difference in zone size between ciprofloxacin-resistant and ciprofloxacin-sensitive strains. Results are presented in table 1.

3.1. Antibacterial effect of Peppermint

The entire subject isolates were exposed to different concentration of two types of peppermint extracts i. e. aqueous extract and phenolic extract. The sensitivity assay showed a wide difference in efficacy of both of these extract against subject isolates of *S. pyogenes.* The ethanol extract of peppermint was found to be more effective as compare to aqueous

extract. Majority of the isolates were inhibited at 100µL/mL. Opposing to this, minimum inhibitory concentration of aqueous extract was 250µL/mL. In addition to this 3 isolates were even more resistant and tolerate 250µL/mL and inhibited at 500µL/mL. The results have shown that 4 isolates of *S. pyogenes*were sensitive to 250µL/mL of aqueous extract of peppermint. Further analyses of the results have indicated that 4 isolates of S*. pyogenes*were resistant to 100 µL/mL of ethanol extract of peppermint and inhibited at 200µL/mL (Results are presented in table-1). The zone size of aqueous and phenolic extract of peppermint were 29mm and 36mm respectively (Presented in Fig 1)

3.2. Antibacterial effect of Garlic

The subject isolates of *S. pyogenes* were exposed to different concentration of aqueous and ethanol extract of garlic. The result showed garlic was highly effective against the entire subject isolates recovered from throat of different patients. The sensitivity assay indicated that 50µL/mL was effective dose to inhibit the growth of *S. pyogenes*. (Results are presented in table-1) Furthermore, the aqueous extract and ethanol extract showed identical inhibitory effect against the subject pathogens of *S. pyogenes.* The disc diffusion assay reveled clearance zone size around garlic disc was 38mm. (Presented in Fig 1). Therefore; these pathogens were categorized as sensitive to garlic extract.

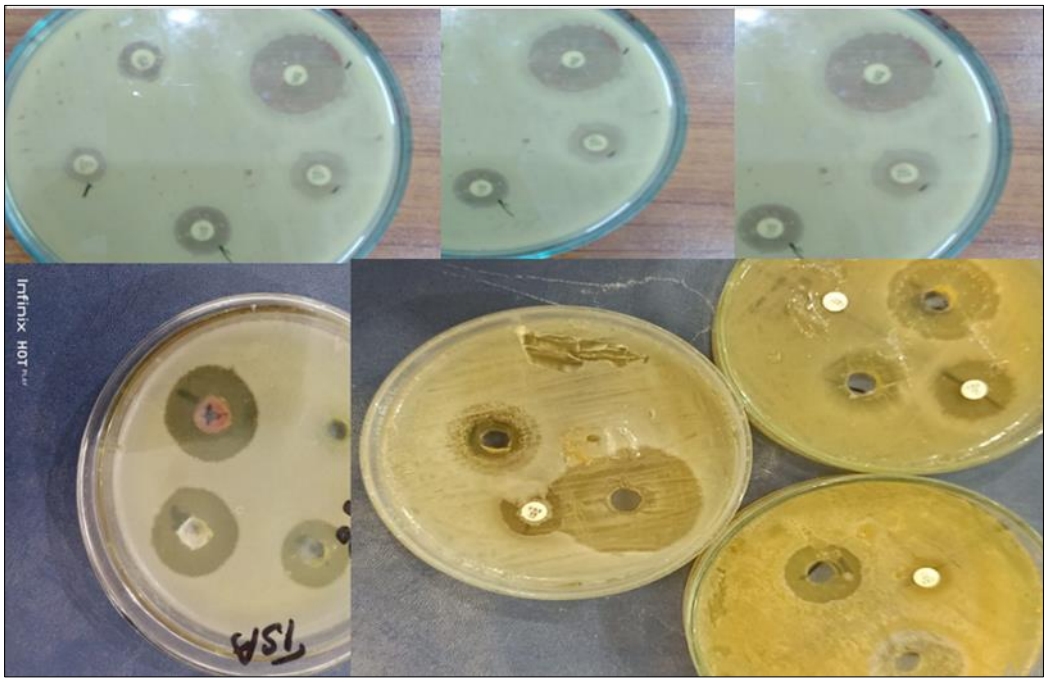

Figure 1 Some representative pictures showing zone of inhibition of Peppermint and Garlic extract effect on isolates of *S. pyogenes*

Table 1 MICs of Garlic and Peppermint extracts and antibiotics sensitivity profile (zone size)

S#	Gander	Age	Peppermint		Garlic		Antibiotic Sensitivity		
			Aqua extract	Ethanol extract	Aqua extract	Ethanol extract	Penicillin	co-amoxiclav	Ciprofloxacin
1.	M	21	250 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	20 mm (S)	24 mm (s)	9 mm (R)
2.	M	43	250 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	27 mm (S)	26 mm (s)	6 mm (R)
3.	F	51	250 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	22 mm (S)	26 mm (s)	6 mm (R)
4	M	23	250 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	25 mm (S)	23 mm (s)	8 mm (R)
5	M	29	250 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	25 mm (S)	25 mm (s)	11 mm (R)
6	M	21	250 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	26 mm (S)	25 mm (s)	13 mm (R)

7	M	42	250 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	29 mm (S)	27 mm (s)	4 mm (R)
8	M	56	250 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	22 mm (S)	22 mm (s)	4 mm (R)
9	F	29	500 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	24 mm (S)	27 mm (s)	3 mm (R)
10	M	25	250 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	25 mm (S)	26 mm (s)	6 mm (R)
11	F	33	100 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	25 mm (S)	27 mm (s)	7 mm (R)
12	M	28	250 µg/mL	200 µg/mL	50 µg/mL	50 µg/mL	25 mm (S)	23 mm (s)	7 mm (R)
13	M	12	250 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	24 mm (S)	24 mm (s)	9 mm (R)
14	M	49	250 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	20 mm (S)	24 mm (s)	4 mm (R)
15	M	11	500 µg/mL	200 µg/mL	50 µg/mL	50 µg/mL	22 mm (S)	22 mm (s)	4 mm (R)
16	M	16	500 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	22 mm (S)	22 mm (s)	18 mm (s)
17.	F	27	250 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	20 mm (S)	23 mm (s)	21 mm (s)
18	M	12	250 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	20 mm (S)	25 mm (s)	9 mm (R)
20.	F	56	250 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	23 mm (S)	25 mm (s)	18 mm (s)
21.	M	8	250 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	24 mm (S)	25 mm (s)	17 mm (s)
22.	M	33	100 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	23 mm (S)	22 mm (s)	9 mm (R)
23.	F	6	100 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	23 mm (S)	25 mm (s)	9 mm (R)
24.	F	27	100 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	26 mm (S)	26 mm (s)	6 mm (R)
25.	F	42	250 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	27 mm (S)	26 mm (s)	4 mm (R)
26.	F	21	250 µg/mL	200 µg/mL	50 µg/mL	50 µg/mL	21 mm (S)	26 mm (s)	12 mm (R)
27.	F	23	250 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	20 mm (S)	24 mm (s)	12 mm (R)
28.	M	45	250 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	20 mm (S)	24 mm (s)	19 mm (s)
29.	F	36	500 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	26 mm (S)	25 mm (s)	10 mm (R)
30.	F	32	250 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	28 mm (S)	23 mm (s)	10 mm (R)
31.	M	23	250 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	25 mm (S)	25 mm (s)	11 mm (R)
32	M	22	250 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	26 mm (S)	26 mm (s)	10 mm (R)
33.	M	7	250 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	29 mm (S)	27 mm (s)	4 mm (R)
34.	F	54	250 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	22 mm (S)	22 mm (s)	5 mm (R)
35.	M	14	500 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	24 mm (S)	27 mm (s)	5 mm (R)
36.	M	25	250 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	25 mm (S)	26 mm (s)	6 mm (R)
37.	M	31	100 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	25 mm (S)	27 mm (s)	7 mm (R)
38.	F	27	250 µg/mL	200 µg/mL	50 µg/mL	50 µg/mL	25 mm (S)	23 mm (s)	7 mm (R)
39.	F	62	250 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	24 mm (S)	24 mm (s)	9 mm (R)
40.	M	47	250 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	20 mm (S)	24 mm (s)	4 mm (R)
41.	M	19	500 µg/mL	200 µg/mL	50 µg/mL	50 µg/mL	21 mm (S)	20 mm (s)	5 mm (R)
42.	M	70	500 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	22 mm (S)	20 mm (s)	18 mm (s)
43.	F	21	250 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	20 mm (S)	23 mm (s)	21 mm (s)
44.	M	22	250 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	20 mm (S)	25 mm (s)	9 mm (R)

45.	M	56	250 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	23 mm (S)	25 mm (s)	18 mm (s)
46.	M	18	250 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	24 mm (S)	25 mm (s)	17 mm (s)
47.	M	43	100 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	23 mm (S)	22 mm (s)	9 mm (R)
48.	F	44	100 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	23 mm (S)	25 mm (s)	9 mm (R)
48.	F	27	100 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	26 mm (S)	26 mm (s)	6 mm (R)
49.	F	22	250 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	27 mm (S)	26 mm (s)	4 mm (R)
50.	F	69	250 µg/mL	200 µg/mL	50 µg/mL	50 µg/mL	21 mm (S)	26 mm (s)	12 mm (R)
51.	M	23	250 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	22 mm (S)	24 mm (s)	11 mm (R)
52.	F	45	250 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	20 mm (S)	24 mm (s)	19 mm (s)
53.	M	26	500 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	26 mm (S)	25 mm (s)	10 mm (R)
54.	F	31	250 µg/mL	100 µg/mL	50 µg/mL	50 µg/mL	27 mm (S)	23 mm (s)	9 mm (R)

4. Discussion

The present study was conducted on throat borne isolate of group a streptococcus particularly *S. pyogenes* collected from patients of Mingora Swat. This is a ubiquitous gram-positive (cocci) pathogen capable to cause a wide range of infection in human. [30]In the present study and literature survey it was noticed that children and old age population are major target of this pathogen. However, adult population is also affected and infected by this pathogen. It may cause minor, acute and self resolving infection like pharyngitis as well as life threatening infections as necrotizing fasciitis [29].

In present study two of the commonly used food additives Garlic and Peppermint were applied against *S. pyogenes*. The results indicated that both of these were highly effective against throat borne isolates of *S. pyogenes.* But the comparative analysis of both of these compounds reveled that garlic is more effective against *S. pyogenes*. The results indicated that mean minimum inhibitory concentration for the garlic extract is lesser than that of the mint's. This is also supported by previous studies and suggested that garlic is a fairly strong natural antibacterial [30]. The active ingredient in garlic is allicin, which interferes with lipid synthesis and RNA production in bacteria. Allicin inhibits growth and leads to the death of bacteria [31]. While, the ethanol extract of peppermint was more effective as compare to aqueous extract against subject isolates of *S. pyogenes.* This highest antibacterial activity of peppermint extract is might be due to solubility of the active substances in organic solvents. This finding is in accordance with previous research published. It is mentioned that the organic (ethanol, methanol, ethyl acetate, chloroform, hexane and petroleum ether) extracts of the leaves were found to possess strong antibacterial activity against a range of pathogenic bacteria [32]. This finding suggested that peppermint different ingredients some of these are water soluble and some are soluble in organic solvents. Also these compounds have different antibacterial effect [32].

On the other hand, Garlic has shown equal effectiveness with water and ethanol extraction. This could be the reason for more efficacy of garlic as compare to peppermint. The active ingredients of Garlic are soluble in water as well as in ethanol. The other possibility is garlic contain high quantity of antibacterial agents that are very effective against the pathogens [33]. The susceptibility has been tested as minimum inhibitory concentration and zone of inhibition. The garlic extract showed that all of these isolates have almost similar response e. g. MIC of all is 50µL/mL and zone size was 38mm. By these results it was confirmed that all of the *S. pyogenes* isolates recovered from the throat of different patients were highly sensitive to garlic.

As compare to garlic, MIC of aqua extract of peppermint was 250µL/mL and ethanol extract was 100µL/mL. The zone size was 29mm and 36mm. This indicates that S. pyogenes are also sensitive to peppermint extract. The antibacterial activity of these compounds may be affected by cell wall composition bacteria as well as solvents. The cell walls of Gram positive bacteria contain <2% lipid [34]. Therefore, it has been assumed that water soluble compounds easily penetrate inside cell wall and damage the bacterial growth. Whereas, hydrophobic compounds may not penetrate cell wall as easy as water soluble. Consequently, these compounds from Peppermint leaves are less effective as compare to garlic extract. The results suggested that *S. aureus* was susceptible to hydrophilic compounds and *E. coli* was susceptible to hydrophobic compounds. This is due to presence of outer membrane in *E. coli* and presence of low lipid contents in cell wall of *S. aureus*. [35]. The water solubility of active antibacterial ingredients of garlic and peppermint make them potential candidate to use as synergistic partner of different antibiotics. Interestingly, up till now there is no report of

Garlic or Peppermint resistant bacteria from any part the world. Therefore, this compound could be potential alternates of antibiotics. In vitro studies have suggested that allicin can inhibit the proliferation of both bacteria and fungi or kill cells outright, including antibiotic-resistant. A recent study further explored its mode of action and suggested that it targets and inhibit DNA gyrase activity by oxidization of sub unit A [35-36].

The Peppermint mainly inhibits microbial growth due the presence of compounds such as l-menthol, menthone, menthyl acetate, and limonene. These compounds are reported to damage the membrane of bacteria. Therefore, it is highly active against Gram positive bacteria e. g. *S. pyogenes.* The Gram negative bacteria like *E. coli* have outer membrane that prevent the entrance of these compounds to cell membrane and protect it from toxic effect [37]. Whereas, Gram positive bacteria do not have outer membrane and these compounds have easy access to cell membrane. This is the reason that these Gram positive bacteria are easy target for Peppermint and Garlic [40]. On the basis of our research results and literature survey on antibacterial activity of Garlic and Peppermint it has been noticed that these medicinal plants have great potential to use as an alternate to antibiotics.

5. Conclusion

In the present study total of 29 isolates of *S. pyogenes* were recovered from the throat sample of patients. All of these isolates were sensitive to amoxicillin and the majority was resistant to ciprofloxacin. The garlic and peppermint were found to be highly effective against all of these isolates of *S. pyogenes*. However, the ethanol extract of peppermint was more effective than compared water extract. The Garlic extract (ethanol and water) was equally effective against these pathogens recovered from throat swabs. The Ethanol extract of Peppermint was found to kill subject isolates of *S. pyogenes* within 30 minutes. The aqueous extract was achieved similar results in 140 minutes and it require high dose as well. Compare to this the ethanol and aqueous extract were equally effective and cleared the complete growth of *S. pyogenes* within 15 minutes. The *S. pyogenes* is one of the major pathogens responsible for throat infections in the Swat region of the KPK province of Pakistan. The present research has suggested that this pathogen infects almost everyone in all age groups of the male and female populations. Moreover, due to the prevalence of multidrug-resistant, it is very difficult to control this infection. Therefore, the natural compound could be the best available option to control and prevent the spread of multidrug resistant pathogens. This study has proved that garlic and peppermint are the best examples of this. However, the results of the present study confirmed that garlic and peppermint are very effective against a wide range of isolates of *S. pyogenes* recovered from throat samples of patients in Swat.

Compliance with ethical standards

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Disclosure of conflict of interest

This manuscript has no conflict of interest with any party.

References

[1] Leung TN, Hon KL, Leung AK. Group A Streptococcus disease in Hong Kong children: an overview. Hong Kong Medical Journal. *2018*.

[2] Bowen AC, Tong SY, Chatfield MD, Carapetis JR. The microbiology of impetigo in Indigenous children: associations between Streptococcus pyogenes, Staphylococcus aureus, scabies, and nasal carriage. BMC infectious diseases. 2014, 14, 1-6.

[3] Al-Harrasi A, Bhatia S. Epidemiology Respiratory Infections: Types, Transmission, and Risks Associated with Co-Infections. InRole of Essential Oils in the Management of COVID-19 2022 Mar 30 (pp. 7-17). CRC Press.

[4] Kilian M. Streptococcus and enterococcus. Medical Microbiology. **2007**,17,178-93.

[5] Perdana RF, Rosalina E. Case Report: Bilateral peritonsillar abscess with complications of upper airway obstruction. F1000Research. 2022**,** 19, 11(550):550.

[6] Zhu X, Chen Z, Shen W, Huang G, Sedivy JM, Wang H, Ju Z. Inflammation, epigenetics, and metabolism converge to cell senescence and ageing: the regulation and intervention. Signal Transduction and Targeted Therapy. 202128, 1-29.

[7] Wali S, Jan HA, Haq SM, Yaqoob U, Bussmann RW, Rahim F. The Traditional phyto-recipes used to cure various ailments by the local people of ShishiKoh valley, Chitral, Pakistan. Ethnobotany Research and Applications. 2021**,** 24, 22.

[8] Abuelgasim H, Albury C, Lee J. Effectiveness of honey for symptomatic relief in upper respiratory tract infections: a systematic review and meta-analysis. BMJ Evidence-Based Medicine. 2021**,**1,57-64.

[9] Sargin SA. Plants used against obesity in Turkish folk medicine: A review. Journal of Ethnopharmacology. 2021**,** 24, 270:113841.

[10] Valussi M, Antonelli M, Donelli D, Firenzuoli F. Appropriate use of essential oils and their components in the management of upper respiratory tract symptoms in patients with COVID-19. Journal of herbal medicine. 2021**,** 1, 28:100451.

[11] Khobragade VR, Vishwakarma P, Dodamani AS, Kshirsagar MM, Raut SN, Deokar RN. Herbal Mouthwash for the Management of Oral Diseases: A Review on the Current Literature. Journal of Oral Health and Community Dentistry. 2021, 15(2):71.

[12] Muhammad MI, Huma Z, Bibi N, Shah SM, Javed A, Ibrahim M. In vitro Antifungal Activity of MentaPiperita Extract AgainstVenturiainaequalis. Phytopharmacology Research Journal. 2022, 7, 1(2):24-37.

[13] Ahluwalia S, Kaur N. Bioactive Compounds in Mint. InSpice Bioactive Compounds 2022,5: 245-268. CRC Press.

[14] Soleimani M, Arzani A, Arzani V, Roberts TH. Phenolic compounds and antimicrobial properties of mint and thyme. Journal of Herbal Medicine. **2022**, 30:100604.

[15] Alam M, Bano N, Ahmad T, Sharangi AB, Upadhyay TK, Alraey Y, Alabdallah NM, Rauf MA, Saeed M. Synergistic Role of Plant Extracts and Essential Oils against Multidrug Resistance and Gram-Negative Bacterial Strains Producing Extended-Spectrum β-Lactamases. Antibiotics. 2022, 26, 11(7):855.

[16] Sharmeen JB, Mahomoodally FM, Zengin G, Maggi F. Essential oils as natural sources of fragrance compounds for cosmetics and cosmeceuticals. Molecules. 2021, 27, 26(3):666.

[17] Rani N, Singla RK, Narwal S, Kumar N, Rahman M. Medicinal Plants Used as an Alternative to Treat Gingivitis and Periodontitis. Evidence-Based Complementary and Alternative Medicine. 2022, 6, 2022.

[18] Joshi VK, Joshi A. Garlic in Traditional Indian Medicine (Ayurveda) for Health and Healing. Herbs and Spices-New Processing Technologies. 2021, May 31.

[19] Forma A, Chilimoniuk Z, Januszewski J, Sitarz R. The potential application of allium extracts in the treatment of gastrointestinal cancers. Gastroenterology Insights. 2021, Mar 27, 12(2):136-46.

[20] Islam AR, Ferdousi J, Shahinozzaman M. Previously published ethno-pharmacological reports reveal the potentiality of plants and plant-derived products used as traditional home remedies by Bangladeshi COVID-19 patients to combat SARS-CoV-2. Saudi journal of biological sciences. 2021 1, 28(11):6653-73.

[21] Yao J, Zhao J, Wen JR, Yang ZJ, Lin YP, Sun L, Lu QY, Fan GJ. Flavonoid-containing supplements for preventing acute respiratory tract infections: A systematic review and meta-analysis of 20 randomized controlled trials. Complementary Therapies in Medicine. 2022, 5:102865.

[22] Khan MM, Khalilullah H, Eid EE, Azam F, Khan MA, Khan A, Siddiqui NA, Mahmood T, Ahsan F, Khan WU, Bhatia N. A Dig Deep to Scout the Pharmacological and Clinical Facet of Garlic (Allium sativum). Current Traditional Medicine. 2022, 1, 8(1):1-9.

[23] Magryś A, Olender A, Tchórzewska D. Antibacterial properties of Allium sativum L. against the most emerging multidrug-resistant bacteria and its synergy with antibiotics. Archives of microbiology. 2021,203(5):2257-68.

[24] Akullo JO, Kiage B, Nakimbugwe D, Kinyuru J. Effect of aqueous and organic solvent extraction on in-vitro antimicrobial activity of two varieties of fresh ginger (Zingiberofficinale) and garlic (Allium sativum). Heliyon. 2022**,**1, 8(9):e10457.

[25] Gandhi G, Thimmappa L, Upadhya N, Carnelio S. Could mouth rinses be an adjuvant in the treatment of SARS-CoV-2 patients? An appraisal with a systematic review. International journal of dental hygiene. 2022, 20(1):136-44.

[26] Mukhtar S, Ghori I. Antibacterial activity of aqueous and ethanolic extracts of garlic, cinnamon and turmeric against Escherichia coli ATCC 25922 and Bacillus subtilis DSM 3256. International Journal of applied biology and pharmaceutical Technology**. 2012** ,3(2):131-6.

[27] CLSI . Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, approved standard Ninth Edition. M07-A9. 2012,32(2): 12- 13.

[28] Shaheen AY, Sheikh AA, Rabbani M, Aslam A, Bibi T, Liaqat F, Muhammad J, Rehmani SF. Antibacterial activity of herbal extracts against multi-drug resistant Escherichia coli recovered from retail chicken meat. Pakistan Journal of Pharmaceutical Sciences. 2015, 1, 28(4).

[29] Wu ZY, Campeau A, Liu CH, Gonzalez DJ, Yamaguchi M, Kawabata S, Lu CH, Lai CY, Chiu HC, Chang YC. Unique virulence role of post-translocational chaperone PrsA in shaping Streptococcus pyogenessecretome. Virulence. 2021, 31, 12(1):2633-47.

[30] Liu M, Pan Y, Feng M, Guo W, Fan X, Feng L, Huang J, Cao Y. Garlic essential oil in water nanoemulsion prepared by high-power ultrasound: Properties, stability and its antibacterial mechanism against MRSA isolated from pork. UltrasonicsSonochemistry. 2022, 1, 90:106201.

[31] El-Saber Batiha G, MagdyBeshbishy A, G. Wasef L, Elewa YH, A. Al-Sagan A, Abd El-Hack ME, Taha AE, M. Abd-Elhakim Y, Prasad Devkota H. Chemical constituents and pharmacological activities of garlic (Allium sativum L.): A review. Nutrients. 2020, 24, 12(3):872.

[32] Mahato TK, Sharma K. Study of medicinal herbs and its antibacterial activity: A review. Journal of Drug Delivery and Therapeutics. 2018**,** 15, 8(5-s):47-54.

[33] Yan JK, Wang C, Yu YB, Wu LX, Chen TT, Wang ZW. Physicochemical characteristics and in vitro biological activities of polysaccharides derived from raw garlic (Allium sativum L.) bulbs via three-phase partitioning combined with gradient ethanol precipitation method. Food Chemistry. 2021, 1, 339:128081.

[34] Rohde M. The Gram-positive bacterial cell wall. Microbiology Spectrum. 2019**,** 24, 7(3):7-3.

[35] Wolde T, Kuma H, Trueha K, Yabeker A. Anti-bacterial activity of garlic extract against human pathogenic bacteria. J Pharmacovigil. 2018, 6(253):2-8.

[36] Banerji R, Saroj SD. Interspecies signaling affects virulence related morphological characteristics of Streptococcus pyogenes M3. FEMS Microbiology Letters. 2021,368(13):fnab079.

[37] Soleimani M, Arzani A, Arzani V, Roberts TH. Phenolic compounds and antimicrobial properties of mint and thyme. Journal of Herbal Medicine. 2022, 30:100604.

[38] Liu J, Mahmood MS, Abbas RZ, Dillawar A, Nawaz Z, Luqman M, Abbas A, Rafique A. Therapeutic appraisal of ethanolic and aqueous extracts of clove (Syzygiumaromaticum) and garlic (Allium sativum) as antimicrobial agent. Pakistan Journal of Agricultural Sciences. 2021**,** 1, 58(1).