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Antibacterial activity of Garlic, Tulsi, Bitter guard and Cinnamon extracts against wound pathogens

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Abstract

In the present study antibacterial properties of fruits harvested from natural medicinal plants garlic, tulsi, bitter guard and cinnamon, were explored against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, which had been extracted from wound infections of sick patients. Antimicrobial components were extracted by solvent extraction using methanol and ethanol as extracts. Agar well diffusion method was used to assess the antimicrobial nature of extracts. The methanolic extract of garlic was found effective against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, which gave an inhibition zone of 27mm and 28mm respectively. Ethanolic extract of cinnamon was effective against *Escherichia coli* by giving an inhibition zone of 27mm. These results were far better than the zone of inhibition given by the standard tetracycline antibiotic used throughout the study. Tulsi and bitter guard were less effective against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* by giving an inhibitory zone which was less as compaired to garlic and cinnamon extracts. The minimum inhibitory concentration of the most effective extracts were determined where by garlic methanolic (GM) extract had 0.14µg/ml against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, while cinnamon ethanolic (CE) extract had 0.12µg/ml against *Escherichia coli*.

Keywords: Antibacterial, Medicinal plants, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*.

INTRODUCTION

Wound infections and consequent mortality reached their peak in the 19th century [1]. Florence Nightingale, after her experience of wound sepsis at Scutari and her reform of army medical service; she turned attention to British wounds in her books "Notes on wounds' where she established important principles of hygiene and nursing [2]. Further confirmation was provided by the survey of the squealer of amputation, which established that sepsis, gangrene and pyeremia were very common in large town areas. At the same time Louis Pasteur introduced his "antiseptic surgery". The breakthrough of pathogenic bacteria in 20th century provided a new phenomenon for the study of wound infection [3, 4]. Wound infections may be endogenous or exogenous [5]. *Staphylococcus aureus* has been among the microorganism most frequently isolated from wound infection of the adult and is the most general cause of newborn-associated wound infections most of strain within the wound are resistant to antibiotics [6]. The commonest pyrogenic bacteria are *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pneumococcus* and coliform bacilli such as *Escherichia coli*, *Proteus* species and *Pseudomonas aeruginosa*, Pus and exudates from an infected wound or open abscess would be expected to have the etiologic agents of the infection [7].

Garlic is one of the edible plants, which has generated a lot of interest throughout human times as a medicinal plant. A broad range of microorganism including bacteria, fungi, protozoa and viruses has been found to be responsive to crushed garlic preparations. Moreover garlic has been reported to reduce blood lipids and to have anticancer effects [8]. Chemical analysis of garlic has revealed unusual concentration of sulphur containing compounds which have antibiosis effect [9].

Ocimum sanctum also known as holi basii is especially advantageous in respiratory tract infections because of its ant-inflammatory, anti-microbial and anti-allergic. It is useful in relieving symptoms of flu like common cold, wet and dry cough. Tulsi works as powerful immunodulator and restores balance of being [10]. *Ocimum sanctum* extract was found to be active against multi drug resistant strains of

of *Neisseria gonorrhea* strains, *Salmonella typhi*, *Klebsiella pneumonia*, *Escherichia coli* and P*roteus vulgaris* [8]. *Momordica charantia* contains gurmarin a polypeptide considered to be alike to bovine insulin, which has been shown in experimental studies to attain a positive sugar regulating effect by suppressing the neuronal response to sweet test stimulus [11].

Cinnamon kills *Escherichia coli*, *Salmonella typhi* and *Campylobacter* also may fight other food poisoning bugs. Scientists have now discovered that cinnamon can fight the cause a deadly form of poisoning. That adding the spice to food is the most effective way after cooking and pasteurization would kill the *Escherichia coli* bug [3, 6]. In this present study we are to identify different etiological pathogens which inhabit the wound of patients' later find the inhibitory activity of the selected medicinal plants against the wound pathogens so as to find the effective natural antibacterial product for local treatment**.**

MATERIALS AND METHODS

Plant fruits

Fresh fruits of Natural medicinal plants; garlic, tulsi, bitter guard and cinnamon were collected using plant material collection guidelines from Ayothiapattinum market, Salem Tamilnadu. Later they were asceptically treated to avoid contaminations by environmental pathogens [9].

Pathogens

Three different pathogens namely *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* available at Bio-gene private laboratory formerly extracted from the wound pus of sick patients admitted at Vinayaka Mission Hospital – Salem using Paniker's method, were subcultured and used in the entire project work [12].

Extraction and preparation of plant materials.

Different medicinal fruits were used for the preparation of the methanolic and ethanolic extract. The outer covering of the fruits were cleaned and sterilized by first washing in distilled water 3 times .Then subjected to washing by the ethanol solution by immersing inside ethanol for a period of 2 minutes, again washed with distilled water to remove the extra ethanol solution.

10 grams of each sterilized fruit were used in the extraction of antimicrobial component. The fruits were cut into small pieces and grind well using the grinder or mixer later was dissolved in 20ml of 70% ethanol and 80% methanol to promote the solvent extraction process. The mixture was filtered and the solid residue material was discarded, the filtrates were kept in the dark for 2-4 days. The solutions were filtered and left in oven at 50ºC till the extract dried [1, 3, 11]. The amounts of evaporated metabolites from methanolic and ethanolic extracts were dissolved in double amount of 100 mM Tris HCl buffer pH 8. Giving the concentration of antimicrobial extracts to be 500µg/ml. All different samples were prepared and the list is shown in Table 1.

Table 1: Different medicinal plants with Methanolic and Ethanolic Extracts.

Garlic	Garlic Methanolic(GM) extract
	Garlic Ethanolic (GE) extract
Tulsi	Tulsi Methanolic(TM) extract
	Tulsi Ethanolic (TE) extract
Bitter guard	Bitter guard Methanolic(BM) extract
	Bitter guard Ethanolic (BE) extract
Cinnamon	Cinnamon Methanolic(CM) extract
	Cinnamon Ethanolic (CE) extract

Antibiogram analysis

Nutrient Agar media were prepared and autoclaved along with petri plates. Media was poured into sterile petri plates under sterile settings and left to solidify. After solidification using micropipette 50 µl test pathogens were spread on the different plates and wells were bored, later 50 µl antimicrobial Samples and tetracycline were loaded in the wells. Petri plates were incubated at 37 ºC for 24 hours and observed in the zone of inhibition. The zone of inhibition of the sample was compared with the standard antibiotic Tetracycline [1].

Determination of minimum inhibitory concentration (MIC)

Twelve test tubes were taken and 3ml of Nutrient Broth was filled in each and autoclaved. Later were left to cool down to room temperature. Afterwards, 500 µl of medicinal plant extract was poured using micropipettes into two test tubes. Two set each containing 6 test tubes were made and the antimicrobial extract was serially diluted. The inoculated set was kept for incubation for 24hrs whereas the blank was preserved. Optical Density was read at 600nm for each inoculated test tube using the uninoculated test tube as blank [13].

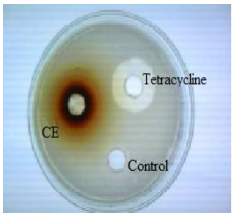
RESULTS

Antibiogram and MIC analysis

Agar well diffusion method of Kirby- bauer was used to check the antimicrobial activity of extracted plant samples. With the help of this test we determined if the culture we were using had antibacterial property or not. Table 2 below shows the results of the zone of inhibitions observed for the antibacterial extracts and the standard antibiotic tetracycline used throughout the study. It can be seen that the zones of inhibitions of the extracts GM and CE are much better than the standard antibiotic Tetracycline, while TM, TE, BM and BE had lower standard when compared to the standard antibiotic Tetracycline. Figure 1, 2 and 3 below show the photographs of the antibiogram performed against available pathogens.

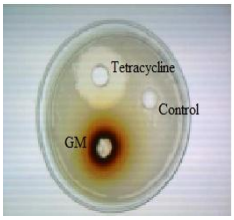
Table 2: Antibiogram of different Samples against different Pathogen

Zone of inhibition in mm			
Medicinal Plants Extracts	***E. coli* / Tetracycline**	***P.aeruginosa* / Tetracycline**	***S. aureus* / Tetracycline**
GM	21/19	28/27	27/22
GE	20/19	27/27	27/22
TM	16/19	14/27	11/22
TE	15/19	14/27	12/22
BM	18/19	18/27	17/22
BE	18/19	17/27	17/22
CM	25/19	20/27	16/22
CE	27/19	19/27	16/22



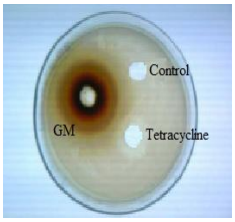
Escherichia coli

Fig 1: Antiboigram of ethanoic Extract of Cinnamon against *E.coli*



Pseudomonas aeruginosa

Fig 2: Antibiogram of methionic extract of garlic against *P. auriginosa*



Staphylococcus aureus

Fig 3: Antibiogram of methionic extract of garlic against *S. aureus*

The MIC for sample CE was determined against *Escherichia coli*, because it gave the best zone of inhibition in antibiogram analysis. GM was determined against *Pseudomonas aeruginosa* and *Staphylococcus aureus* Table 3 shows the MIC of the extracts against the respective pathogens against which maximum zones of inhibition were observed. The MIC of the ethanolic extract of cinnamon was determined against *Escherichia Coli*. It was seen that MIC is 0.12µg/ml and the MIC of the methanolic extract of garlic was determined against *Staphylococcus aureus* and *Pseudomonas aeruginosa* which were found to be 0.14µg/ml.

Table 3: Minimal Inhibitory Concentration (MIC) in µg/ml

	Garlic		Cinnamon		Tulsi		Bitter guard	
	GM	**GE**	**CM**	**CE**	**TM**	**TE**	**BM**	**BE**
S. aureus	0.14	0.28	0.33	0.41	0.51	0.44	0.42	0.76
P. aeruginosa	0.14	0.30	0.22	0.63	0.30	0.42	0.55	0.52
E. coli	0.50	0.63	0.17	0.12	0.38	0.75	0.44	0.53

DISCUSSION

Medicines from natural plants are valuable and readily available resources for primary health care system. Certainly the plant kingdom still holds many species of the plant containing substances of medicinal value that are still to be discovered, though a large number of plants are continually being screened for this antimicrobial properties .This plant may prove to be a rich source of compounds with possible antibacterial properties. But more pharmacological exploration is necessary.

Medicinal plant extracts were prepared in this research work using the Plant Materials Collection Guideline as reported earlier [9]. Both the ethanolic and methanolic extracts were taken for the antibiogram analysis in the present research work for the study of antimicrobial properties of garlic, tulsi, bitter guard and cinnamon which was reported earlier by [1, 3, 14]. The antibacterial properties of the different extracts against the test organisms were investigated using agar well diffusion method as used earlier by Bhatia for antibacterial assay [14]. Ethanoic extracts of cinnamon gave the zone of inhibition of 27 mm,against *Escherichia coli*. The methanolic extract of garlic shows the zone of inhibition of 27mm, 28mm, against *Staphylococcus aureus* and *Pseudomonas aeruginosa* respectively. Earlier also a zone of inhibitions of 30mm against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* have been reported by Bharathi [11]. Also according to Mukhtar it was reported of finding a zone of inhibition of 27 mm against *Escherichia coli,* 26mm against *Pseudomonas aeruginosa,* and 25 mm against *Staphylococcus aureus* [3].

The Minimum Inhibitory concentration was also performed in order to know the minimum inhibitory concentration of all the extracts by broth dilution method and the MIC for garlic was found to be ranging between 0.14 - 0.63µg/ml, tulsi was between 0.30 – 0.75 µg/ml, bitter guard was between 0.42 - 0.76µg/ml and cinnamon was between 0.12 - 0.63µg/ml this can be compaired to several reported MIC research done on garlic, tulsi and cinnamon which ranged between 0.12- 0.65 µg/ml, 4.0 – 12.0 mg/ml and 0.14- 0.50 µg/ml [15,16]. The MIC value of Samples CE and GM, which showed the best zones in antibiogram analysis after being tested against the pathogens. MIC for ethanolic extract (CE) ranged from 0.12 - 0.63µg/ml as well as methanolic extract (GM) ranged from 0.14 - 0.63µg/ml. It is apparent from the result that the MIC values are lower thus indicating that they are effective and active in very low concentration which is a merit for a good antimicrobial agent.

The future prospects of present investigated work include isolation, purification and pharmacological evaluation of the active antimicrobial extracts of several methods such as Thin Layer Chromatography, High-Performance Liquid Chromatography and Nuclear Magnetic Resonance. The purified antimicrobial can also be characterized for the effect of various cations in order to check their effects on the total effectiveness of the antimicrobial.

CONCLUSION

The result of the present research showed the antibacterial nature of extracts of different medicinal plants i.e., garlic, tulsi, cinnamon and bitter guard. Each of the plant parts was extracted in its fresh and raw form using ethanol and methanol. The data obtained demonstrated that the antimicrobial activity depends on the source plant, solvent used for extraction and the test organism tested for susceptibility assay. Methenolic extracts of garlic was found to be most effective against two of the pathogens (*Staphylococcus aureus* and *Pseudomonas aeruginosa*). The ethanolic extract of cinnamon was found to be most effective against one of the pathogens (*Escherichia coli*). Other test plants also had antibacterial properties but were less effective compared to tetracycline which was the standard antibiotic. So it can be concluded that Methenolic extraction procedure is the best in order to get antibacterial components from garlic while ethanol is the best extract of antibacterial component from cinnamon.

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Conflict of Interest: Nil

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