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SYNERGISTIC ANTIBACTERIAL ACTIVITY OF CLOVE, CUMIN AND TURMERIC AGAINST PATHOGENIC BACTERIA

A. Prabakaran*[1]

[1*]Department of Biotechnology, Sankaralingam Bhuvaneswari College of Pharmacy, Sivakasi, Tamilnadu, India.

ABSTRACT

Nowadays majority of world population rely on the plant preparations as medicines to cure diseases, as they are considered safe and as effective as allopathic preparations without any side effects. Spices are plant products having aroma. They are mainly used during cooking to impart flavor and taste to the dish. They also possess medicinal values. The present study was designed to evaluate the individual and combined antimicrobial activity of clove, cumin and turmeric against Gram positive and Gram negative pathogenic bacteria viz., *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli*, *Shigella dysenteriae* using ethanolic extracts. Among all extracts tested, the combined extracts showed inhibition zones ranging from 13 to 34 mm in diameters and the diameters of inhibition zones of individual extracts from different spices ranging from 12 to 22 mm against tested bacteria. The combined extract of clove and cumin was found to be most effective in inhibiting the microbial growth among tested drugs. The results confirmed that the combinations of extracts provide additive/synergistic inhibitory effects making them more effective as antimicrobial agents.

KEYWORDS

Antibacterial activity, Clove, Cumin, Herbal, Spices, Synergistic effect and Turmeric.

Author for Correspondence:

Prabakaran A,

Department of Biotechnology,

Sankaralingam Bhuvaneswari College of Pharmacy,

Sivakasi, Tamilnadu, India.

Email: aprabakaran95@gmail.com

INTRODUCTION

A spice may be a seed, fruit, root or bark[1]. Spices are different from herbs, which are the leaves, flowers, or stems from plants used for flavoring. Traditionally India was known as the legendary land of spices[2]. In ancient India, natural herbs and spices were consumed either in food or used as medicines in order to maintain proper sanitation, health and hygiene[3]. In this respect, spices such as clove (toothache, fever and pain), cinnamon

(nervous problems, stomach infections), garlic (antiseptic, diuretic), ginger (digestive, cold) etc., have been reported to possess very good medicinal properties. The phytochemicals are antimicrobial substances present in the spices which are capable of repelling harmful organisms[4]. Spices might have a great potential to be used as antimicrobial agents[5]. Most of the spices have been tested for their antibacterial effects but fewer studies on the synergistic or antagonistic antibacterial effects. Combinations of extracts can also lead to the additive/synergistic or antagonistic effects[6]. Numerous classes of phytochemicals including the isoflavones, anthocyanins and flavonoids are found associated with the spices[7]. Antibacterial agents are used in the treatment of bacterial infections. However in the last two decades, indiscriminate use of such agents has led to the development of drug resistance in many bacterial species[8]. This necessitates the constant development of newer agents which can inhibit the growth of the pathogen or kill it[9]. Thus there is a need to identify newer drugs for bacterial diseases. The present study was undertaken specifically to investigate the individual and combined extracts of clove, cumin and turmeric as a potential antimicrobial agent against some human pathogenic bacteria. The plants used for this study are listed in following Table No.1.

MATERIAL AND METHODS

Plant materials

The plant materials used in this study were collected from local market in Sivakasi. The fresh plant materials were collected, dried, powdered and used for extraction.

Preparation of extracts

Maceration technique was used in this study[10]. The powdered drug was soaked with ethanol 95% v/v in a stoppered container and allowed to stand at room temperature for a period of minimum 3 days with frequent agitation. After 3 days, the mixture was pressed or strained by filtration. After solvent extraction step, the materials were concentrated by evaporation. The resulting concentrated extracts were stored in a refrigerator.

Determination of antimicrobial activity

Test microorganisms

The bacterial species used for the antibacterial activity are listed below.

Gram Positive Organisms

1. *Staphylococcus aureus.*
2. *Streptococcus pneumoniae.*

Gram Negative Organisms

1. *Escherichia coli.*
2. *Shigella dysenteriae.*

Preparation of working solution

The dried plant extract was dissolved in Dimethyl sulfoxide (DMSO) to final extract of 50 mg/ml.

Standard drug

The standard drug used for this study was Tetracycline (0.5mg/ml).

Control drug

The control drug used for this study was Dimethyl sulfoxide (DMSO).

Agar well diffusion method

The antimicrobial activities of the extracts were carried out by well diffusion method using suspension containing 10^8 CFU/ml of bacteria spread on Mueller Hinton Agar (MHA) medium[11]. A sterile 8 mm diameter cork borer was used to prepare a well in the MHA agar. Each well was filled with 100 microliters extract of plant material. The inoculated plates were incubated at 37°C for 24 hours. The antibacterial activity was measured as the diameter in mm of clear zone of growth inhibition around the well.

Relative Percentage Inhibition of Drugs[12]

Percentage of relative inhibition zone diameter= [(IZD Sample- IZD negative control) / IZD antibiotic standard] X 100

Where, IZD - Inhibition zone in diameter (mm).

RESULTS AND DISCUSSION

Spice extracts were prepared as per standard procedures. The antimicrobial activity of individual and their combined extracts against the selected microorganisms were assessed by presence or absence of inhibition zone. The zone of inhibition was estimated by agar well diffusion method and the diameters of zones are as shown in Table No.2.

The entire individual extracts and their different combinations showed broader antimicrobial activity against bacteria. The diameters of inhibition zones of individual extracts from different spices ranging from 12 to 22 mm against bacteria. The agar well diffusion method of antimicrobial activity yielded the inhibitory zones of 16 to 22 mm in diameters for clove, 14 to 18 mm in diameters for cumin and 12 to 20 mm in diameters for turmeric extract. The combined spices extracts showed inhibition zones ranging from 13 to 34 mm in diameters against tested bacteria. The combined extract of clove and cumin was found to be most effective in inhibiting the microbial growth among tested drugs.

The three drugs combination (clove, cumin and turmeric) did not show the greatest antimicrobial activity.

Standard drug (Tetracycline 0.5 mg/ml) showed the highest antibacterial activity. The Control drug (DMSO) did not show any antibacterial activity.

The combined extract of clove and cumin was found to be most effective in inhibiting the microbial growth. Differential antimicrobial activity of extracts against different microbes might be due to the presence of different active constituents present in the plants[13,14].

Table No.1: Plants used for this study

S.No	NAME OF SPICE	*BOTANICAL NAME*	FAMILY	PART USED
1	CLOVE	*Eugonia caryophyllata*	Myrtaceae	Bud
2	CUMIN	*Cuminum cyminum*	Umbelliferae	Fruit
3	TURMERIC	*Curcuma longa*	Zingiberaceae	Rhizome

Table No.2: Antimicrobial activities as indicated by inhibition zones of selected spice extracts and their combinations against bacteria

S.No	Name of Organism	Name of Drug(s)								
		Cl	Cu	T	Cl+Cu	Cl+T	Cu+T	Cl+Cu+T	Std	C
1	*Staphylococcus aureus*	20	18	12	26	24	18	20	52	0
2	*Streptococcus pneumoniae*	20	16	14	24	22	16	18	40	0
3	*Escherichia coli*	18	18	20	34	26	18	20	46	0
4	*Shigella dysenteriae*	22	14	12	24	16	13	16	56	0

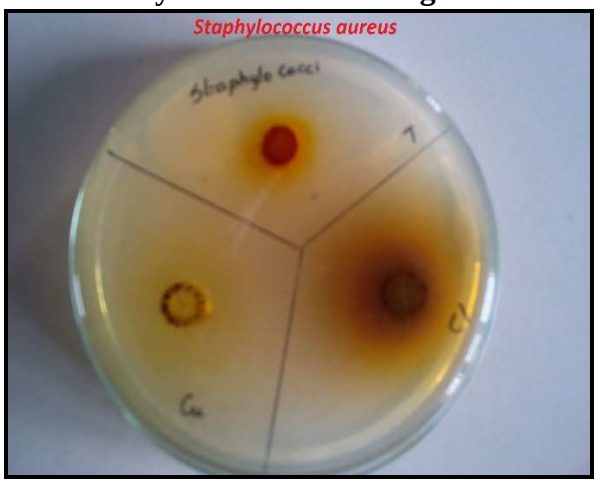
Cl – Clove, Cu- Cumin, T – Turmeric, Std - Standard drug (Tetracycline), C-Control (DMSO) and Inhibition zone in diameter (mm)

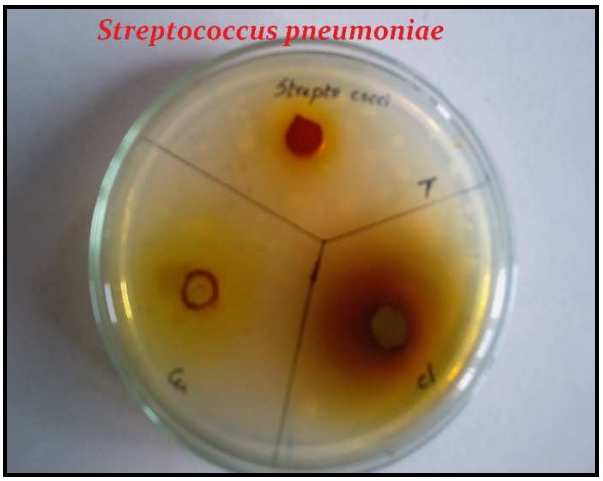
Table No.3: Relative Percentage Inhibition (%) of Test Drugs

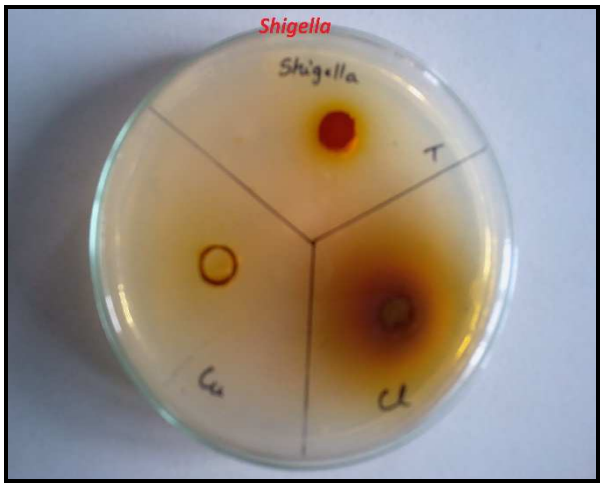
S.No	Name of Organism	Name of Drug(s)						
		Cl	Cu	T	Cl+Cu	Cl+T	Cu+T	Cl+Cu+T
1	*Staphylococcus aureus*	38.46	34.16	23.07	50.00	46.15	34.16	38.46
2	*Streptococcus pneumoniae*	50.00	40.00	35.00	60.00	55.00	40.00	45.00
3	*Escherichia coli*	39.13	39.13	43.47	73.91	56.52	39.13	43.47
4	*Shigella dysenteriae*	39.28	25.00	21.42	42.85	28.57	23.21	28.57

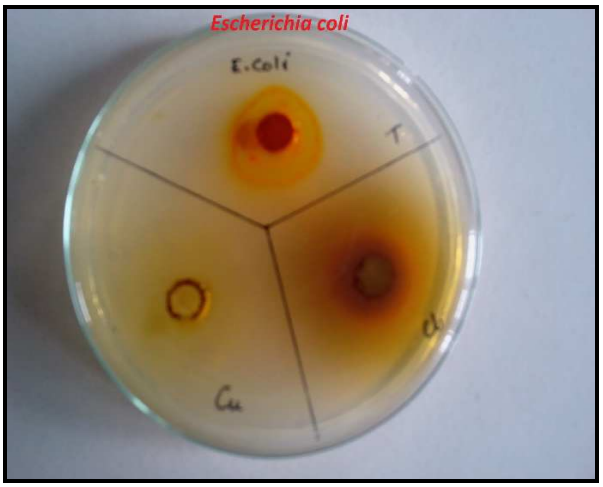
Cl – Clove, Cu- Cumin, T – Turmeric and Relative Percentage Inhibition (%)

Antibacterial activity of Individual Drugs



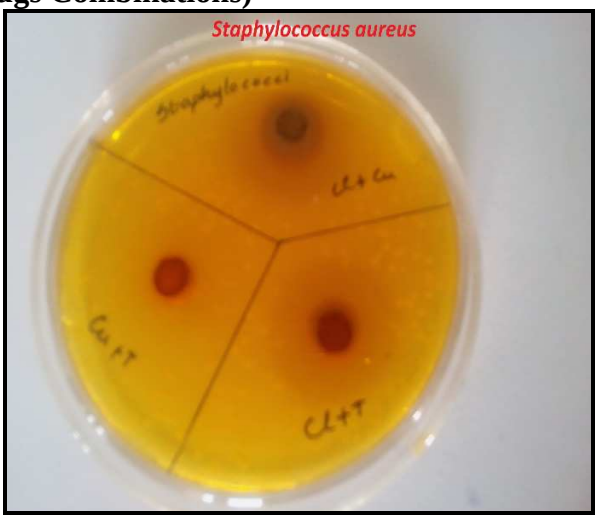


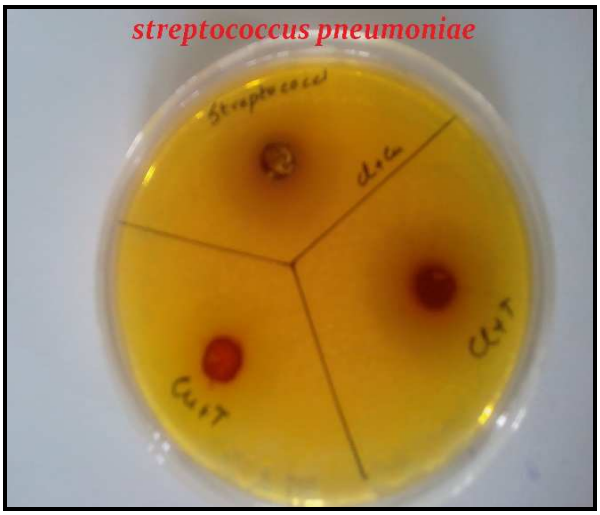


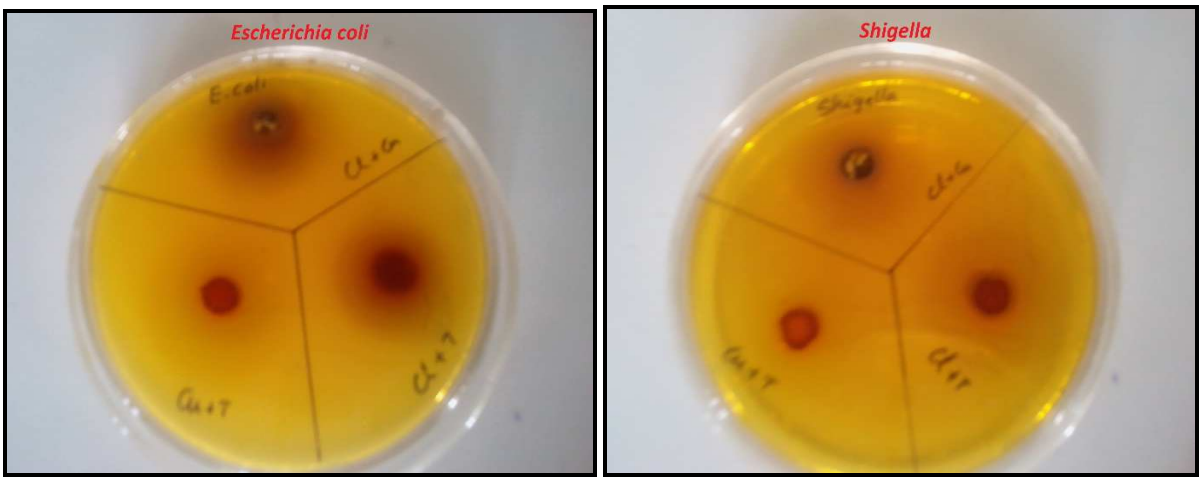


Cl – Clove Cu- Cumin T – Turmeric

Antibacterial activity of Combined Drugs (Two Drugs Combinations)

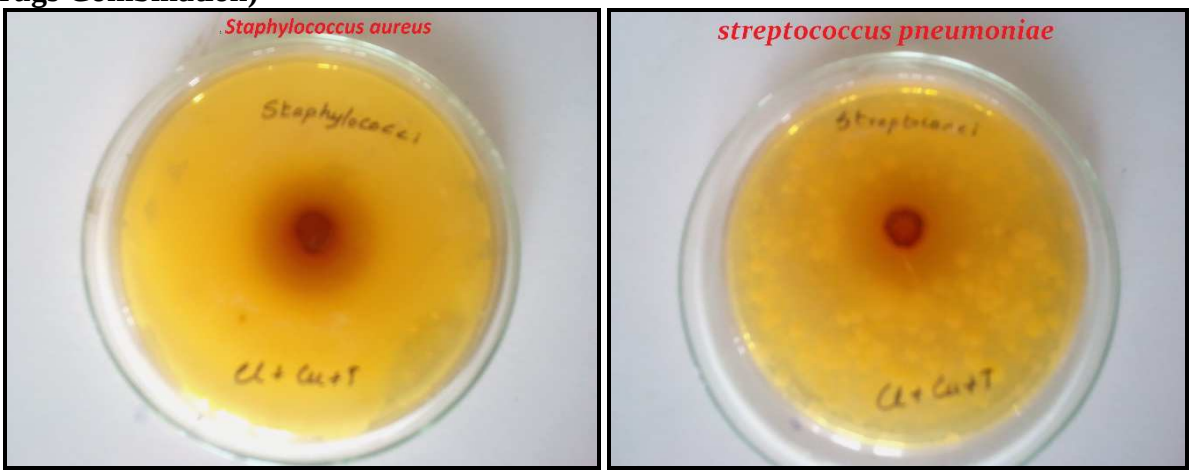


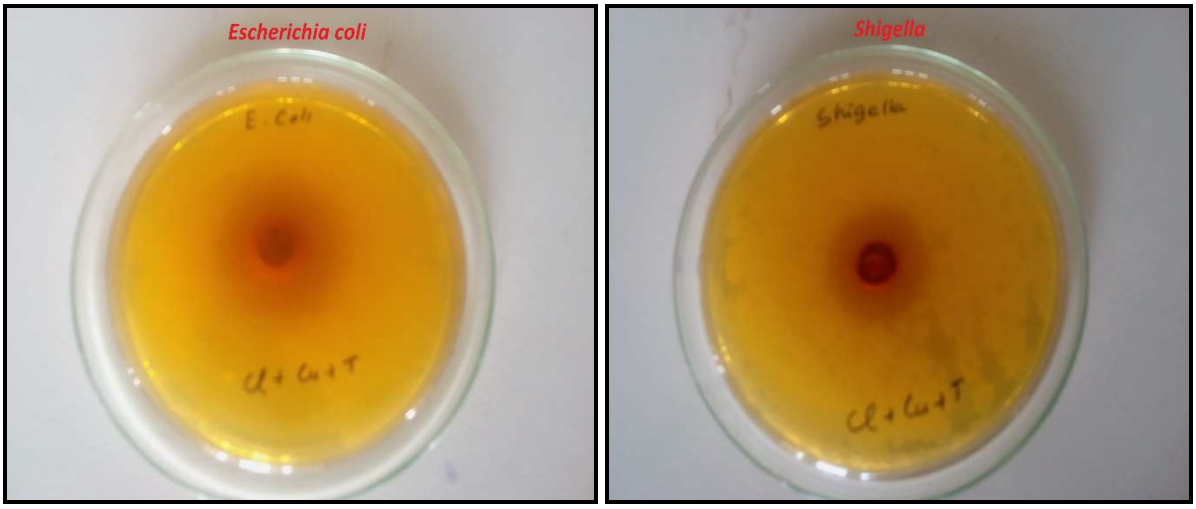




Cl – Clove Cu- Cumin T – Turmeric

Antibacterial activity of Combined Drugs (Three Drugs Combination)





Cl – Clove Cu- Cumin T – Turmeric

Antibacterial activity of Standard and Control Drugs

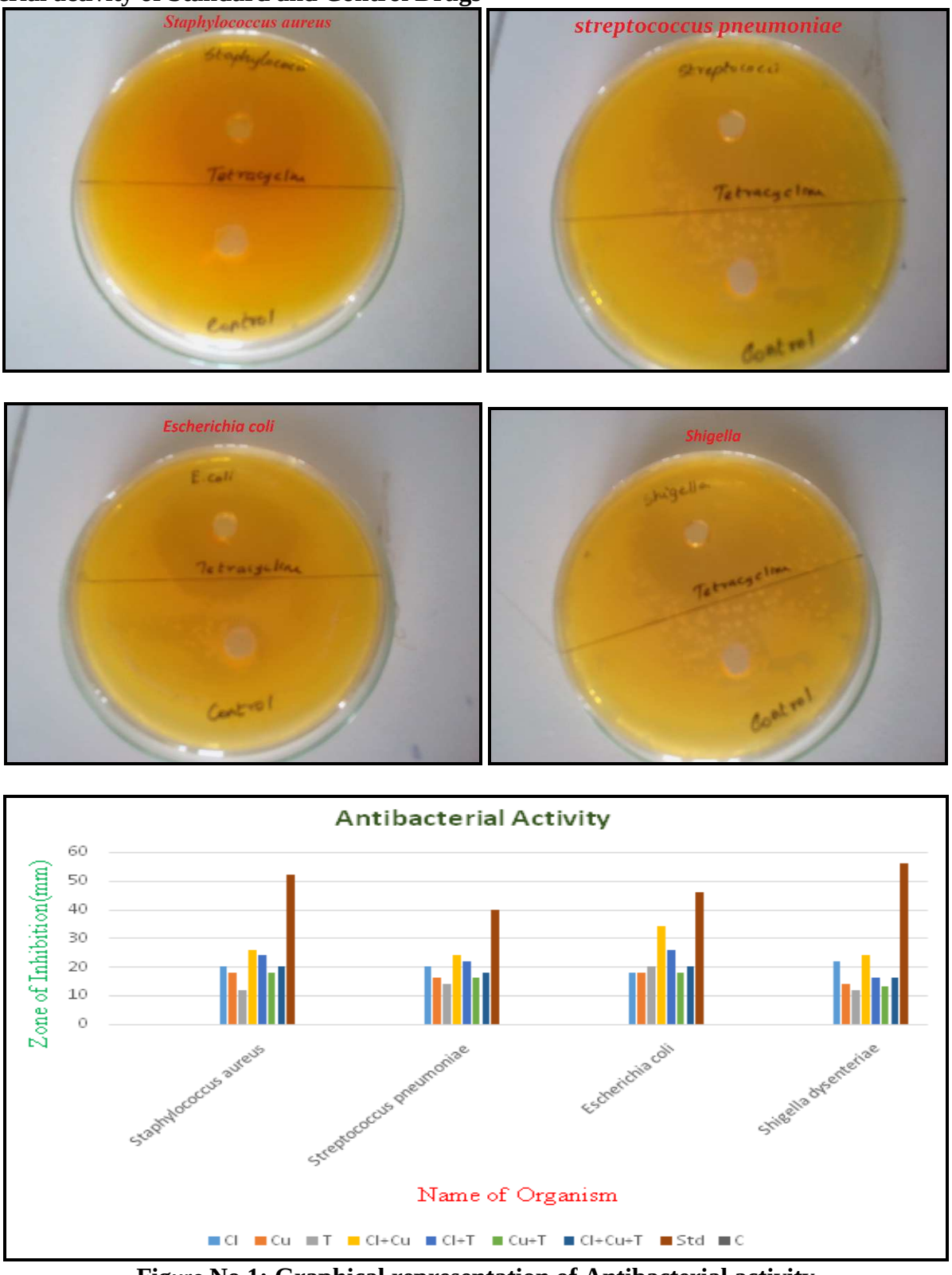


Figure No.1: Graphical representation of Antibacterial activity

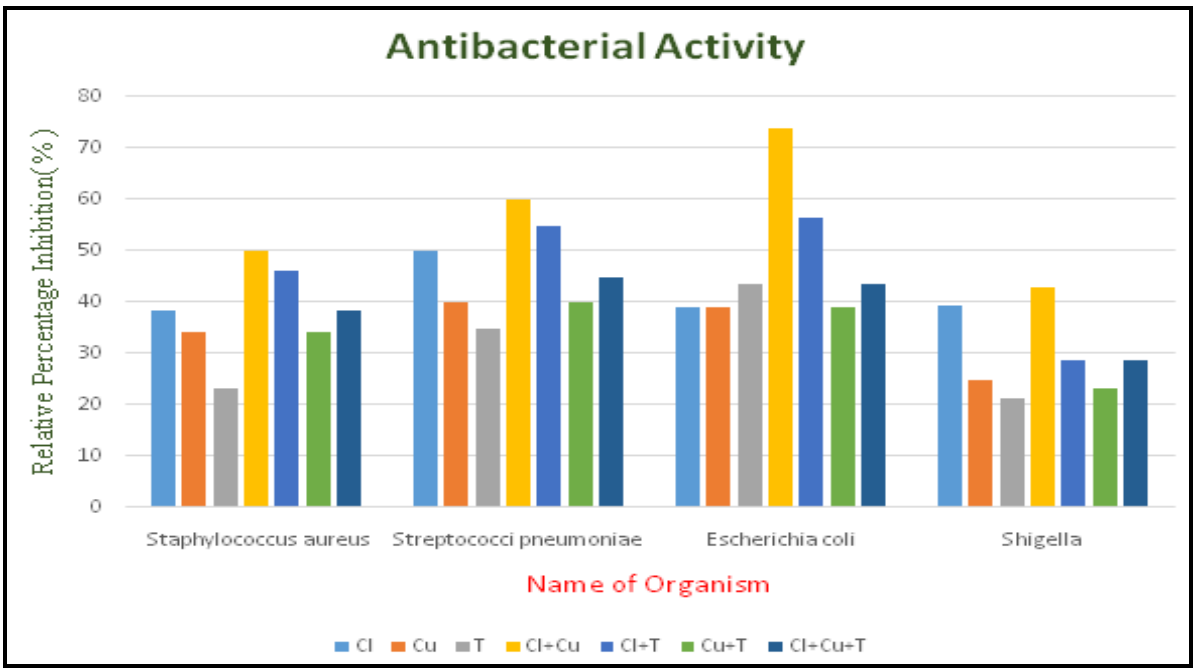


Figure No.2: Graphical representation of Antibacterial activity

CONCLUSION

The plant materials were collected and subjected to extraction using ethanol. Based on observations of this study on antimicrobial activities of clove, cumin and turmeric, it can be concluded that these spices can be used as effective antimicrobial agents against both Gram +ve and Gram –ve bacteria. The combinations of extracts provide additive/synergistic inhibitory effects making them as potent antimicrobial agents. For optimum inhibitory effect, it is necessary to establish their antimicrobial properties by standardizing their concentrations on the combined extracts. Further studies are required to identify the mechanism of interaction of different phytochemicals from the spices and their mechanism of microbial growth inhibition.

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CONFLICT OF INTEREST

I have no conflicts of interest to declare.

REFERENCES

1. https://en.wikipedia.org/wiki/Spice
2. http://24caratspices.com/the-general-introduction-of-indian-spices/
3. De A K. Spices: Traditional uses and medicinal properties, *Asian books pvt. Ltd, Dargaganj, Delhi*, 2004, 25-35.
4. John De Brito A, Herin Sheeba Gracelin D, Benjamin P and Jeya Rathna Kumar. Antimicrobial Potency and Synergistic effect of some South Indian Spices against antibiotic resistant bacteria, *Indian journal of natural products and resources,* 3(4), 2012, 557-562.
5. Daljit S Arora, Jasleen Kaur. Antimicrobial activity of spices, *International Journal of Antimicrobial Agents*, 12(3), 1999, 257-262.
6. Baljeet S Y, Simmy G, Ritika Y and Roshanlal Y. Antimicrobial activity of individual and combined extracts of selected spices against some pathogenic and food spoilage microorganisms, *International*

Food Research Journal, 22(6), 2015, 2594-2600.

7. Shan B, Cai Y Z, Brooks J D, Corke H. The *in vitro* antibacterial activity of dietary spice and medicinal herb extracts, *International Journal of Food Microbiology*, 117(1), 2007, 112-119.
8. Arora D S and Kaur J. Antimicrobial activity of spices, *International Journal of Antimicrobial Agents*, 12(3), 1999, 257-262.
9. Gold S G and Moellering R C. Antimicrobial drug resistance, *N. Engl. J. Med*, 335(19), 1996, 1445-1453.
10. Azwanida N N. A review on the extraction methods used in medicinal plants, principle, strength and limitation, *Med Aromat Plants*, 4(3), 2015, 1-6.
11. Irshad S. *et al. In-Vitro* Anti-Bacterial Activities of Three Medicinal Plants Using Agar Well Diffusion Method, *Research Journal of Biology*, 02(01), 2012, 1-8.
12. Raja A *et al, In vitro* Studies on Efflux pump Inhibition of *Cathranthusroseus* and Piperine against Ofloxacin resistant *M.tuberculosis, International Journal of Pharmaceutical science Invention*, 4(9), 2015, 32-37.
13. Das S, Anjeza C and Mandal S. Synergistic or additive 259 antimicrobial activities of Indian spice and herbal extracts against pathogenic, probiotic and food-spoiler micro-organisms, *International Food Research Journal*, 19(3), 2003, 1185-1191.
14. Hould J R and Paya M. Pharmacological and biochemical action of simple coumarins: natural products with therapeutic potential, *General Pharmacology*, 27(4), 1996, 713-22.

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