Comparative Study of Antibacterial Potential of Solvent Extracts of Some Spices Plants Against Common Pathogenic Bacteria

D. K. Shrivastava
Department of Botany and Microbiology,
Govt. E. Raghavendra Rao Postgraduate Science College, Bilaspur (Chhattisgarh) India 495001

Abstract: Traditionally spices are used as flavoring agent, coloring agent, preservatives, food additives and medicines in our country. In the present study the antibacterial properties of some spices have assed focusing on the need of alternative sources of antibiotics. Antibacterial potentials of crude extracts (Ethanol, Methanol and Hot water) of four spices plants *Eugenia caryophyllata (L.), Cinnamonum zeylanicum (L.), Zingiber officinale (L.)* and *Coriandrum sativum (L.)* have been examined against common pathogenic bacteria like *Bacillus magaterium*, *Escherichia coli* ATCC10536, *Staphylococcus aureus* ATCC 25923 employing disc diffusion method. The result indicated that various extracts of these spices plants have antibacterial properties compared with standard antibiotics. The zone of inhibition of ethanol extracts was observed with the range of 6.0 – 15.5 mm; whereas flower & bark of *Eugenia caryophyllata* (Clove) showed 6.8 -15.5mm ZOI, root of *Zingiber officinale* (Ginger) had 6.5 -13.6 mm ZOI, while fruit of *Cinnamonnun zeylanicum* (Cinnamon) & *Coriandrum sativum* (Coriander) exhibited 6.2 – 13.2 mm of inhibition zone in culture plates of such pathogenic bacteria comparing 17-19mm ZOI due to standard antibiotics.

IndexTerms **- Spices plants, Antibacterial properties, Pathogenic bacteria. ZOI**

I. INTRODUCTION

Nature has been a source of medicinal agents for thousands of years. Many of the natural sources were based on the uses of the agents in traditional medicine. This plant-based, traditional medicine system continues to play an essential role in health care (Owolabi. *et al*., 2007). According to world Health Organization medicinal plants would be the best source to obtain a variety of drugs. Extraction of bio-active compounds from medicinal plants permits the demonstration of their anti-microbial activity (Alzoreky *et al*. 2003). It also facilitates pharmacology studies leading to the synthesis of a more potent drug with reduced toxicity (Ebana *et al*., 1991, Toroglu, 2007).

An anti-microbial is a substance that kills or inhibits the growth of micro-organism such as bacteria, fungi, protozoan, etc. On the basis of mode of action, antimicrobials are classified into two broad categories as micro-biocidal that kill microbes without leaving any option for their survival and micro-biostatic that cease all the metabolic activities of microbes that are important for their survival so they are called as growth inhibitors of microbes (Khan, 2011).

The widespread use of commercially available antimicrobials led to the consequence of emergence of antimicrobial resistant pathogens that ultimately led to the threat to global public health (Davis, 2009, 2013). All commercially available antibiotics with prolonged use may have negative effect on human because they kill gut flora, so human beings need to take probiotics to replace the killed gut flora. Therefore, there is a constant and urgent need to develop new infectious disease from medicinal plants (Collins, 10=989; Baradshaw, 1997; Cordell, 2000, Prasannabalaji *et al*., 2012).

The aim of this study was to compare the inhibiting effect of spices plant products on the growth of some pathogenic bacteria *Escherichia coli* and *Staphylococcus aureus*. In the present study, the antibacterial activities of Ethanol extracts (EE), Methanol extracts (ME) and Hot water extracts (HE) of 4 plant species were studied in vitro against 2 bacterial strains by the disc diffusion method and the results are discussed.

II. MATERIALS AND METHODS

Spice samples were taken from local market noticing the fresh and bold material as commonly used in our kitchen. Simultaneously the pathogenic bacterial strains was borrowed from local recognized pathological centre and incubated in Nutrient Broth (NB) at 36 ±2.

2.1. Preparation of extraction

Plants material of selected spices was washed under running tap water, air dried and an oven dried at 40^{o}c for 24hrs for crude extract separately under aseptic condition and stored in airtight bottles. Two separate samples of the plant material (1g of each sample) were air dried powdered and extracted with hot water (2ml), ethanol 100% (2 ml) and methanol 100% (2 ml) dissolved at

aseptic condition. In this way, three different solvent extracts were obtained - hot water extract (HE), ethanol extract (EE) and methanol extract (ME) that graded further into 25%, 50%, 75%, 100% concentration with double distilled water.

2.2. Assessment of antimicrobial nature of spices plant

To evaluate the in-vitro antibacterial efficacy of spices plant product extract; bacteria were used as test system for the characterization of its toxin producing nature. Characterization of phytotoxicity as antimicrobial properties of spices plants *Eugenia caryophyllata* (Clove), *Cinnamonun zeylanicum* (Cinnamon), *Zingiber officinale* (Ginger) and *Coriandrum sativum* (Coriander) was done with the help of measurement ZOI.

2.3. Antibacterial bioassay

The antibacterial potentiality was screened by Zone Of Inhibition. Overnight cultures (at 37^{o}C for 24 hours) of each bacterial strain (*E. coli* - ATCC10536 and *Staphylococcus aureus* - ATCC 25923) were spread with glass rod on the surface of Nutrient Agar Plates. The antimicrobial activity was screened using the cork borer well (4mm in diameter) diffusion method well were saturated with 50µl (1gm/2ml) of the spices extracts of *Eugenia caryophyllata* (Clove), *Cinnamonun zeylanicum* (Cinnamon), *Zingiber officinale* (Ginger) and *Coriandrum sativum* (Coriander) and kept under laminar air flow. Agar well diffusion method was used for determining antibacterial activity. Petri plates were prepared by pouring 25 ml of seeded nutrient agar and allowed to solidify. The plates were placed in incubator for 24 hours. After 24 hours culture with spread on agar plates, a standard cork borer of 4mm diameter was used to cut uniform wells on the surface of the agar plate and 2ml extract of 3 dilutions prepared, were introduced in wells. The plates were incubated at 37^{o}C for 24hrs. After incubation, the diameter of clear zones around each well is measured and compared against zone of inhibition produced by solution of known concentration of standard antibiotic Gentamycin (10 mg) and Kanamycin (10 mg). Different concentrations (25%, 50%, 75% &100%) of extracts were used and results were observed.

III. RESULTS AND DISCUSSION

Antibacterial potentialities of crude extract of spices plant product against bacterial growth have been found variously *in-vitro* culture plate. In the present investigation, to evaluate the antibacterial efficacy of solvent extracts of *Eugenia caryophyllata* (Clove), *Cinnamonun zeylanicum* (Cinnamon), *Zingiber officinale* (Ginger) and *Coriandrum sativum* (Coriander) against the pathogenic bacterial strains (*E. coli* -ATCC10536 and *Staphylococcus aureus* - ATCC 25923) were observed by measuring the zone of inhibition (Plate 1: Fig. i –x) .

The zones of inhibition at different concentrations (25%, 50%, 75% & 100%) of the extracts against specific test organism were measured. Antibacterial potentialities of crude extracts were recorded when the zone of inhibition was found greater than 6mm. The extracts restricted the growth of the bacteria on media around the well. The zones of inhibition as observed were measured have been computed in Table 1 to 4 and shown in figure (Plate 2 & 3, Fig. - 1 to 4). The most significant an antibacterial potentiality was noticed in ethanol extracts (Plate 1: Fig. i –x) as mentioned in Table 5 and shown Fig. 5.

Crude extract of Clove exhibited the toxicity against both the bacteria during present experiment, in case of ethanol extract against *E. coli*, ZOI was found significant in higher concentration i.e. 9.2mm in 50%, 12.8mm in 75% and 15.5mm in 100%; effect of methanol extract was found lesser while the hot water extract affected more or less similar to methanol extracts. More or less similar result was observed against *S. aureus*, however lesser than E. coli in all concentrations (Table 1, Plate 2: Fig 1).

Extracts of Cinnamon was showed toxicity against both the bacteria, but lesser than Clove (Table 2, Plate 2: Fig. 2). Crude extracts of Ginger exhibited the toxicity against both the bacteria whereas ethanol extract against *E. coli* was noticed better inhibitory potential in higher concentrations while in case of *Staphylococcus aureus*, it was found slightly lesser (Table 3, Plate 3: Fig. 3), more or similar to clove. Ssimultaneously Coriander was also showed toxicity against both the bacteria (Table 4, Plate 3: Fig. 4) during present experiment but potentiality was observed lesser than other three spices.

Ethanol extracts all four spices showed more significant toxic potentialities against both pathogens, whereas in case of *E. coli*, it was noticed better effect. The zone of inhibition in 100% concentration / crude extracts was found nearer to the ZOI made by the effect of standard antibiotics (Gentamycin and Kanamycin ranges 14.5 - 17.6 mm ZOI). The findings of present investigation have correlated with the observation of other workers in case of another herbaceous / medicinal plant (Abu-Shanad*et al.*, 2004; Krishnaraju *et al.*, 2005; Ganjewala *et al.*, 2009; Shrivastava *et al.*, 2012).

IV. CONCLUSION

The present investigation justifies the classified uses of these four spices plants as the traditional system of medicine to treat various infectious diseases caused by microbes. Further chemical investigation may be carried out to isolate and identified the chemical constituents in the selected plants responsible for the antibacterial activity. The extract of these spices could be possible source to effective herbal medicines to treat the diseases caused by such bacteria as multi-drug resistant strains in community. However, it is necessary to isolate the active constituents and determine their toxicity, side effects and pharmacokinetic properties. The whole plant of these spices may be screened for other potential biological activities as well. Such screening of

various natural organic compound of plant origin and identifying active agent is the need of the hour to meet the present therapeutic need and development of new drug.

Table 1: Antibacterial effect of leaf extracts of *Eugenia caryophyllata*(Clove) on *E. coli* & *Staphylococccus aureus* and its comparison with standard antibiotics (Mean ± SD).

Plant product Extracts & Standard Antibiotics		Concentrations	Zone of inhibition (mm.) (Mean ± SD)	
			E. coli ATCC10536	*Staphylococcus aureus* ATCC 25923
Control		0%	00	00
Solvent Extract	Ethanol	25%	7.2±0.22	6.8±0.25
		50%	9.2±0.52	8.2±0.42
		75%	12.8±0.55	12.2±0.45
		100%	15.5±0.65	14.6±0.55
	Methanol	25%	7.0±0.35	6.5±0.32
		50%	8.5±0.65	8.2±0.36
		75%	11.0±0.56	10.4±0.47
		100%	14.0±0.55	13.0±0.54
	Hot Water	25%	6.5±0.65	6.0±0.56
		50%	8.2±0.54	8.5±0.55
		75%	9.8±0.66	10.0±0.58
		100%	11.0±0.72	10.5±0.60
Antibiotics	Gentamycin	10 mg. / disc	16.4±0.45	14.5±0.30
	Kanamycin	10 mg. / disc	17.06±0.35	16.2±0.73

Table 2: Antibacterial effect of leaf extracts of *Cinnamonun zeylanicum* (Cinnamon) on *E. coli* & *Staphylococccus aureus* and its comparison with standard antibiotics (Mean ± SD).

Plant product Extracts & Standard Antibiotics		Concentrations	Zone of inhibition (mm.) (Mean ± SD)	
			E. coli ATCC10536	*Staphylococcus aureus* ATCC 25923
Control		0%	00	00
Solvent Extract	Ethanol	25%	6.8±0.32	6.2±0.36
		50%	8.6±0.56	8.0±0.52
		75%	11.5±0.52	10.5±0.48
		100%	13.2±0.45	12.6±0.58
	Methanol	25%	6.4±0.45	6.0±0.42
		50%	8.5±0.62	8.0±0.52
		75%	10.4±0.46	9.4±0.45
		100%	12.0±0.35	11.0±0.48
	Hot Water	25%	6.0±0.54	6.4±0.52
		50%	8.2±0.48	8.0±0.45
		75%	9.5±0.46	9.0±0.52
		100%	10.6±0.52	9.4±0.56
Antibiotics	Gentamycin	10 mg. / disc	16.4±0.45	14.5±0.30
	Kanamycin	10 mg. / disc	17.06±0.35	16.2±0.73

Table 3: Antibacterial effect of leaf extracts of *Zingiber officinale* (Ginger) on *E. coli* & *Staphylococccus aureus* and its comparison with standard antibiotics (Mean ± SD).

Plant product Extracts & Standard Antibiotics		Concentrations	Zone of inhibition (mm.) (Mean ± SD)	
			E. coli ATCC10536	*Staphylococcus aureus* ATCC 25923
Control		0%	00	00
Solvent Extract	Ethanol	25%	7.0±0.42	6.5±0.45
		50%	9.4±0.38	8.2±0.48
		75%	12.2±0.46	12.0±0.35
		100%	14.6±0.55	13.2±0.45
	Methanol	25%	7.2±0.45	6.2±0.38
		50%	8.4±0.54	8.0±0.46
		75%	10.6±0.52	10.0±0.44
		100%	13.0±0.46	12.5±0.54
	Hot Water	25%	6.0±0.55	6.0±0.36
		50%	7.6±0.52	7.5±0.55
		75%	8.8±0.54	8.0±0.64
		100%	10.2±0.64	10.0±0.45
Antibiotics	Gentamycin	10 mg. / disc	16.4±0.45	14.5±0.30
	Kanamycin	10 mg. / disc	17.06±0.35	16.2±0.73

Table4: Antibacterial effect of leaf extracts of *Coriandrum sativum* (Coriander) on *E. coli* & *Staphylococccus aureus* and its comparison with standard antibiotics (Mean ± SD).

Plant product Extracts & Standard Antibiotics		Concentrations	Zone of inhibition (mm.) (Mean ± SD)	
			E. coli ATCC10536	*Staphylococcus aureus* ATCC 25923
Control		0%	00	00
Solvent Extract	Ethanol	25%	6.8±0.32	6.2±0.36
		50%	8.6±0.56	8.0±0.52
		75%	11.5±0.52	10.5±0.48
		100%	13.2±0.45	12.6±0.58
	Methanol	25%	6.4±0.45	6.0±0.42
		50%	8.5±0.62	8.0±0.52
		75%	10.4±0.46	9.4±0.45
		100%	12.0±0.35	11.0±0.48
	Hot Water	25%	6.0±0.54	6.4±0.52
		50%	8.2±0.48	8.0±0.45
		75%	9.5±0.46	9.0±0.52
		100%	10.6±0.52	9.4±0.56
Antibiotics	Gentamycin	10 mg. / disc	16.4±0.45	14.5±0.30
	Kanamycin	10 mg. / disc	17.06±0.35	16.2±0.73

Table 5: Antibacterial effect of leaf extracts of *Eugenia caryophyllata, Cinnamonun zeylanicum, Zingiber officinale* and *Coriandrum sativum*on *E. coli* & *Staphylococcus aureus* and its comparison with standard antibiotics 100% concentration (Mean ± SD).

Solvent Extracts & Standard Antibiotics		Solvent	Zone of inhibition (mm.) (Mean ± SD)	
			E. coli ATCC10536	*Staphylococcus aureus* ATCC 25923
Solvent Extracts	*Eugenia caryophyllata*	Ethanol	15.5±0.65	14.6±0.55
		Methanol	14.0±0.55	13.0±0.54
		Hot water	11.0±0.72	10.5±0.60
	Cinnamonun zeylanicum	Ethanol	13.2±0.45	12.6±0.58
		Methanol	12.0±0.35	11.0±0.48
		Hot water	10.6±0.52	9.4±0.56
	Zingiber officinale	Ethanol	14.6±0.55	13.2±0.45
		Methanol	13.0±0.46	12.5±0.54
		Hot water	10.2±0.64	10.0±0.45
	Coriandrum sativum	Ethanol	13.2±0.45	12.6±0.58
		Methanol	12.0±0.35	11.0±0.48
		Hot water	10.6±0.52	9.4±0.56
Antibiotics	Gentamycin	10 mg. / disc	16.4±0.45	14.5±0.30
	Kanamycin	10 mg. / disc	17.06±0.35	16.2±0.73

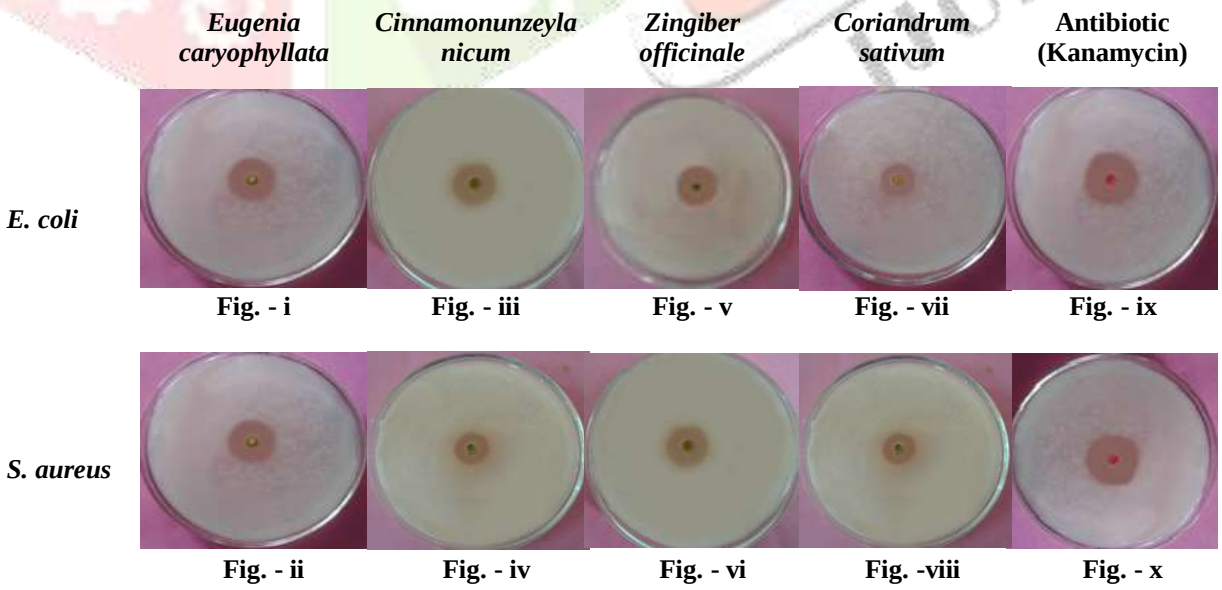

Plate1: Fig.-i-x: Showing comparative view of inhibition zone made by Ethanol extracts of spices plants

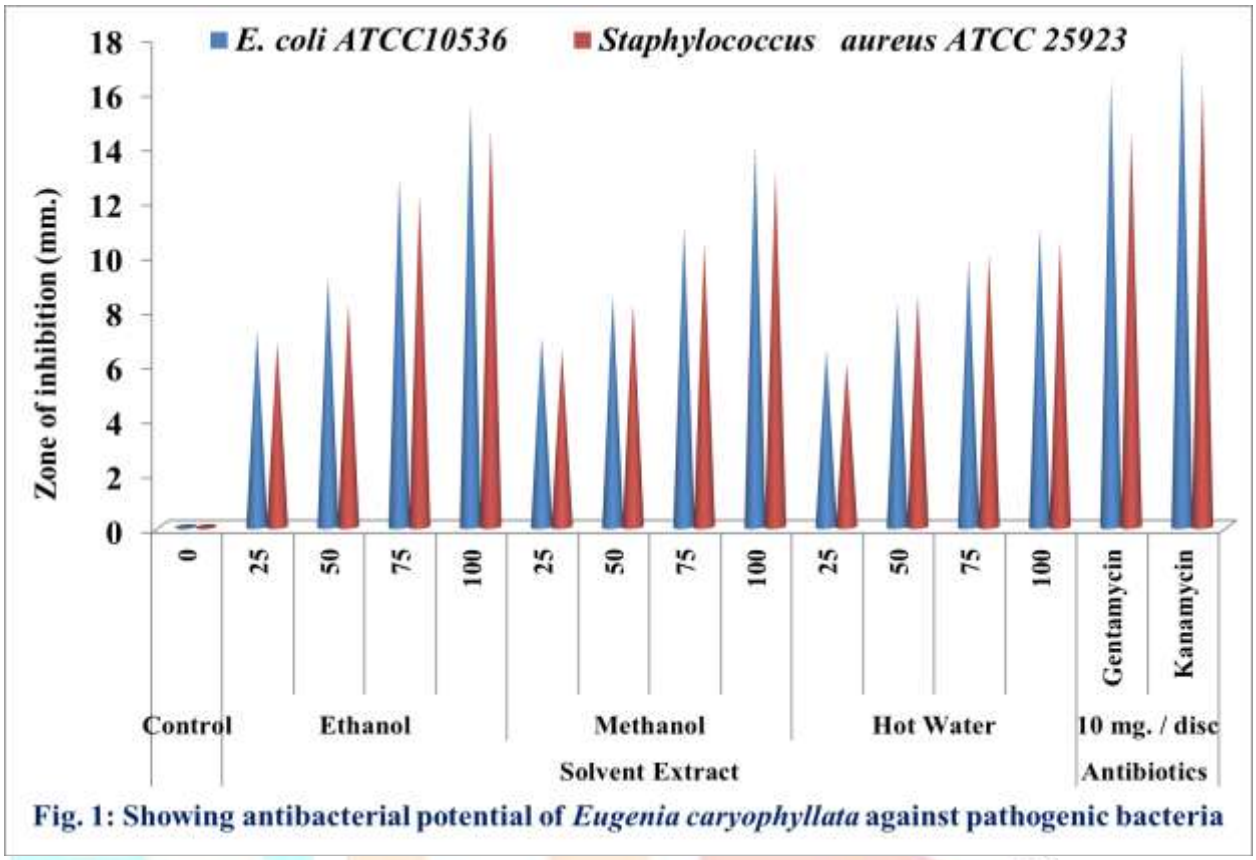

Fig. 1: Showing antibacterial potential of *Eugenia caryophyllata* against pathogenic bacteria

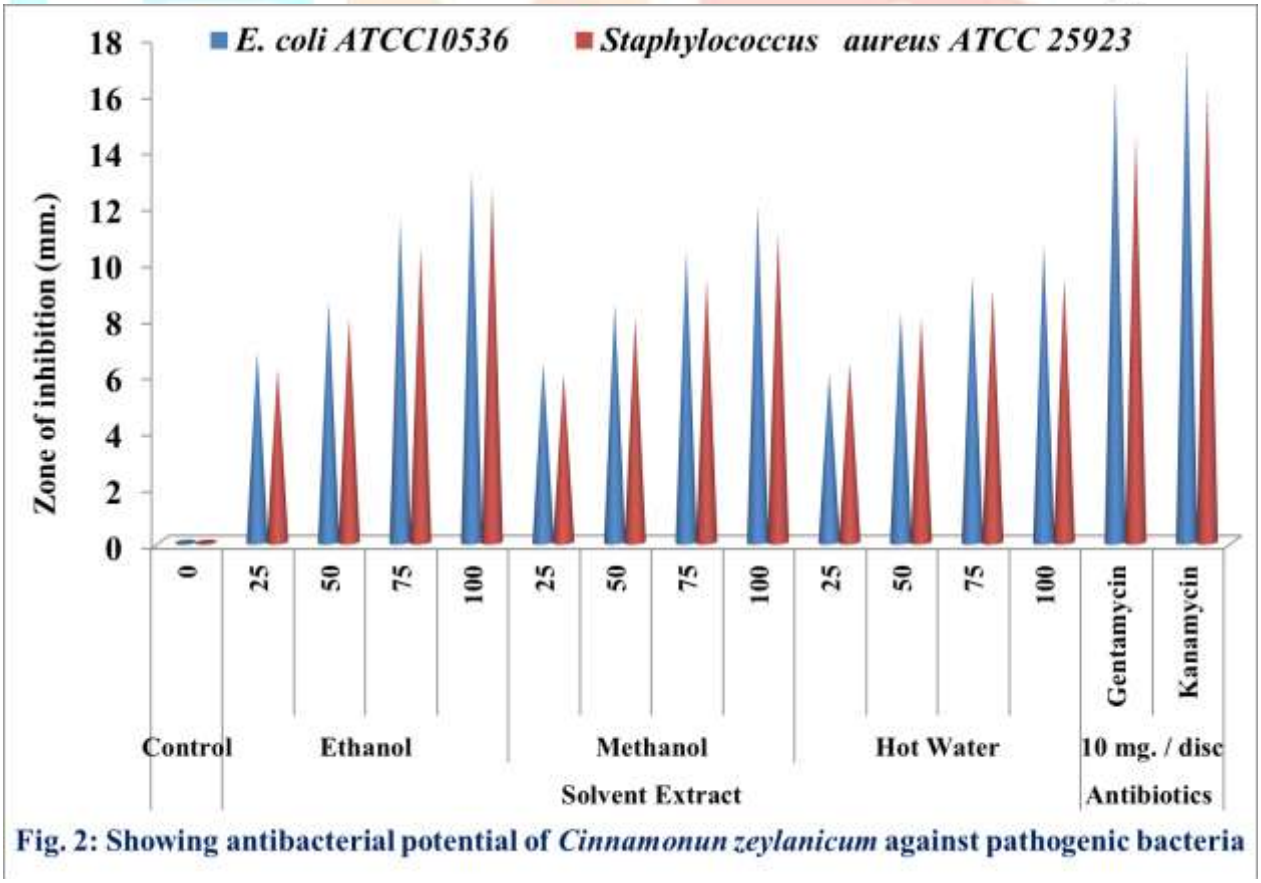

Fig. 2: Showing antibacterial potential of *Cinnamonun zeylanicum* against pathogenic bacteria

Plate 2: Fig. – 1&2: Showing antibacterial potentialities of Clove and Cinnamon extracts

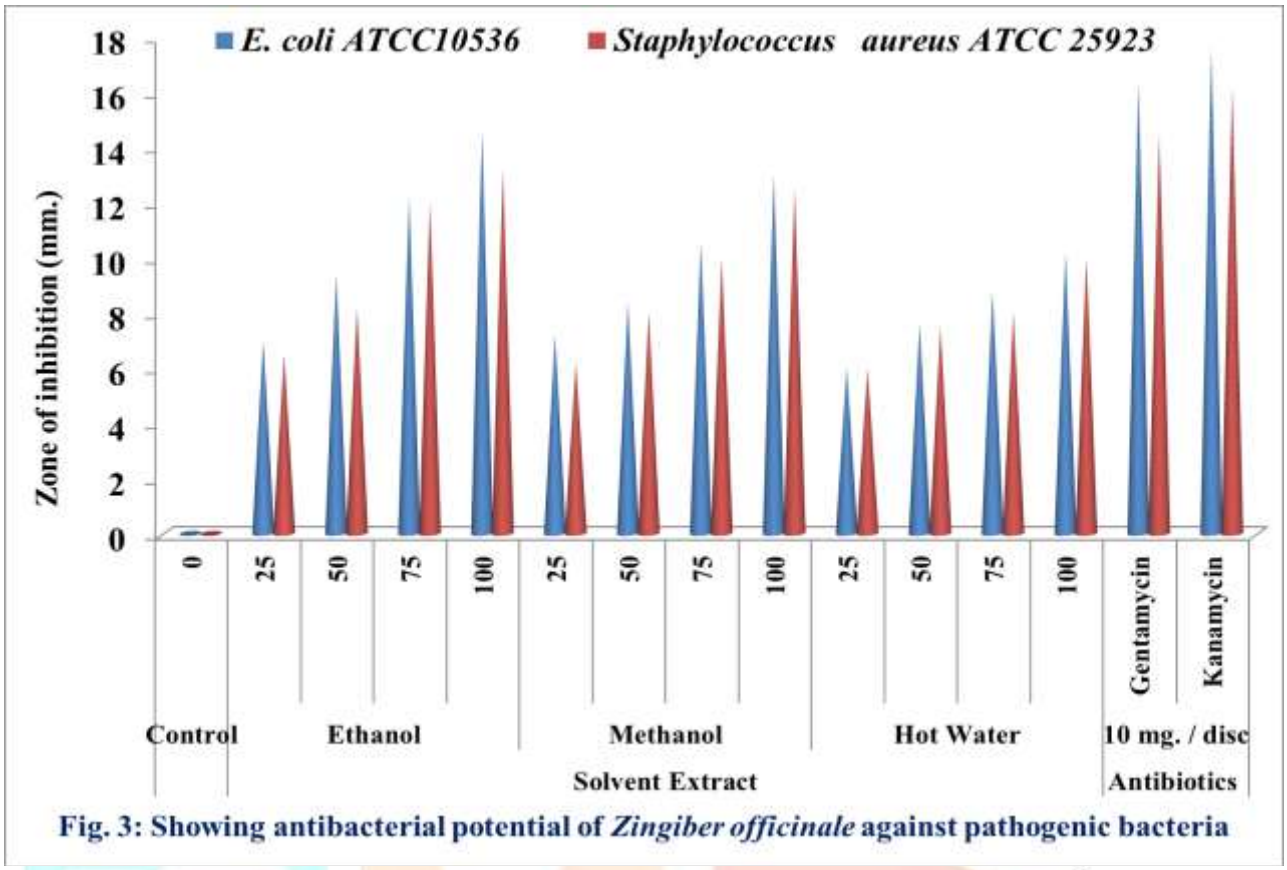

Fig. 3: Showing antibacterial potential of *Zingiber officinale* against pathogenic bacteria

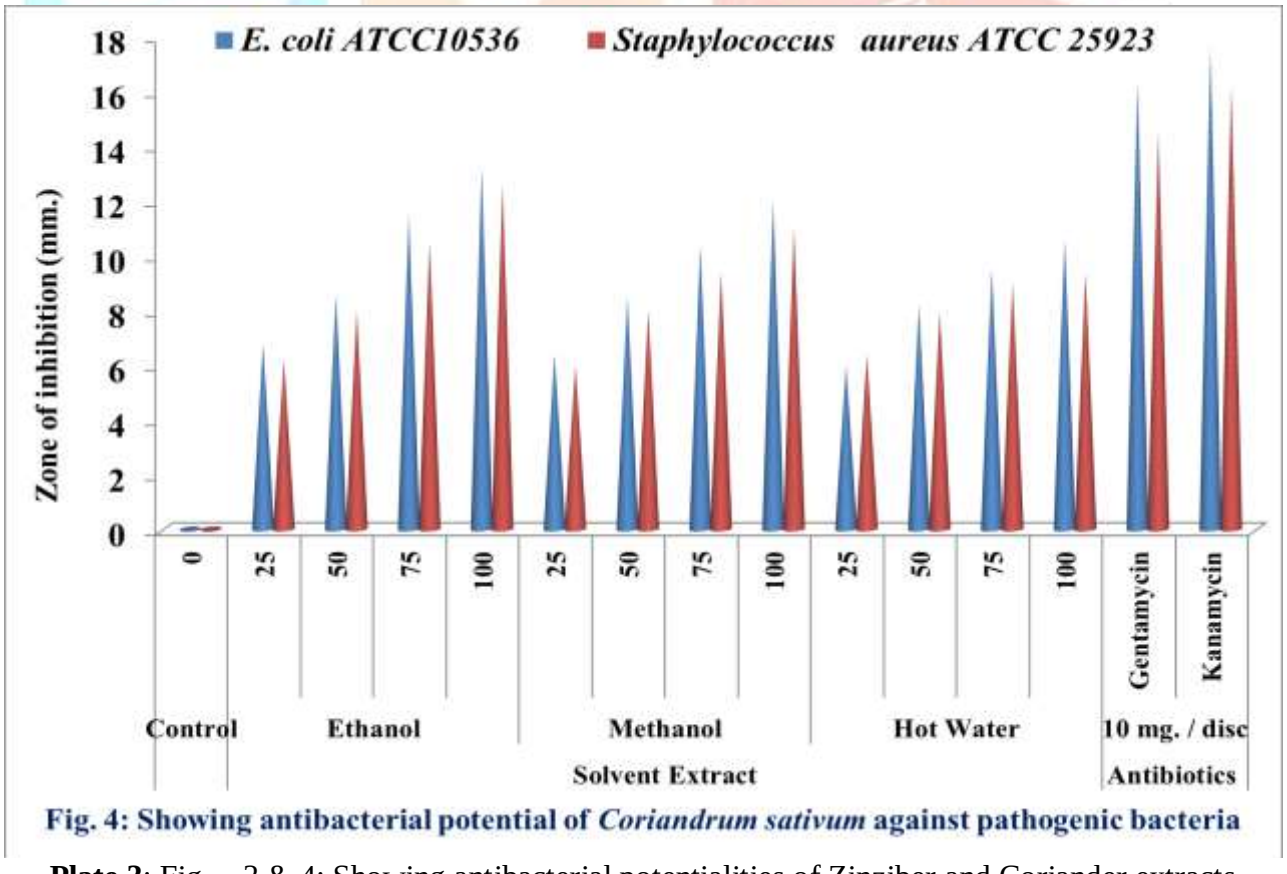

Fig. 4: Showing antibacterial potential of *Coriandrum sativum* against pathogenic bacteria

Plate 3: Fig. – 3 & 4: Showing antibacterial potentialities of Zinziber and Coriander extracts

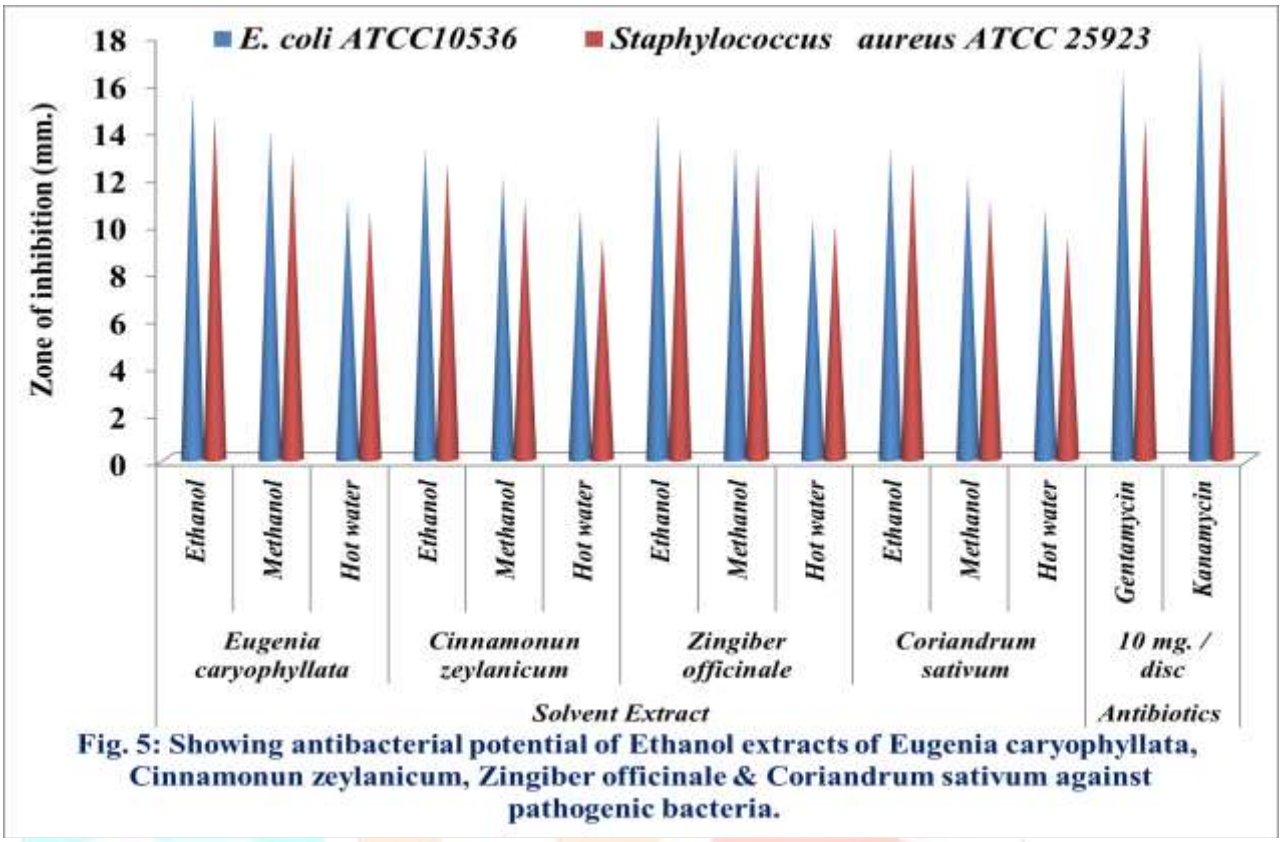

Fig. 5: Showing antibacterial potential of Ethanol extracts of Eugenia caryophyllata, Cinnamonun zeylanicum, Zingiber officinale & Coriandrum sativum against pathogenic bacteria.

V. ACKNOWLEDGMENT

Authors are thankful to Pathological center, Bilaspur city and the Principal, Govt. E. Raghavendra Rao Postgraduate Science College, Bilaspur (C.G.) for providing necessary facilities and encouragement.

REFERENCES

[1]. Abu-Shanad, B., Adwan, G., Abu-Safiya, D., Jarrar, N., Adwan, K., (2004) Antibacterial activities of some plant extracts utilize in popular medicine in palestine. Turkish Journal of Biology, Vol. –28, Pp. 99- 102.
[2]. Alzoreky, N. S. and K. Nakahara (2003) Antibacterial activity of extracts from some edible plants commonly consumed in Asia. Int. J. Food Microbiol, 80, 223-230.
[3]. Bradshaw, L. J. (1997) Laboratory microbiology. 4th Edn., Saunders College Publishing. Fort Worth.
[4]. Collins, C. H., P. M. Lyne and J. M. Grange (1989) Microbiological methods. 6thEdn.,Butterworhs, London. p. 410.
[5]. Cordell, G. A, (2000) Biodiversity and drug discovery – a symbiotic relationship. *Phytochemistry*, 55, 463-480.
[6]. Davies S., Grant J., Catchpole M. (2013) The drugs don't work. A Global Threat. London: Penguin Specials.
[7]. Davis, J. (2009): *Science*, 264, 375-82.
[8]. Ebana, R. U. B., Madunagu, B. E., Ekpe, E. D. and Otung, I. N. (1991) Microbiological exploitation of cardiac glycoside and alkaloids from *Garcinia kola*, *Borreriaocymoides*, *Kolanitida*and *Citrus aurantifolia*. *Journal ofApplied Biotechnology* 71: 398 – 401.
[9]. Ganjewala, D., Silviya, S. and Khan, K. H. (2009) Biochemical compositions and antibacterial activities of Lantana camara plants with yellow, lavender, red and white flowers. EurAsian Journal of BioSciencesEurAsia J BioSci 3, 69-77.
[10]. Khan, J. A. and Tewari, S. (2011)*Asian Journal of Plant Science and Research*, 2011, 1(1), 22-30.
[11]. Krisharaju, A.V., Rao T.V. and Sundararaju. A. (2005) Assessment of bioactivity of Indian medicinal plants using Brine shrimp (Altenariasalania) lethality assay. Int. J. Appl. SciEngg. 2: 125- 134.
[12]. N Prasannabalaji, N., Muralitharan, G., Sivanandan, R. N., Kumaran, S. and Pugazhvendan, S. R. (2012) Antibacterial activities of some Indian traditional plant extracts. Asian Pacific Journal of Tropical Disease, S291-S295
[13]. Owolabi, J., Omogbai, E. K. I. and Obasuyi, O. (2007) Antifungal and antibacterial activities of the ethanolic and aqueous extract of Kigeliaafricana (Bignoniaceae) stem bark. Afr. J. Biotechnol. 6 (14): 882-85.
[14]. Shrivastava, D. K., Swarnkar, K. and Chandra, T. P. (2012) Fungi-toxic Properties of Leaf Extracts of Some herbaceous wild Plants. International Journal of Science and Research (IJSR); Volume 3 (6), Pp. 1852-1856.
[15]. Toroglu, S. (2007) In-vitro antimicrobial activity and antagonistic effect of essential oils from plant species. J. Environ. Biol., 289, 551-559.