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STUDIES ON THE ANTIMICROBIAL ACTIVITY OF CLOVE OIL AGAINST *E.COLI* AND *KLEBSIELLA* SPECIES

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ABSTRACT

Clove oil can be used as an antimicrobial agent as it contain euginol as agent which shows very high antimicrobial activity. The present study was carried out to check the antimicrobial activity of clove oil against the two member of Enterobacteriaceae family which have been found to be resistance against more than 5 antibiotics isolated from clinical sample. Total 85 sample of urine and blood were examined out of which 18 isolates of *Klebsiella* spp and 20 isolates of *E.coli* were found to be highly susceptible. Among the 20 isolates of *E.coli* 17 isolates very high susceptibility to clove oil on the other hand out of 18Isolates of *Klebsiella* spp 13 isolates showed highly significant susceptibility to clove oil.

INTRODUCTION

Herbal oil represents one of the most important fields of traditional medicine all over the world. To promote the proper use of herbal oils and to determine their potential as source for new drugs, it is essential to study the medicinal plant, which has folklore reputation in more intensified way (Jigna&Sumitra, 2007). Over the past 20 years, there has been an increased interest in the antibacterial agents. Different oils from traditional medicinal plants have been approved as new antibacterial drugs. But there is still an urgent need to identify novel substances that are active toward pathogens with high resistance (Recio, 1989 and Srivastava&Musaddiq, 2011).

Recently, multiple drug resistance has been developed in microorganisms due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases (Service, 1995) making it a new source of antimicrobial agent with possibly novel mechanisms of action. Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious disease (lwu et al., 1999). Isolation of microbial agents less susceptible to regular antibiotics and recovery of increasing resistant isolates during antibacterial therapy is rising throughout the world, which highlights the need for development of new principles (Shahidi&Nik, 2004). In the present study the antibacterial activity of clove oil was analyzed to combat the multidrug resistant *E.coli* and *Klebsiella* spp.

MATERIAL AND METHOD

Microbial culture processing

The test organism i.e*E.coil* and *Klesiella*spp were isolated from the clinical samples. The present piece of work involved the processing of total 85 clinical samplesobtains from various pathological laboratories of Akola city. All the collected Samples were enriched and transferred to selective media as well as differential media like EMB agar, MaCconkey agar, CLED agar for preliminary screening the suspended colonies for *E.coli*and*Klebsiella*spp were screened culture on EMB agar and CLED agar. Obtained isolated were further confirmed based on biochemical characterization by standard conventional methods.

ANTIBIOTIC SUSCEPTIBILITY TESTING

All the isolates of *E.coli* and *Klesieela*spp were then analyzed for in vitro antibiotic susceptibility testing by using disc diffusion method 12hrs old culture of *E.coli* and *Klebsiella*spp isolate were inoculated on the surface of Muller-Hinton Agarby using sterile cotton swab to ensure the complete inoculation. Antibiotics used in this study were

Ampicillin Chloramphenicol, Co-trimoxazole, Kenamycin, Ciprofloxacian, Tetracyclin, Norfloxacin, Ofloxacin,Methicillin, Cefepime, Gentamycin, Amoxyclave, supplied by Hi-Media Laboratories, Mumbai the zone of inhibition around the discs were measured and the sensitivity resistance pattern was determined by using interpretation chart supplied by the antibiotic disc manufacturers(Hi-Media Mumbai).

Antimicrobial activity of Clove Oil (Agar well diffusion method)

Antimicrobial activity of clove oil was determined by agar well diffusion method. A loop full culture of each isolates of *E.coli* and *Klebsiella*spp were inoculated in nutrient broth in the test tube and inoculated for 12 hrs to activate the strain. The 12 hrs old culture was inoculated on Muller-Hinton Agar plates by using sterile cotton swab to ensure proper homogenization after inoculation well was punched by using sterile cork borer of 6mm diameter and 500ul clove oil was introduced in each well the plates were incubated for 24 hrsat 37C.

The clove oil was purchase from the medical store of Akola city 3ml vial manufactured by Deepti pharmaceutical Nagpur. all the isolates were tested in triplicates plates without test compound were used as control antimicrobial activity of clove oil was determine by measuring the diameter of zone of inhibition in mm compound which produce zone of inhibition more than 8mm considered as susceptible.

RESULT AND DISCUSSION

In the present study a total 20 isolates of *E.coli* and 15 isolates of *Klebsiella*85 samples of urine and blood. After the successful isolation all the isolates were subjected to in vitro antimicrobial susceptibility resistance pattern study against 12 different usually recommended antibiotics and the result was elaborated in table 1. About 60% isolates of *E.coli* shows resistance to Ampicillin, Tetracycline, Norfloxacin, Methicillin, Gentamycin, and Amoxyclave, Similarly about 55% isolates of *Klebsiellaspp* shows resistance to Ampicillin, Methicillin, and Gentamycin, and about 40% isolates shows resistance to Kanamycin, Cefepime, Amoxyclave, and Chloremphenicol, all the isolates of *Klebsiella*spp and *E.coli* was found to have resistance against more than four antibiotics.

All the isolates of *E.coli* and *Klebsiella*spp were then subjected to in vitro susceptibility testing of Clove oil on Muller-Hinton Agar. Out of 20 Multidrug resistant isolates of *E.coli* 17 isolates found susceptible to clove oil showing the zone of inhibition more than 12 mm as shown in table 2.

Table 1: Antimicrobial resistance pattern of *E.coli* and *Klebsiellaspp* to individual antibiotics

Sr. No	Antibiotics	% Resistance E.coli (n=20)		% Resistance Klebsiella (n=18)	
		Resistance No	% Resistance	Resistance No	% Resistance
1.	Ampicillin	16	80	13	72.22
2.	Chloramphenicol	3	15	5	27.77
3.	Co-trimoxazole	5	25	5	27.77
4.	Kanamycin	2	10	7	38.88
5.	Ciprofloxacin	2	10	3	16.66
6.	Tetracyclin	8	40	8	44.44
7.	Norfloxacin	6	30	2	11.11
8.	Ofloxacin	4	20	3	16.66
9.	Methicillin	17	85	14	77.77
10.	Cefepime	7	35	7	38.88
11.	Gentamycin	15	75	12	66.66
12.	Amoxyclave	10	50	6	33.33

Table 2: Antimicrobial activity of Clove oil against *E.coli* and Klebsiellaspp isolates.[Zone of inhibition in mm]

Sr. No	*E.coli* isolates	Clove oil	*Klebsiella* isolates	Clove oil
1	E1	25	K1	21
2	E2	18	K2	23
3	E3	14	K3	20
4	E4	17	K4	14
5	E5	16	K5	13
6	E6	20	K6	8
7	E7	19	K7	7
8	E8	20	K8	15
9	E9	18	K9	1
10	E10	18	K10	20
11	E11	20	K11	18
12	E12	7	K12	18
13	E13	15	K13	15
14	E14	9	K14	17
15	E15	14	K15	6
16	E16	14	K16	13
17	E17	12	K17	8
18	E18	8	K18	14
19	E19	17	--	--
20	E20	16	--	--

Out of 18 Klebsiellaspp isolates 13 were found susceptible to the zone of inhibition more than 12mm (Table 2). The maximum zone of inhibition was produced by *E.coli* isolate was 25mm and minimum 7mm. Similarly the maximum zone of inhibition for Klebsiellaspp isolate was 23mm and minimum 6mm. In Indian traditional medical system a number of plants and plant products are known to possess potent medicinal properties, suggesting the plants plant product and there extract may be use full for specific medical condition. Hence in our study to identify the new compounds which can inhabit the Pathogenic microorganisms

we tested the clove oil against the Multidrug resistant isolates of two pathogenic bacteria *E.coli* and *Klebsiella* Spp.

The antibacterial activity of clove may be attributed to its antimicrobial compound, eugenol, which may be responsible for cell wall destruction and a high degree of cell lysis, (Thoroski*et al*., 1989). The results obtained from this study are comparable with that of Moreira *et al*., (2007) who observed significant bactericidal action of clove oil against *Escherichia coli*, Blaszyk and Holley, (1998), have concluded that eugenol inhibited the growth of *Escherichla coli* O157:H7 Similarly Abdel Suliman*et al*., (2007) reported that clove oil has potential antimicrobial activity against *Escherichia coli*, *Staphlococcusaureus* and *Salmonella typhimurium*. According to Seenivasan*et al*., (2006), Clove oil possesses significant antibacterial activity against the *Klebsiellapneumoniae*.

This compound has already been found to have antibacterial activity different multidrug antibacterial activity different multidrug resistant bacteria. (Ingle *et al*., 1992), in the present study all the isolates of *Klebsiella*spp and *E.coli* showed multidrug resistance found susceptible to the Clove oil. Thus from present study it can be concluded that essential oil of clove possesses antibacterial activity. We believe that the present investigation together with previous studies provide support to the antibacterial properties of clove oil. It can be used as antibacterial supplement in the development of new therapeutic agents. Additional in vivo studies and clinical trials would be needed to justify and further evaluate the potential of this oil as an antibacterial agent in topical or oral applications.

REFERENCES

Abdel M E S, Iman M O E L Bashra and EI Amin A. and EI Khalifa (2007): "Nutritive value of clove 180-165.(*Sazygiumaromaticum*) and detection of antimicrobial effect of its bud oil". *Res. J. Microbiology* 2(3): 266-2781.

Bauer AW, Kirby WM, Sherris JC, TurckM(1966):"Antibioltic susceptibility testing by a standardized single disk method".*Am J ClinPathol*. Apr;45(4):493496

Blaszyk M. and Holley R. A. (1999): "Interaction of monolaurineugenol and sodium citrate on growth of common meat spoilage and pathogenic organisms". *Int. J. Food Microbiology* 39: 175-183.

Iwu, MW, Duncan, A.R and Okunji (1999): "New antimicrobial of plant origin In Janik J ASHS press, *Alexandria VA*, PP457-462.

Jigna p and Sumitra C (2007): "Invitro antibacterial activity of the crude methanol extract of *Woodfordiafructicosa* flower Brazilian".*J. Microbiology* 38:204-207.

Moreira R., Alejandra G Ponce, Carlos E Del valle and Sarai (2007): "Effects of clove and tea tree oils on *Escherichia coli* O157:H7 in blanched spinach and minced cooked beef". *J. Food Processing and Preservatrion* 31(4):379-391.

Parze C, Paul M and Bazerque P (1990)."Antibiotic assay by agar-well diffusion method".*Acta. Biol. Med. Exp*. 15113-115

Recio MC (1989). "A review of some antimicrobial compound isolated from Medicinal Plants Phytotherape".*Res. 3*: 117-125.

Service RF (1995):"Antibiotics that resist". *resistance Science*, 270: 724-727.

Shahidi, B.G.H and Nik KA (2004):"Antibacterial activity of some medicinal plant of Iran against *Pseudomonas aeruginosa". Asian J Plant Science* 3:61-64.

SeenivasanPrabuseenivasan, ManickkamJayakumar and SavarimuthuIgnacimuthu (2006): "In vitro antibacterial activity of some plant essential oils".*BMC Complementary and Alternative Medicine*, 1186/1472-6882-6-39.

Suresh P, Ingle VK, Vijaya Lakshmi V(1992):"Antibacterial activity of Eugenol in comparison with other antibiotics". *J FdSciTechnol*, 29:254-256.

Thoroski J., Blank G., and Biliaderis C. (1989): "Eugenol induced inhibition of extracellular enzyme production by *Bacillussubtilis". J. Food Protection* 52:399-403.