JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

"ASSESSMENT OF ANTI-BACTERIAL ACTIVITY OF TULSI (O.TENUIFLORUM) AGAINST KLEBSIELLA PNEUMONIAE MTCC 109"

[1]Anjali kumari, [2]Dr. Agam Gupta

Department of Biotechnology

Raja Balwant Singh College, Agra – 282002, Uttar Pradesh (India)

ABSTRACT

Ocimum tenuiflorum (krishna tulsi) is most sacred plant of the India.It is cultivated for medicinal and religious purposes. It has many beneficial properties namely anti-oxidative, antimicrobial, antistress, antiviral and many others that's why this plant is also given the term *"Queen of Herbs"*.Tulsi extract shows inhibitory effects against pathogens such as *Klebsiella pneumoniae MTCC 109*. The aim of this study was to assest the antimicrobial effects of ethanol and distilled water (aqueous) leaf extracts of O.tenuiflorum against pathogenic bacteria name *K. pneumoniae MTCC 109* to determine their potentials as antibacterial agent. Leaves were separated from the stem then washed in clear water and dried (for 6 days) under the shady area and crushed in an Mortar and Pestle until a homogenous powder was obtained. Ethanolic and distilled water (aqueous) extract was prepared using *soxhlet extraction method*. The culture media was Muller Hinton Agar (MHA) Both extracts were serially diluted at quantities at the concentration of 1, 1/10, and 1/100 and for positive control *antibiotic Gentamicin* used. Disc diffusion method was done by soaking discs in these five various concentration and then placing on both cultured plates respectively.Both plates were left in BOD incubator for 24 hours. The activity of ethanol and distilled water Tulsi extract against *K. pneumoniae MTCC 109* was found at a concentration of 55.25% and 50% respectively. The ethanol as well as distilled water(aqueous) both shows similar zone of inhibition against the bacteria *Klebsiella pneumoniae MTCC 109*. This study would be useful for the pharmacist for preparation of specific and more effective antimicrobial formulations by using *Krishna tulsi* species . in ancient medicinal traditions and suggest that some of the plant extracts possess compounds with antimicrobial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens. It is safer to use as an herbal medicine as compare to chemically synthesized drug.

Keywords: Ocimum tenuiflorum, Ethanol extract, Distilled water extract, Klebsiella pneumoniae MTCC 109, Disc Diffusion .

I. INTRODUCTION

O. Tenuiflorum (KRISHNA TULSI) is also known as tulsi in Hindi or Tulasi in Sanskrite (Holy Basil in English) . It is widely used in Hinduism practices , it got its name from the manifestation of the goddess Lakshmi (Tulasi).It is an aromatic plant native to the tropics of Asia and Africa being medicinally important plant in the *family lamiaceae*. It is described as *'Queen of Herbs'* and *'Mother of Medicine of Nature'* because of many useful medicinal properties. According to Indian *"Padmottara Purana"*, Tulsi has

antimicrobial activities against many pathogens and can be used as mouth wash agent, for wound healing, and preservation of food stuff. *O.Tenuiflorum* is antibacterial, antiviral, antifungal, antiprotozoal, antimalarial, and can be used also for killing mosquitoes . where a garden of Tulsi plant exists is itself a centre of pilgrimage; neither servants of *"Yama"* nor disease can enter there and wherever Tulsi plant fragrance goes, the air get purified. The use of medicinal plants in traditional medicine has been described in literature dating back several 1000 years ***(Chang et al., 2016).*** The leaves are a nerve tonic and also sharpen memory. They promote the removal of the catarrhal matter and phlegm from the bronchial tube. The leaves strengthen the stomach and induce copious perspiration. Tulsi is ideal source of adaptogenic properties that controls the frequent mood swings and provide the mental peace and clarity. It is very vital to have some immuno-modulator in the body that stabilizes, recovers and maintains the proper balanced functioning of the immune system. Polyphenol Rosmarinic acid present in the Tulsi chemical composition acts as the powerful antioxidant. It protects the cells in the body from smash up due to the presence of free radicals. Excess of oxidation in the body also causes the cell damage. This acid prevents the formation of excess oxidation**. (*Devi et al.*)** investigated that the O. sanctum leaves extract is excellent antimicrobial agent; it is highly effective against gram positive and gram negative bacteria such as Staphylococcus aureus, Klebsiella pnuemoniae, P. putida, B. subtilis and E. coli. **(*Agarwal et al.*)** investigated in his research Tulsi leaves extract had been found maximum antibacterial potential against Streptococcus mutans. Antimicrobial properties of O. tenuiflorum (krishna Tulsi) have been found to be higher as compared to commonly available other species i.e. O. gratissimum, O. canum and O. basilicum etc. in India . The aqueous extract, seed oil and alcoholic extract of O. tenuiflorum exhibited antimicrobial properties against enteric pathogens. Tulsi extract has also shown significant antimicrobial properties against some of the multi-drug resistant and clinical isolates of Neisseria gonorrhoeae30 . Antibacterial properties O. tenuifloram fixed oil contains higher content of Linolenic acid which contributed towards its antibacterial activity. Fresh leaves extract of O. sanctum and oil are more effective against bacterial strains as compared to dried leaves extract.

II. MATERIALS AND METHOD :

Source of Tulsi;

Tulsi (*Ocimum tenuiflorum*), obtained from my house yard at the lawyers colony, Agra. The fresh plant leaves material was washed and then dried under the shady area for 6 days and dried leaves then grind with the help of the mortar and pestle in the powdered form. After that the tulsi powder was weigh using weighing machine 50g of the powdered sample was weighed.

Test organism- Klebisella pneumonae MTCC 109.

Klebisella pneumonae is used to evaluate the antibacterial activity. Bacterial culture was maintained in nutrient agar slants stored at 4^{O}C. *K.pneumonae* are bacteria that normally live in your intestines and faeces. Experts refer to them as *Gram-negative*, encapsulated, and non-mobile bacteria. They also have a high tendency to become antibiotic resistant. These bacteria are harmless when they are in your intestines stool.

Preparation of extract

Firstly prepared the 50g of dried tulsi powdered placed into thimble of filter paper , which is placed inside the Soxhlet extractor. Solvent used for extraction is Ethanol (60°C) and Distilled Water (60°C) . Both the extraction is carried out for 22 hrs and the extracts were concentrated by keeping the round bottom flask on hot plate at respective temperature as their solvent.

Fig.1.Tulsi Extraction during Soxhlet extraction

These extracts were heated until they become solvent free. The solvent free extracts were weighed, and stored in refrigerator at 4^{O}C. During antibacterial sensitivity test these are diluted as per the requirements. And then we weigh the tulsi (O.tenuiflorum) sample on the weighing machine .

PREPARATION OF MHA MEDIA :

We take the MHA media and then weigh of 38 gm MHA media on the the weighing machine.Then,38g of MHA dissolved in lukewarm water about 250 ml conical flask than stirred until it dissolved completely.

Fig.2.Media solidified

Then it is placed in autoclave for 20-25 min at 15 psi pressure. petri plates were taken from the Hot Air Oven and then we poured the MHA media in petriplates under the laminar Air flow.The time taken to solified the media is 35 min and solidified completely.

Preparation of Antibiotic discs and Antibiotic sensitivity test

The Discs (5mm) were prepared with whatman's filter paper and then These discs were left in Hot Air Oven for sterilisation at 170^{O}C for 30 min. After that the some discs are dipped in to 20µg/ml antibiotic (Gentamicine) for 15 min and some discs are dipped in Conc.1, Conc.1/10, and Conc.1/100 for 15 min .We took 1 ml Tulsi extract and then dissolved in 9 ml distilled water, and further on. Swab a Mueller-Hinton plate with of the Klebsiella pneumonia. Dip a sterile swab into the broth and express any excess moisture by pressing the swab against the side of the tube. Swab the surface of the agar completely. Allow the surface to dry for about 5 minutes before placing antibiotic disks on the agar and Gentamicine antibiotic disk is used. Lightly touch each disc with your sterile inoculating loop. Incubate upside down and incubate at 37^{o} C. The disc is placed on the petri plate and marked as the disc are placed then put it in the incubator for 24 hrs and the results were recorded by measuring the zones of inhibition i.e. the clear area around the disc. Record the results in table form.

RESULTS AND DISCUSSION:

RESULTS

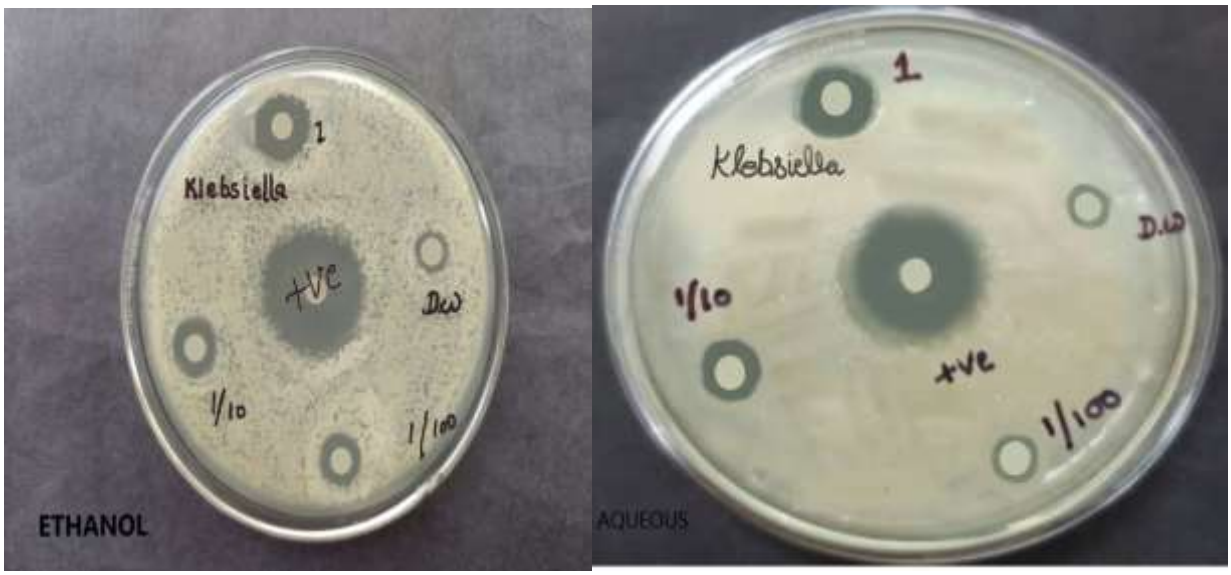


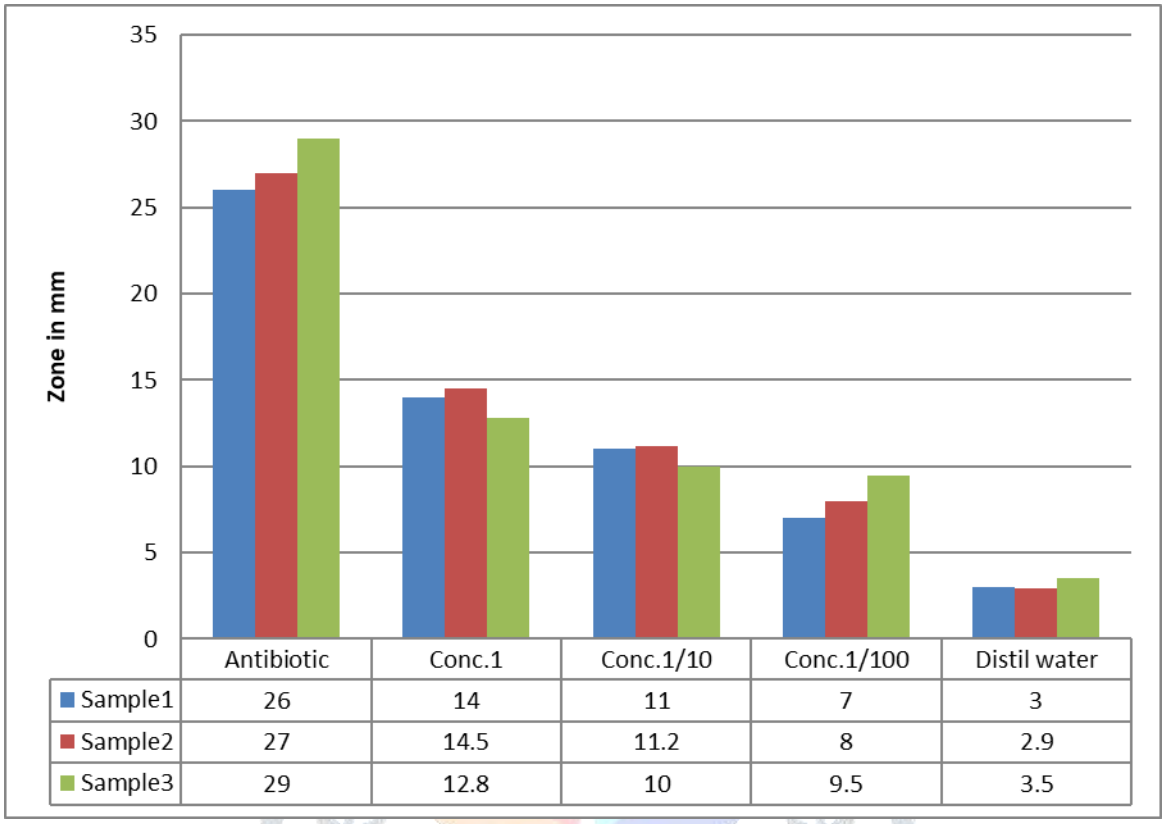
Fig.3.Result of ethanol extract and aqueous(D.W) tulsi extract on Klebsiella pneumonia MTCC 109

TABLE FORM OF THE RESULTS:

For Ethanol extract:

	Sample1	Sample2	Sample3
Antibiotic	26 mm	27mm	29mm
Conc.1	14mm	14.5 mm	12.8 mm
Conc.1/10	11mm	11.2 mm	10 mm
Conc.1/100	7 mm	8mm	9.5 mm
Distil water	3 mm	2.9 mm	3.5 mm

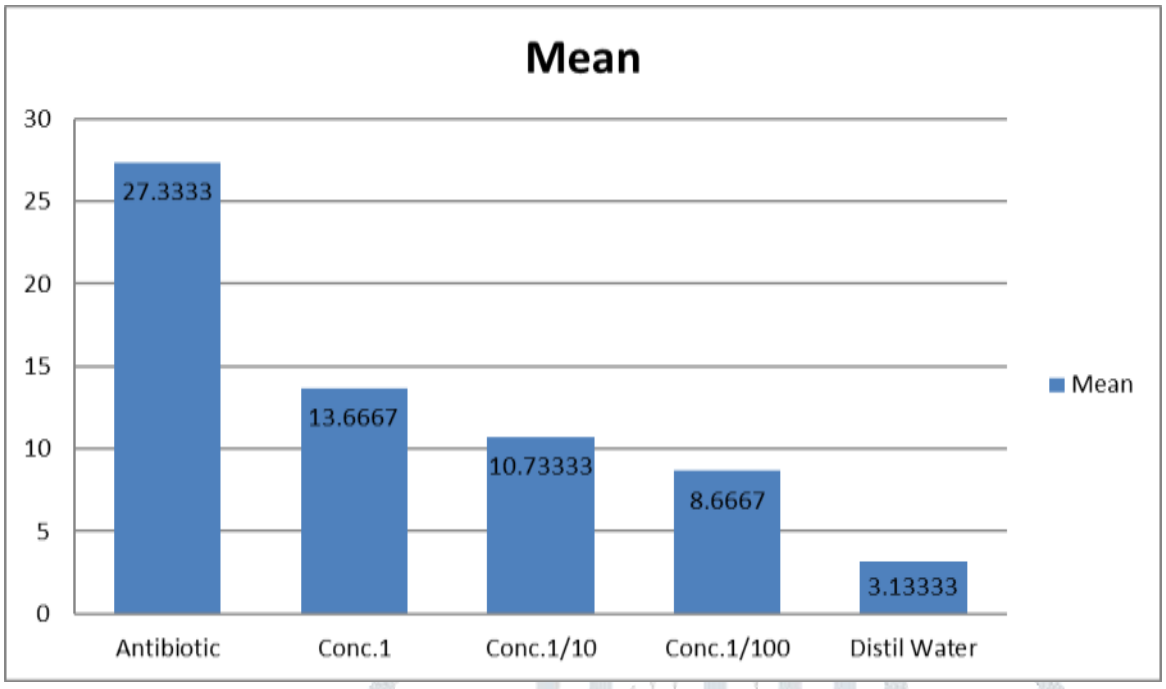
Table 1, Graph 1, for Ethanol



	Antibiotic	Conc.1	Conc.1/10	Conc.1/100	Distil water
Sample1	26	14	11	7	3
Sample2	27	14.5	11.2	8	2.9
Sample3	29	12.8	10	9.5	3.5

Statical analysis (mean);

	Antibiotic	Conc.1	Conc.1/10	Conc1/100	Distil Water
Mean	27.3333	13.6667	10.73333	8.6667	3.13333



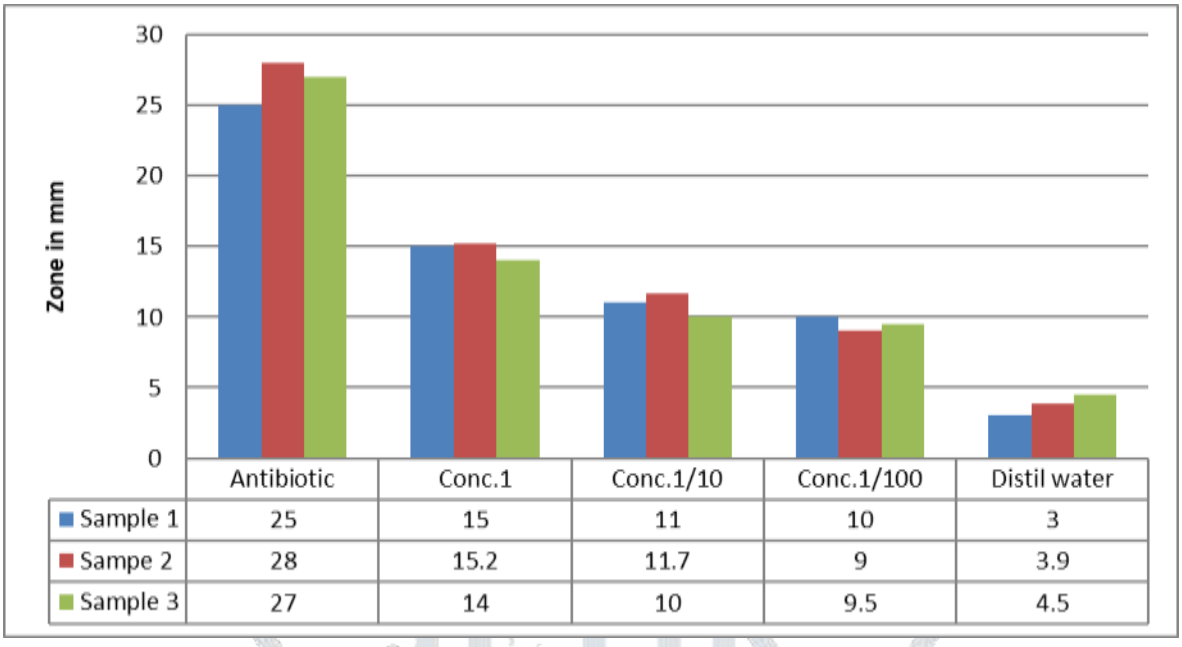
When comparison against antibiotic **conc.1** 50%, **Conc. 1/10** 39.27%, **Conc. 1/100** 31.71 %, and **Distilled Water** 11.47%.

Graphical Representation

For Aqueous extract:

	Sample1	Sample2	Sample3
Antibiotic	25 mm	28 mm	27mm
Conc.1	15mm	15.2 mm	14mm
Conc.1/10	11 mm	11.7 mm	10 mm
Conc.1/100	10mm	9 mm	9.5 mm
Distil water	3mm	3.9mm	4.5mm

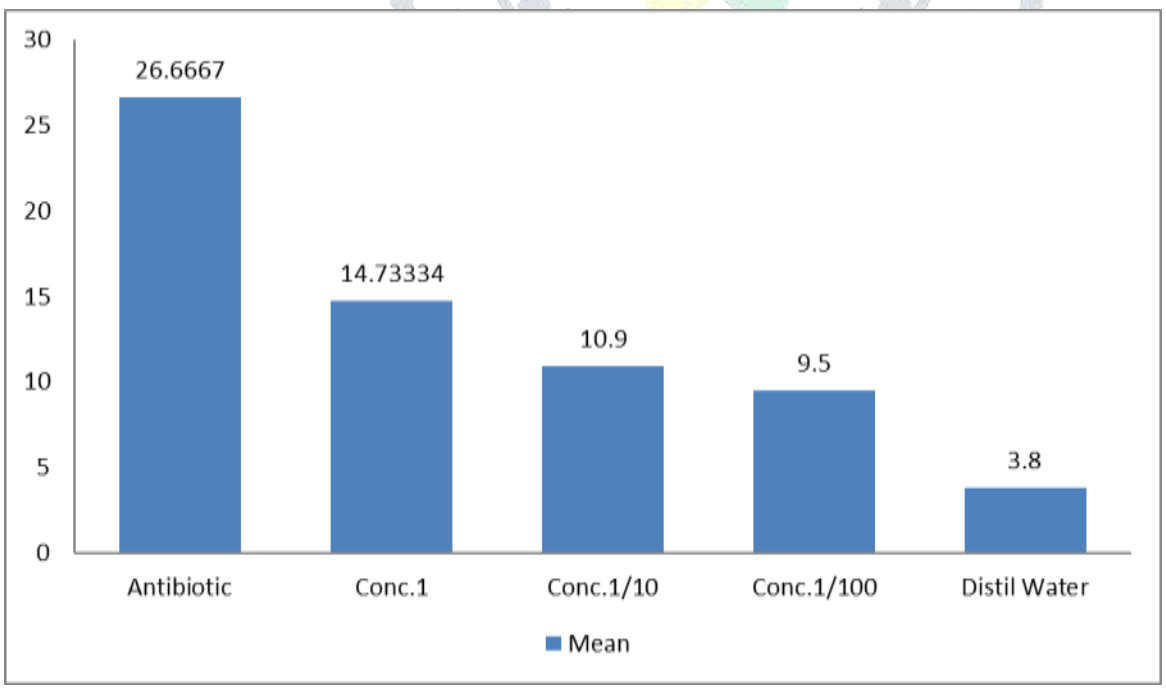
Table 1, Graph 1, for Aqueous;



	Antibiotic	Conc.1	Conc.1/10	Conc.1/100	Distil water
Sample 1	25	15	11	10	3
Sampe 2	28	15.2	11.7	9	3.9
Sample 3	27	14	10	9.5	4.5

Statical analysis (mean);

	Antibiotic	Conc.1	Conc.1/10	Conc.1/100	Distil Water
Mean	26.6667	14.73334	10.9	9.5	3.8



When comparison against antibiotic **conc.1** 55.25%, **Conc. 1/10** 37.5%, **Conc. 1/100** 35.63 %, and **Distilled Water** 14.25%.

Graphical Representation

one of inhibition displayed by Tulsi extract of ethanol and aqueous (at different concentrations), positive and negative controls against *K.pneumoniae MTCC 109* . The least zones of inhibition were displayed by the negative control. Tulsi leaves' extract showed increasing zones of inhibition with increasing concentration against K.pneumoniae MTCC 109. Mean zone of inhibition for each concentration was calculated for analysis .

When comparison against antibiotic conc.1 50%, Conc. 1/10 39.27%, Conc. 1/100 31.71 %, and Distilled Water 11.47%. When comparison against antibiotic conc.1 55.25%, Conc. 1/10 37.5%, Conc. 1/100 35.63 %, and Distilled Water 14.25%.

DISCUSSION;

In this project work my test organism on a bacteria named as **K.pneumoniae MTCC 109** . which is a gram negative bacteria that lives in the human intestines. It can cause infections that are very dangerous. Up to 50% of people with pneumonia caused by Klebsiella pneumoniae die.

I used aqueous as well as ethanol as solvent for O. tenuiflorum extract but there is no such different for zone of inhibition. It is almost same zone of inhibition with little different. **I have used 20 ml gentamicin antibiotc and 1, 1/10, 1/100, conc. Of tulsi extract, for negative control we used distill water**. For minimal inhibitory concentration i used little amount of antibiotic as compared to tulsi extract. Test show maximum zone of inhibition for positive control i. e. For antibiotic (gentamicin) and zone of inhibition decreasing as the concentration of the turmeric extract decreasing.

Zone of inhibition of antbiotic for aqueous sample 1 – 25 mm, sample 2 - 28 mm, sample 3 - 27 mm. **zone of inhibition ethanol** sample 1 – 26 mm, sample 2 – 27 mm, sample 3 – 29 mm so the zone of inhibition decrease.

In **concentration 1 zone of nhibition for aqueous** sample 1- 15 mm, sample 2 – 15.2 mm, sample 3 – 14 mm, **and zone of inhibition for ethanol** is sample 1 – 14 mm, sample 2 – 14.5 mm, so here and sample 3 – 12.8 mm, zone of inhibition increasing.

Concentration 1/10 zone of inhibition for aqueous sample 1 – 11 mm, sample 2 – 11.7 mm, sample 3 – 10 mm, **and zone of inhibition for ethanol** is sample 1 – 7 mm, sample 2 – 8 mm, andsample 3 – 9.5 mm zone of inhibion increasing.

In **concentration 1/100 zone of inhibition for aqueous** sample 1 –10 mm, sample 2 – 9 mm, and sample 3 – 9.5 mm and **zone ofinhibition for ethanol** sample 1 – 7 mm, sample 2 – 8 mm, and sample 3 – 9.5 mm, zone of inhibition increasing.

In **Distilled water zone of inhibitioin for aqueous** sample 1 – 3mm, sample 2 – 3.9 mm, and sample 3 – 4.5 mm and **zone ofinhibition for ethanol** is sample 1 – 3 mm, and sample 2 – 2.9 mm, and sample 3 – 3.5 mm zone of inhibition decreases.

Antibacterial activity of different O.tenuiflorum extracts against Kleibsiella pneumonia MTCC 109 (Gram negative becteria) were studied.

According to the results, two types of extracts obtained from Ocimum tenuiflorum leaves shown to be with antibacterial activity against tested microbial pathogen. Ocimum extract has found to be with antimicrobial properties against Kleibsiella pneumonia MTCC 109 .Highest antibacterial activity was shown by ethanol extracts against gram-negative bacteria Kleibsiella pneumonia MTCC 109 and the least antibacterial activity was shown by aqueous extracts against gram-negative bacteria Kleibsiella pneumonia MTCC 109 .Comparisons with previous studies are not justified here due to variation in the organisms tested against Tulsi for its antimicrobial effect. Since there were scarce literature available that could depict the efficacy of Tulsi against periodontal microbes specifically, the present study encourages researchers to carry out further studies assessing toxicity, durability and other assessments followed by clinical trials to provide an insight into the activity of Tulsi against periodontal pathogens on a transient as well as longitudinal basis to establish clear implications of Tulsi in periodontal disease management. With the basic limitations of the study design, generalizability is a possibility with further accumulation of evidence in this regard. However, within the limitations of the present study it could be concluded that Tulsi, as an adjunct, "if" found effective and safe on further research would be considered as a potential "adjunct" along with the standard care in the management of periodontitis to overcome the side effects of synthetic drugs, especially in this era of ever advancing clinical dentistry.

CONCLUSIONS;

The present study clearly indicates that Ocimum tenuiflorum a rich source of phyto-chemical constituents.The antimicrobial efficacy of Ocimum tenuiflorum leaves indicates that the plant possesses potent antimicrobial properties as well as tulsi is widespread in India, it can be recommended as an easily available and renewal source of antimicrobial agent instead of synthetic chemicals. During the period of project work and hands on experience over the laboratory machinery such as autoclave, laminar air flow, hot plate, BoD incubator, colony, counter vortax mixture, microscope, weight machines, centrifugation and water bath observed .During the project work only one media was use muller Hinton Agar (MHA). And two types solvent aqueous and ethanol. • From the above study it could be concluded that **TULSI** has anti bacterial activity. Zone of inhibition are (almost same) some miner change for both solvent aqueous and ethanol.Zone of inhibition and decreases as concentration for tulsi extract decreasing. Minimal inhibition conc. For antibiotic is less as compared to extract.Tulsi has recieved worldwide attention for its multiple health benefits, which appear to act primarily through its antioxidant and anti infilamentary mechanism Tulsi extract could be a valuable topical antimicrobial agent for management of skin infections caused by this organisms or as a wound dressing to prevent infection.

REFERENCES:

1.Agarwal P, Nagesh L, Murlikrishnan Evaluation of the antimicrobial activity of various concentrations of Tulsi (Ocimum sanctum) extract against Streptococcus mutans: An in vitro study. Indian J Dent Res. 2010;21:357–9. [PubMed] [Google Scholar]

2. Agarwal P, Nagesh L. Comparative evaluation of efficacy of 0.2% chlorhexidine, listerine and tulsi extract mouth rinses on salivary Streptococcus mutans count of high school children – RCT. ContempClin Trials. 2011;32:802–8. [PubMed] [Google Scholar]

3.Chang J. D., Mantri N., Sun B., Jiang L., Chen P., Jiang B., et al. (2016). Effects of elevated CO2 and temperature on Gynostemma pentaphyllum physiology and bioactive compounds. J. Plant Physiol. 19 41–52. 10.1016/j.jplph.2016.02.020 [PubMed] [CrossRef] [Google Scholar]

4. Devi PU, Ganasoundari A. Modulation of glutathione and antioxidant enzymes by *Ocimum sanctum* and its role in protection against radiation injury. *Indian J Exp Biol.* 1999;37:262–8. [PubMed] [Google Scholar]