"TULSI" *OCIMUM SANCTUM* L. AS GERMICIDAL

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Abstract : Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. There are more than thousand known phytochemical and some of are lycopene in tomatoes, isoflavones in soy and flavanoids in fruits. "Holy Basil" (*Ocimum sanctum*) or "Tulsi" is undoubtedly the best medicinal herb ever known. It has endless miraculous and medicinal values and is being worshipped in India since thousands of years. It is an excellent germicidal herb. They are quite effective in treatment of dysentery and can rid toxins from the body. Our studies have shown that extract of tulsi leaves kills *Salmonella* and *E.coli* tests clearly depicted zone of inhibition of 3mm to 14mm for *Salmonella* and 0mm to 14mm for *E.coli*. On the basis of the results obtained for the MIC determination by disc diffusion assay on LB agar media, it was observed that for the Protein Fraction up 1/10000 dilution factor in fraction against *Salmonella typhi* and *E. coli.* For protein fraction the MIC was found to be 1/10000 dilution factor against *S. typhi* and *E. coli*.

Keywards : "Tulsi" (*Ocimum sanctum),* Phytochemicals, Antibiotic, Germicidal, *Salmonella typhi* and *E.coli.*

INTRODUCTION

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. They are nonessential nutrients, as these are not required by the human body for sustaining life. It is well-known that plants produce these chemicals to protect themselves but recent research has demonstrated that they can also protect human against diseases. There are more than thousand known phytochemical and some of are lycopene in tomatoes, isoflavones in soy and flavanoids in fruits. Medicinal plants have been used for centuries before the advent of orthodox medicine. These complex materials are referred to as secondary metabolites. Plants secondary metabolites have recently been referred to as phytochemicals. Phytochemicals are naturally occurring and biologically active plant compounds that have potential diseaseinhibiting capabilities. It is believed that phytochemicals may be effective in combating or preventing disease due to their antioxidant effect (Halliwell and Gutteridge, 1992; Farombi et al., 1998). "Holy Basil" (*Ocimum sanctum*) or "Tulsi" is undoubtedly the best medicinal herb ever known. It has endless miraculous and medicinal values and is being worshipped in India since thousands of years. Even going closer to a Tulsi plant alone can protect from many infections. It is an excellent antibiotic, fungicidal, germicidal, disinfectant, and very efficiently protects the body from nearly all sorts of day to day ailment. Every part of the plant is beneficial, stem worn around neck as rosary beads generates electric energy and fortifies the immune system. Seeds are rich in demulcent substances and aid digestion and are most suitable in genito-urinary disorders. They are quite effective in treatment of dysentery and can rid toxins from the body. In present paper its germicidal properties against *Salmonella typhi* and *E.coli* are being given.

MATERIAL AND METHOD

1. Cultivation of *Ocimum sanctum* (Tulsi) : *Ocimum sanctum* (Tulsi) is commonly cultivated in the gardens. There are two varieties, first is the green type Sri Tulsi which is most common. The second type Krishna Tulsi bears purple leaves. Both varieties were cultivated on well irrigated lands in the kitchen garden of the candidate residence at Remount Training School and Depot, Saharanpur. The irrigated land was 15 X 10 feet. Tulsi plants were grown in the month of August to November at 15^0C - 30^0. In the experiment the Sri tulsi bearing green leaves was used.

2. Plants parts used : Fresh leaves of the Tulsi (Os) were used.

3. Plucking the leaves: - The Tulsi leaves were plucked by hand and washed thoroughly in the water.

4. Preparation of the extracts

Following extracts of Tulsi leaves were prepared for detection of pH and phytochemicals : -

(A) **Preparation of aqueous extract (sample no 1)** : Aqueous extract of Tulsi leaves was obtained by grinding 200 g of fresh leaves of the plant with 100 mL of distilled water. The extract was sonicated and centrifuged at the rate of 10,000 rpm for 10 minutes. The supernatant was used for further studied. The protein was precipitated from this extract by using cold acetone.

(B) **Preparation of acetone extract (sample no 2) :** 250 g fresh leaves were kept with 100 mL of acetone for 7 days at room temperature (25^0C) in the dark. Then, the leaves were crushed and filtered through whatmann filter paper. The filtrate was sonicated and centrifuged at the rate of 10,000 rpm for 10 minutes. The supernatant was acetone extract. It was kept in the incubator at 30^0C for 5 minutes, to evaporate the acetone. Thus, the acetone extract was prepared.

(C) **Eugenol oil by steam distillation (sample no 3):** The eugenol oil was extracted by steam distillation method.

(D) **Pure eugenol oil (sample no 4) :** The pure eugenol was used for the further studied.

(E) **Preparation of crude extract (sample no 5)** : 300 g fresh leaves were crushed in mortar pestle (kharal). Then, crushed leaves were squeezed through a fine muslin cloth and it was filtered. The crude extract was ready to use.

(F) Separation of eugenol :

It was carried into following steps :

Preparation of crude extract :

1- 250 Tulsi (Os) fresh leaves

2- 50 mL acetone

3- A Grinder

Tulsi leaves were plucked and grinded in a grinder with acetone for 2 minutes till Tulsi leaves were crushed. The mixture was kept for 7 days. After filtering, the filtrate was placed in the dryer at 25^0C to evaporate the acetone. Thus 170 mL crude extract was prepared. By this crude extract eugenol oil was separated by fractional distillation.

5. Bacterial strain – The *E. coli* and *Salmonell typhi* sampals were collected from human volunteer wastes and culture; these were identified by already present mounted slides in the lab and cultured. The single colony of the sample (*E. coli* and *Salmonella typhi*) picked and inoculates in the 100 ml LB broth.

6. Preparation of the solution for antibacterial activity : Five types of samples were used crude extract, water extract, acetone extract and pure eugenol. The solution in prepared in the acetone. The ratio of the extract and acetone was 2:3. The sample measured by a micropipette. The serial dilution was made. Then bacterial cultured plate was taken, the well were made in the plate at different position as 1A, 1B, 1C, 1D, 1E, 1F, antibacterial drug and for a control in the isolation chamber. This plate was kept for 24 hrs in incubator. The anti bacterial activity was recorded by measuring the zone of inhibition by vernier caliper. This method was repeated for different samples. The treated area was compare with the ampicillin and with the control (acetone) and tabulated.

7. Media preparation (for bacteria culture) : For the MIC disc method was used, in this experiment the best result was shown by the method and presented in the tables 1 and 2 as well as in the plates. 5mm diameter discs size were used which were dipped in the different extracts. The LB-Agar culture media was prepared as per standard protocol. For making media for 100 mL, following material was used yeast (0.5g) ,NaCl (1g) Agar (1.5g) and Peptone (1g) were mixed in 80 mL of distilled water and finally make up to 100 mL. The plastic wares and glass wares were sterilized by autoclave at 121 ^{0}C at 15 psi for 20 minutes. After autoclaving the media was allowed to cool upto 50 ^{0}C and then poured into the Petridishes and allowed to solidify at room temp for 1 hrs. The bacterial strain was prepared by spreading 200 µl of broth culture of *Salmonella typhi* and *Escherichia coli* separately on different plates. *Ocimum* extract was poured in each well. Acetone was used as control and ampicillin was used as a reference antibiotic.

For germicidal activity, the crude extract was used for MIC. Then bacterial cultured plate was taken, the well were made in the plate at different position, antibacterial drug and for a control in the isolation chamber. This plate was kept for 24 hrs in incubator. The anti bacterial activity was recorded by measuring the zone of inhibition by vernier caliper. The treated area was compare with the ampicillin and with the control (acetone) and tabulated.

RESULT AND DISCUSSION

Salmonella typhi and *Escherichia coli* are facultative intracellular pathogens. These are obligate anarobes bacteria. Antibiotics used for controlling these sometimes interact with other drugs, raising or covering sercum levels of other drugs by increasing or decreasing the metabolic activity. The bactericidal drugs kill bacteria, slow or stop in vitro bacterial growth. It also affects the human health by their side effects. For the present study, these bacteria (*Salmonella typhi* and *Escherichia coli)* samples were collected from the human as well as from pathological lab. and cultured in the laboratories on the culture media in plates. The serial dilutions of the extract were made and these dilutions were poured in the bacterial plates to see antibacterial activities.

For *Salmonella typhi***,** the sample no 4 showed a zone of inhibition ranging from 25mm when used crude extract ,while on dilution with acetone from 33% to 0.1% as shown in the table, the zone of inhibition ranged from 20mm to 5mm in comparison to the control acetone which showed a zone of inhibition of 8 mm while for the *E.coli.* the zone of inhibition ranged from 25mm to 10mm in comparison to the control acetone which showed a zone of inhibition of 8 mm (table-2 and 4, plate 1 and 2).

The results of the experiments showed that the sample no 5 had the minimum antibacterial activity against *Salmonella typhi.* For *Salmonella typhi,* the sample no 5 showed a zone of inhibition ranging from 5mm when used crude extract , while on dilution with acetone from 33% to 0.1% as shown the table, the zone of inhibition ranged from 2mm to 0mm in comparison to the e.control acetone which showed a zone of inhibition of 8 mm while for the *E.coli.* zone of inhibition was 4mm in comparison to the control acetone which showed a zone of inhibition of 8 mm (table-1 and 3, plate 1 and 2).

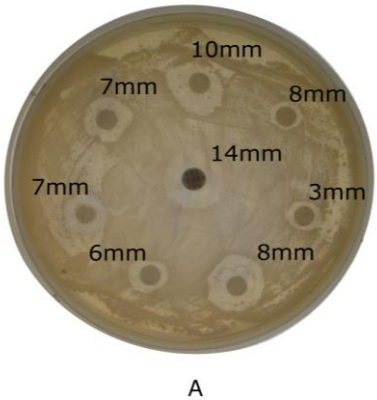
The results of the experiments showed that the sample no 1 had antibacterial activity against *Salmonella typhi.* For *Salmonella typhi,* the sample no 1 showed a zone of inhibition ranging from 20mm when used crude extract, while on dilution with acetone from 33% to 0.1% as shown in the table. the zone of inhibition ranged from 10mm to 3mm in

comparison to the control acetone which showed a zone of inhibition of 8 mm while for the *E.coli.* the zone of inhibition ranged from 5mm to 3.3mm in comparison to the control acetone which showed a zone of inhibition of 8 mm (table-2 and 4, plate 1 and 2).

The results of the experiments showed that the sample no 2 had antibacterial activity against *Salmonella typhi.* For *Salmonella typhi,* the sample no 2 showed a zone of inhibition ranging from 0mm when used crude extract, while on dilution with acetone from 33% to 0.1% as shown in the table, the zone of inhibition ranged from 8mm to 5mm in comparison to the control acetone which showed a zone of inhibition of 8 mm while for the *E.coli.* the zone of inhibition ranged from 8mm to 5mm in comparison to the control acetone which showed a zone of inhibition of 8 mm (table-1 and 3, plate 1 and 2).

The results of the experiments showed that the sample no 3 had antibacterial activity against *Salmonella typhi.* For *Salmonella typhi,* the sample no 3 showed a zone of inhibition ranging from 6mm when used crude extract, while on dilution with acetone from 33% to 0.1% as shown in the table, the zone of inhibition ranged from 9mm to 0mm in comparison to the control acetone which showed a zone of inhibition of 8 mm while for *E.coli.* the zone of inhibition ranged from 3mm to 6mm in comparison to the control acetone which showed a zone of inhibition of 8 mm (table-2 and 4, plate 1 and 2) .

On the basis of the results obtained for the MIC determination by disc diffusion assay on LB agar media, it was observed that for the Protein Fraction up 1/10000 dilution factor in fraction against *Salmonella typhi* and *E. coli.* For protein fraction the MIC was found to be 1/10000 dilution factor against *S. typhi* and *E. coli*. The experiment showed that sample no 4 i.c, pure eugenol showed maximum zone of inhibition against both bacterial strain (tables and plate). Bhaskarwar *et al* (2008) worked on the different plant extract with some bacterial strain and find out the evaluation of antimicrobial activity of medicinal plant *Jatropha podagrica* (Hook). He prepared the hexane extract of stem bark and found to be active on most of clinical isolates of *S. aureus, E.coli* and *Candida albicans*. Phytochemical test confirms the presence of steroids and triterpenes. In most cases 15mg/ml concentration shows the maximum activity, which revealed *Jatropha podagrica* as novel antimicrobial agent. However, he did not find out the zone of inhabitation of the bacterial strains. Omwenga *et al* (2009) observed less antibacterial activity of the methanol extract of *Boscia angustifolia.* Sharma (2009) studied comparative screening of antibacterial activity of weed extract *Dathura stromonius* and *Azadiractta indica*. The present study corresponds with their results. The antimicrobial activity of the essential oils of *Ocimum sanctum* L could, in part, be associated with theirs major constituents such as eugenol that compounds in *Ocimum sanctum* L. which were active against all strains were soluble in all used solvent. Mothana et al. (2009) reported pronounced antimicrobial activity of C. sempervirens against Gram-positive. Terpinyl acetate and cedrol. These components have been reported to display antimicrobial effects (Yang et al., 2007; Demirci et al., 2007). The antibacterial activity of essential oil obtained from *Ocimum sanctum* L. was in vitro tested by the LB agar-disk diffusion method. The microorganisms used in this study were: *S. typhi* and *E. coli* against which significant antibacterial activity of *Ocimum sanctum* L was demonstrated.



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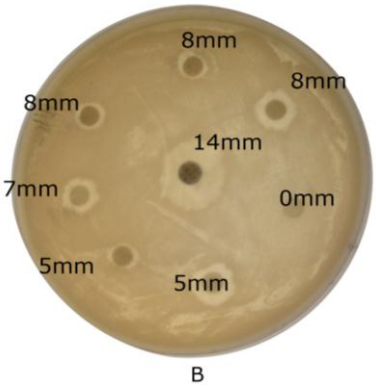

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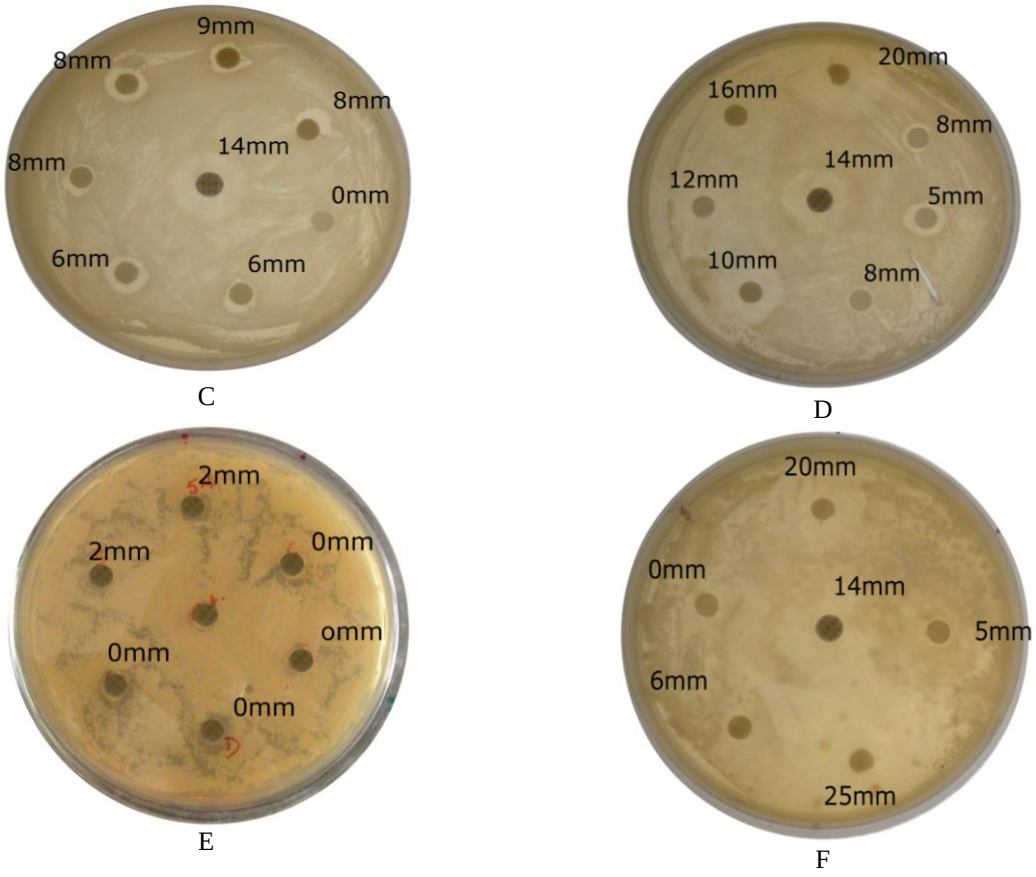
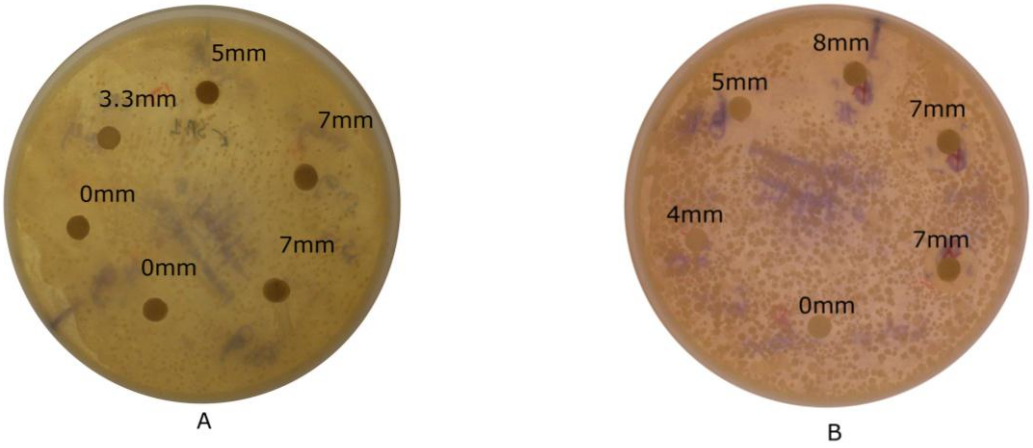


Plate -1 Serial dilution of tulsi leaves showing the zone of inhibition (in mm) against *S. typhi* (A) with aqueous extract (B) with acetone extract (C) with eugenol oil by steam distillation (D) with pure eugenol oil (E) with crude extract (F) with out fraction of dilution



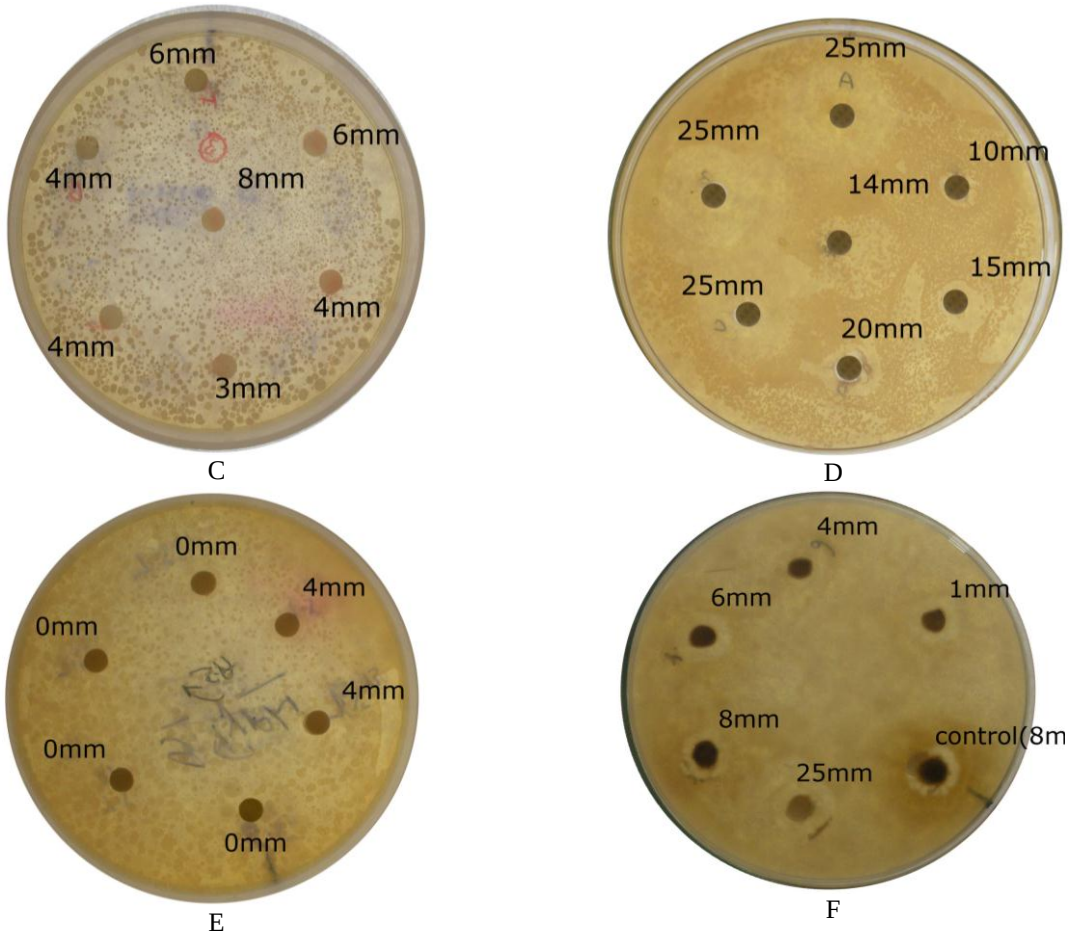


Plate -2 Serial dilution of tulsi leaves showing the zone of inhibition (in mm) against *E.colii* (A) with aqueous extract (B) with acetone extract (C) with eugenol oil by steam distillation (D) with pure eugenol oil (E) with crude extract (F) with out fraction of dilution

CONCLUSION

It is inferred from the current findings that *Ocimum sanctum* L. possess antimicrobial properties against *S.typhi* and *E.coli*, which can be used as natural antimicrobial agents for human consumption and cure of infectious diseases. The effect of the plants on more pathogenic organisms and toxicological investigations and further purification, however, needs to be carried out.

Table-1 Tulsi leaves serial dilution showing the zone of inhibition (in mm) against *Salmonella typhi*

	Dilution 1	**Dilution 2**	**Dilution 3**	**Dilution 4**	**Dilution 5**	**Dilution 6**	**Control (Acetone)**	**Ampicillin**
Sample 1	10±0.5	9±0.5	7±0.5	7±0.5	6±0.5	3±0.5	8±0.5	14±0.5
Sample 2	8±0.5	8±0.5	7±0.5	5±0.5	5±0.5	-	8±0.5	14±0.5
Sample 3	9±0.5	8±0.5	8±0.5	6±0.5	6±0.5	-	8±0.5	14±0.5
Sample 4	20±0.5	16±0.5	12±0.5	10±0.5	8±0.5	5±0.5	8±0.5	14±0.5
Sample 5	2±0.5	2±0.5	-	-	-	-	8±0.5	14±0.5

Table-2 Tulsi leaves crude samples showing the zone of inhibition(in mm) against *Salmonella typhi*

	Aqueous extract	**Acetone extract**	**Extract (steam distillation)**	**Pure eugenol**	**Crude extract**	**Ampicillin (mm)**
zone of inhabitation (mm)	20±0.5	Nil	6±0.5	25±0.5	5±0.5	14±0.5

Table-3 Tulsi leaves serial dilutions showing the zone of inhibition (in mm) against ***Escherichia coli***

	Dilution 1	**Dilution 2**	**Dilution 3**	**Dilution 4**	**Dilution 5**	**Dilution 6**	**Control (Acetone)**	**Ampicillin**

Sample 1	5±0.5	3.3±0.5	-	-	7±0.5	7±0.5	8±0.5	14±0.5
Sample 2	8±0.5	5±0.5	4±0.5	-	7±0.5	7±0.5	8±0.5	14±0.5
Sample 3	6±0.5	4±0.5	4±0.5	3±0.5	4±0.5	6±0.5	8±0.5	14±0.5
Sample 4	25±0.5	25±0.5	25±0.5	20±0.5	15±0.5	10±0.5	8±0.5	14±0.5
Sample 5	-	-	-	-	4±0.5	4±0.5	8±0.5	14±0.5

Table-4 Tulsi leaves crude extract showing the zone of inhibition against ***Escherichia coli***

	Aqueous extract	**Acetone extract**	**Extract (steam distillation)**	**Pure eugenol**	**Crude extract**	**Control**	**Ampicillin (mm)**
zone of inhabitation (mm)	4±0.5	8±0.5	6±0.5	25±0.5	1±0.5	8±0.5	14±0.5

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