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Evaluation of *Ocimum tenuiflorum* and *Syzygium aromaticum* phenolic ethereal oils for *In-vitro* anti-inflammatory and anti-bacterial activities

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ABSTRACT

Aim: The present study is aimed to evaluate *in-vitro* anti-inflammatory and anti-bacterial activity of phenolic ethereal oils like Tulsi *(Ocimum tenuiflorum)* and Clove (*Syzygium aromaticum)*.
Materials and methods: A total of 500 g of fresh leaves and dried flower buds of Tulsi and Clove were subjected to hydro-distillation method for 6 h using Clevenger's apparatus. The isolated ethereal oils were used for testing the *in-vitro* anti-inflammatory activity by using albumin denaturation assay, proteinase inhibitory activity at a concentration of 20, 40, 60, 80 and 100 µl/ml and anti-bacterial activity against two gram positive microorganisms (*Bacillus subtilis and Staphylococcus aureus*) and two gram negative microorganisms (*Salmonella typhi* and *Escherichia coli*) at concentrations 50 µl/ml, 100 µl/ml and 200 µl/ml by adopting cup plate method.
Results: The isolated ethereal oils exhibited significant *in-vitro* anti-inflammatory effect and also inhibited the growth of both Gram positive and Gram negative microorganisms at 50 µl/ml, 100 µl/ml and 200 µl/ml concentrations.
Conclusion: The findings of this study showed that the effectiveness of Phenolic ethereal oils isolated from Clove and Tulsi. Clove (*Syzygium aromaticum)* showed enhanced anti-inflammatory and anti-bacterial activity compared to Tulsi *(Ocimum tenuiflorum)*. The present study provides evidence that *Ocimum tenuiflorum* and *Syzygium aromaticum;* Phenolic ethereal oils contain medicinally important bioactive components justifying its traditional use.

Keywords: Phenolic ethereal oils, anti-inflammatory activity, anti-bacterial activity, *Ociumm tenuiflorum, Syzygium aromaticum.*

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INTRODUCTION

Ethereal oils are the volatile fractions of aromatic and medicinal plants. They are a good source of several bioactive compounds, which possess anti-inflammatory, anti-microbial, anthelmintic**,** anti-viral, anti-cancer, anti-oxidant and insecticidal properties. Phenolic ethereal oils are a unique class of aromatic oils due to the presence of considerable amounts of volatile phenolic constituent's. They are of great important as anti-bacterial, anti-inflammatory and anti-oxidant properties. Examples of phenolic ethereal oils include eugenol, thymol, and carvacrol which are usually the major constituents of Clove, Tulsi and Thyme oils respectively[1].

MATERIALS AND METHODS

Plant material

The Fresh leaves of Tulsi (*Ocimum tenuiflorum)* are collected from Venkatapur Village, Medchal District, Telangana, India.

The Dried unripe flower buds of *Syzygium aromaticum* was procured from local market Hyderabad. Voucher specimens' were preserved in the herbarium of School of Pharmacy, Anurag Group of Institutions, Venkatapur, Ghatkesar, Telangana, India.

Ocimum tenuiflorum, (holy basil) is cultivated on a large scale in India and in the Southeast Asian. It is cultivated for medicinal use and for religious belief. It has long been documented as a diverse and rich source of ethereal oils. At the same time, it is used in cooking for its flavor and fragrance, so the fresh or dried leaves were add to many

foods, such as rice, pasta, and salads in addition the, herb has medicinal and cosmetic uses. Traditionally the various parts like leaves and flowers are being used to treat various disorders such as skin disorders, cold, fevers, vomiting, and cough swelling[2]. The ethereal oil of *Ocimum tenuiflorum* is characterized by remarkably high concentrations of methyl eugenol (82.9%). The other minor constituents were β-caryophyllene (4.1%), borneol (2.4%), germacrene-D (2.3%) and α-copaene (1.9%). Phenyl derivative (83.8%) constituents were the prominent group of compounds followed by sesquiterpene hydrocarbons (11.1%), oxygenated monoterpenes, (3.1%), monoterpene hydrocarbons (0.6%) and oxygenated sesquiterpene (0.3%) [3].

Clove (*Syzygium aromaticum*) is one of the most valuable spices that have been used for centuries as food preservative and for many medicinal purposes. Clove is native of Indonesia but nowadays is cultured in several parts of the world. The plant represents one of the richest sources of phenolic compounds such as eugenol, eugenol acetate and gallic acid and posse's great potential for medical, cosmetic, food and agricultural applications[4]. The secondary metabolites produced by the plant are used for anti-bacterial, anti-inflammatory, and anti-oxidant properties[5].

The ethereal oil of *Clove* is contains high concentrations of 70.1% of eugenol, β-caryophyllene (4.8%), α-humulene (0.55%), α-terpenyl acetate (0.1%), methyl eugenol (0.2%), humulene epoxide (0.2%), and chavicol (0.3%). On the other hand, a number of compounds like sesquiterpenic hydrocarbons, alcohols and oxides, methyl ketones, aliphatic alcohols, and esters are present in trace amounts[6].

Extraction of the ethereal oils

Leaves and flower buds were subjected to hydro-distillation for duration of 6 hours using 500ml of distilled water in Clevenger apparatus. The obtained yielded was 1.0% (Tulsi) and 1.4% (Clove). It was then stored in a screw capped glass vials in a refrigerator maintained at 4–5 ^{0}C until use.

***In- vitro* anti-inflammatory screening**

Inhibition of albumin denaturation

The reaction mixture (5 ml) consisted of 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate buffered saline (PBS, pH 6.4) and 2 ml of varying concentrations of the essential oils was added to reach final concentrations (20, 40, 60, 80, and 100 µl/ml). Similar volume of double distilled water served as control. Then the mixtures were incubated at 37±2 ^{0}C in an incubator for 15minutes and then heated at 70 ^{0}C for 5 minutes. After cooling down, their absorbance was measured at 660 nm using vehicle as blank. The Diclofenac sodium at the final concentration of (20, 40, 60, 80, and 100 µl/ml) was used as reference drug and treated similarly for determination of absorbance[7].

Anti-proteinase action

The reaction mixture (2 ml) include 0.06 mg trypsin, 1 ml 20Mm Tri HCL buffer (pH 7.4) and 1 ml test sample of different concentrations (20-100 µl/ml).The mixture was incubated at 37 ^{0}C for 5 minutes and then 1ml of 0.8% casein was added. The mixture was incubated for an additional 20 minutes. 2ml of 70% perchloric acid was added to arrest the reaction. The cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm against buffer as blank. The experiment was performed in triplicate. The percentage inhibition of proteinase inhihibitory was calculated[8].

***In vitro* anti-bacterial activity screening**

Test organisms

Two strains of gram-negative bacteria *Escherichia coli*, *Klebsiella pneumoniae*, and two strains of gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus* were used in our experiment to evaluate the anti-bacterial activity.

Disc diffusion Method

Disc diffusion method for anti-microbial susceptibility testing was carried out to assess the presence of anti-bacterial activities of the isolated volatile oils from Clove and Tulsi[9-11].

RESULTS AND DISCUSSION

***In-vitro* anti-inflammatory activity**

Table 1: Effect of isolated ethereal oils on heat induced protein denaturation

Concentration (µl/ml)	% inhibition of protein denaturation		
	Diclofenac sodium (standard)	Tulsi	Clove
20	60.25±3.56	34.44±3.06	35.98±5.16
40	70.02±1.84	36.98±2.65	36.71±4.27
60	71.49±2.94	68.93±2.07	69.00±2.4
80	76.35±3.84	72.01±1.45	74.11±3.6
100	84.10±2.16	78.64±2.93	80.10±1.48

*Each value represents the mean ± SD. N=3, Experimental group were compared with control **p <0.01considered extremely significant; *p < 0.05, non significant*

***In-vitro* anti-inflammatory activity**

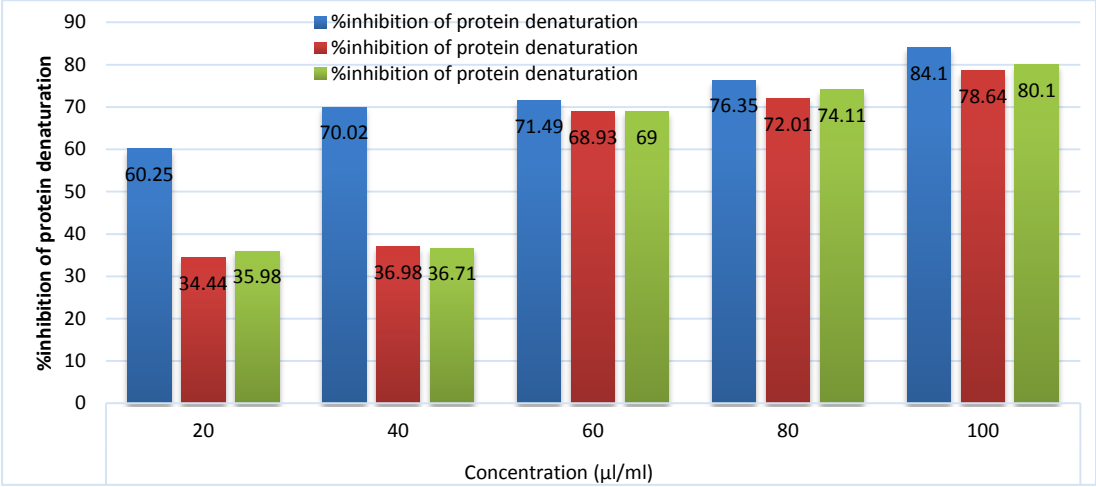


Figure 1: Effect of isolated ethereal oils on heat induced protein denaturation

Table 2: Effect of isolated ethereal oils on proteinase inhibitory action

Concentration (µl/ml)	% inhibition of proteinase action		
	Diclofenac sodium (standard)	Tulsi	Clove
20	60.25±3.64	34.44±2.52	35.98±.84
40	70.02±4.62	22.4±1.48	26.5±2.88
60	71.49±2.54	31.6±3.62	33.00±2.84
80	76.35±4.12	42.2±2.84	48.1±2.60
100	84.10±2.20	54.6±1.84	58.8±2.64

Each value represents the mean ± SD. N=3, Experimental group were compared with control **p <0.01considered extremely significant; *p < 0.05, non significant

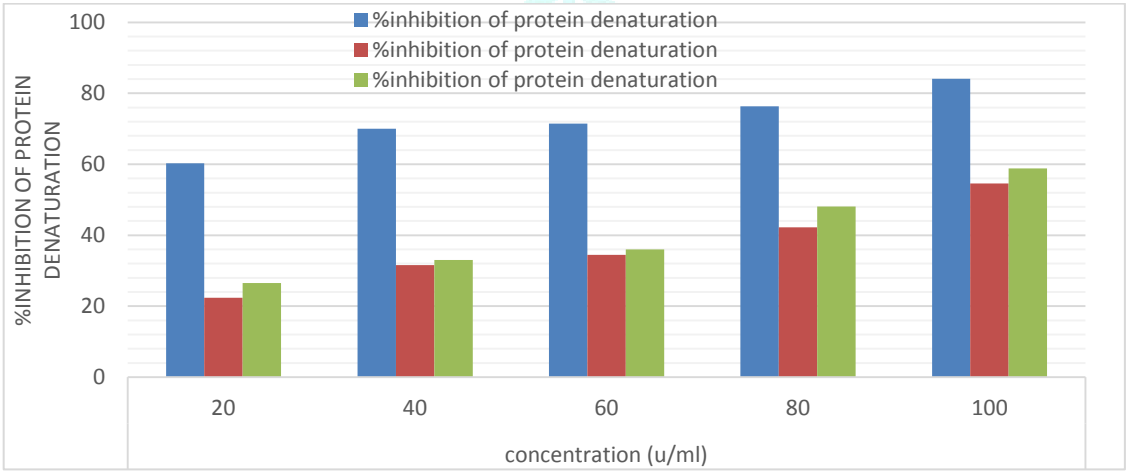


Figure 2: Effect of isolated ethereal oils on proteinase inhibitory action

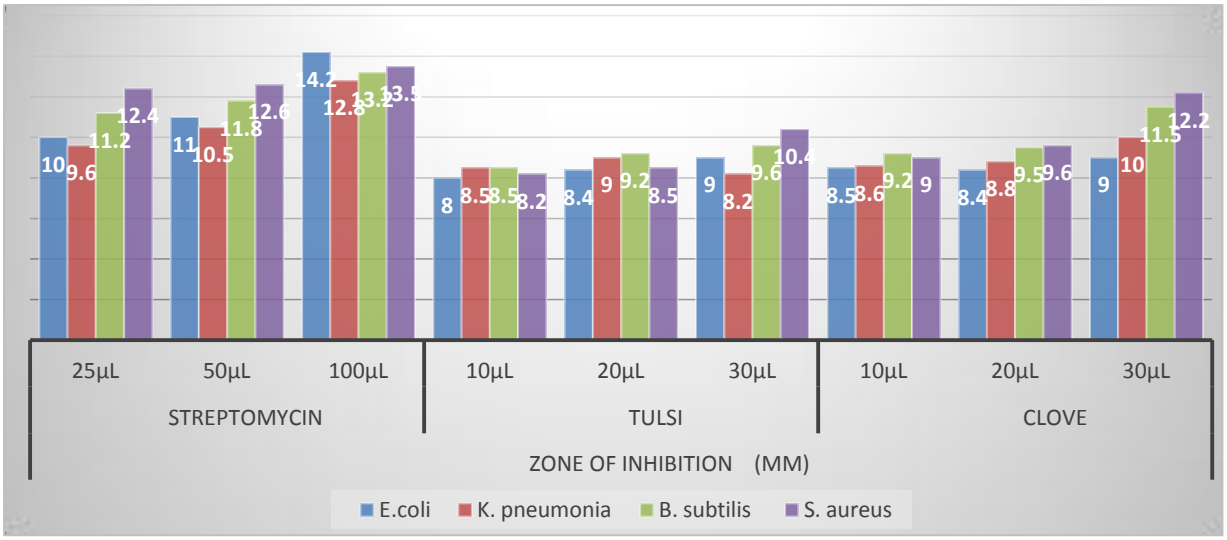
Statistical analysis

Results are expressed as Mean ± SD. The difference between experimental groups was compared by One Way Analysis Of Variance (ANOVA) followed by Dunnet Multiple comparison test (control Vs test).

Table 3: *In-vitro* anti-bacterial activity of isolated ethereal oils

Name of organism	Zone of inhibition (mm)								
	Streptomycin			Tulsi			Clove		
	25µl	50µl	100µl	10µl	20µl	30µl	10µl	20µl	30µl
E. coli	10±0.35	11±0.20	14.2±0.77	8.0±0.45	8.4±0.20	9.0±0.15	8.5±0.22	8.4±0.42	9.0±0.56
K. pneumonia	9.60±0.52	10.5±0.5	12.8±0.17	8.5±0.68	9.0±0.54	8.2±0.35	8.6±0.4	8.8±0.62	10.0±0.52
B. subtilis	11.2±0.66	11.8±0.52	13.2±0.15	8.5±0.66	9.2±0.22	9.6±0.42	9.2±0.44	9.5±0.42	11.5±0.46
S. aureus	12.4±0.12	12.6±0.70	13.5±0.42	8.2±0.71	8.5±0.12	10.4±0.36	9.0±0.52	9.6±0.72	12.2±0.38

ZI were expressed as Mean±Standard deviation of three replicates, low activity (1-6 mm), moderate activity (7-10mm), high activity (11-15 mm).



Represents mean ± S.D. mm; p < 0.05

Figure 3: *In-vitro* anti-bacterial activity of isolated ethereal oils

CONCLUSION

The above results of anti-inflammatory and anti-bacterial activity of *Ocimum tenuiflorum* and *Syzygium aromaticum* confirmed us as a useful anti-inflammatory and anti-bacterial agent. The present study provides evidence that *Ocimum tenuiflorum* and *Syzygium aromaticum* isolated ethereal oils contains medicinally important bioactive compounds and this justifies the use of plant species as traditional medicine for treatment of inflammation and bacterial infections. Furthermore, a detailed and systematic approach can be done in exploiting and identifying the phytopharmacology to explore in knowing the maximum potentiality of the plant which will be useful to mankind.

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