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Research Article

Evaluation of Immunostimulating Activity in Ethanol, Ethyl Acetate, Methanol and Chloroform Extracts of *Ocimum sanctum* L

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ABSTRACT

Immuno stimulating and cytotoxicity activities of *Ocimum sanctum* are evaluated in different solvent extracts to understand bioactive natures. Chloroform extracts (O-C) of *Ocimum sanctum* exhibited highest free radical scavenger activity (FRSA) followed by ethanol (O-E), ethyl acetate(O-EA) and methanol(O-M) respectively with IC_{50} values of 42.39µg/ml,58.45 µg/ml,42.99µg/ml,76.56 µg/ml. O-M exhibited highest anti inflammatory activity (5-Lox assay) followed by O-EA,O-E and O-C with IC_{50} values 30.76 µg/ml,16.73 µg/ml,15.99 µg/ml,14.19 µg/ml respectively. Ethanol, ethyl acetate, methanol and chloroform extracts of *Ocimum sanctum* also show anti diabetic activities (α- amylase property assay) and anti microbial activity. Cytotoxicity studies or Brine Shrimp Lethality Test (BSLT) of O-E, O-EA, O-M and O-C exhibited ED_{50} values 468.40 µg/ml, 116.25 µg/ml, 198.04 µg/ml, 157.24 µg/ml respectively. Based on the cytotoxicity studies, it is evidenced that O-E has highest anti-tumor property**,** compared to O-EA, O-M and O-C. These results suggest that phytochemicals are specific to a particular solvent and yielded varied IC_{50} and ED_{50} values of immunostimulatory activities. Also show significant anti oxidant, anti inflammatory, anti diabetic, anti microbial and anti tumor activities which could be used as a potential source of pharmaceutical materials.

Keywords: *Ocimum sanctum*, anti-oxidant, anti-inflammatory, anti-obesity, anti-microbial, anti-tumor.

INTRODUCTION

India is a varietal emporium of medicinal plants and it is one of the richest countries in the world as regards genetic resources of medicinal plants. It exhibits a wide range in topography and climate, which has a bearing on its vegetation and floristic composition. More over the agro climatic conditions are conducive for introducing and domesticating new exotic plant varieties. At present majority of the people are relying for their primary health care on traditional medicine[1]. The importance of medicinal plants and traditional health systems in solving the health care problems of the world is gaining increasing attention. Most of the developing countries have adopted traditional medical practice as an integral part of their culture. Historically, all medicinal preparations were derived from plants, whether in the simple form of raw plant materials or in the refined form of crude extracts, mixtures, etc. Medicinal plants are gaining global attention owing to the fact that the herbal drugs are cost effective, easily available and with negligible side effects. Plant based natural constituents can be derived from any part of plant like bark, leaves, flowers, fruits, roots, seeds etc .The compounds derived from medicinal plants form the ingredients of analgesic, antibiotics, laxatives ulcer treatment etc. In recent years, the quest for the isolation of new compounds from medicinal plants has become a fascinating area of research. Plants with ethno pharmaceutical importance are being exploited because of their healing properties[2]. Most antioxidants isolated from medicinal plants are polyphenols, which show biological activities include anti bacterial, anti inflammatory, anti obesity, antiviral, anti carcinogenic and immunostimulating effect. Epidemiological studies have revealed that polyphenols provide a significant protection against development of several chronic diseases such as cardiovascular diseases (CVDs), cancer, diabetes, infections, aging, asthma etc.

Ocimum sanctum Linn. (*O. sanctum*), commonly known as Holy Basil or ‘‘Tulsi’’ belonging to the family Labiatae (Lamiaceae), is considered a sacred plant in India and grown in every rural house hold. It has a versatile role to play in traditional medicine including Ayurveda, Siddha,

Figure 1: Role of polyphenols

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Figure 1: DPPH activity of O-E, O-EA, O-M, O-C extracts of *Ocimum sanctum*

O-E O-EA O-M O-C STANDARD (Ascorbic acid)

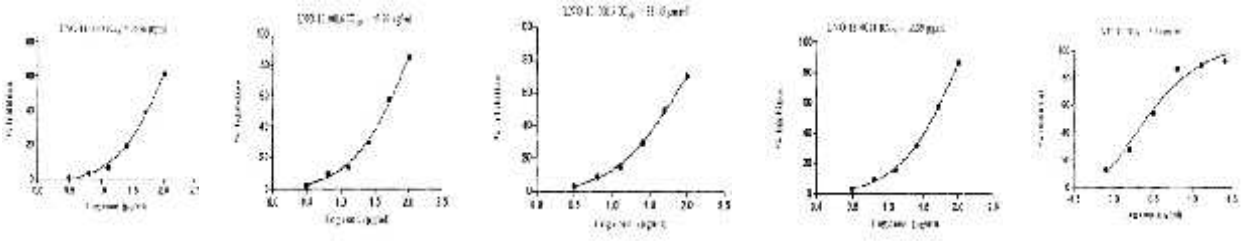
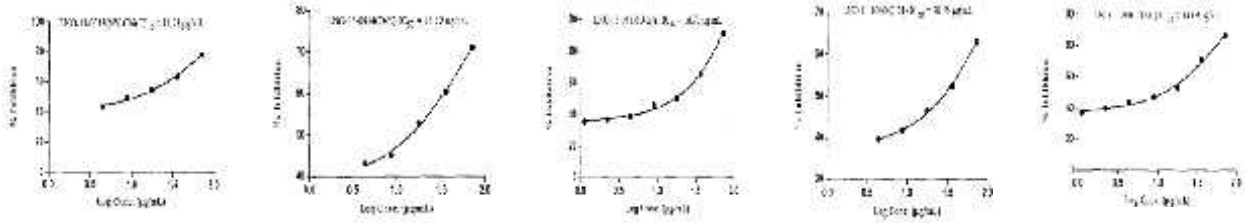


Figure 2: 5-LOX activity of O-E, O-EA, O-M, O-C extracts of *Ocimum sanctum*

O-E O-EA O-M O-C STANDARD (Curcumin)



Unani, Greek and Roman for vast therapeutic applications[3-5]. Several investigations using these extracts have indicated that *Ocimum sanctum* possesses significant anti-inflammatory[6] antioxidant[7] immunomodulatory[8], antistress[9], radioprotective and anticarcinogenic[10] and neuroprotective properties[11]. The major phenolic compounds of *Ocimum sanctum* include phenolic di- and tri-terpenes, flavonoids and phenolic acids and sterols[12]. It is clearly proved from the crude extracts of *Ocimum sanctum* that phytochemicals extracted are specific to a particular solvent and yielded varied IC_{50} and ED_{50} values.

MATERIALS AND METHODS:

Plant material: The leaves of *Ocimum sanctum* were collected from Ravikampadu, West Godavari district. The leaf samples were air dry and weighed. The dried leaves were then, ground using mortar and pestle into coarse powder. Leaves of the plant extracted in polar solvent (methanol), semi polar solvent (chloroform) and non polar solvent (ethanol) by following cold percolation method[13]. The obtained extract was then filtered through Whatmann no.1 filter paper. Extract solution and was transferred into 250ml round bottom flasks which were previously weighed. Then the extract solution was evaporated using Buchi Rotavapor R-210, Switzerland to concentrate the exract. Concentrated extracts were allowed to dry in fume cupboard, weighed again and were kept in 4°C for bioassays evaluation. Their volume was made up to obtain respective concentrations.

Free radical scavenging activity (FRSA): DPPH scavenging radical activity was determined on the basis of reduction of coloured methanolic solution of DPPH[14, 15]. FRSA of the test substances added to the methanolic solutions of DPPH is inversely proportional to the differences in initial and final absorption of DPPH solution at 570 nm. Drug activity was expressed as that 50% inhibitory concentration (IC_{50}). The reaction mixture contained 1X 10^{-4} M methanolic solution of DPPH and various concentrations of the test substances. Percentage inhibition was determined by comparing the absorbance values of test and control tubes. IC_{50} values were obtained from the best fit line drawn concentration (µg) vs. percentage inhibition.

α -Amylase activity: Twenty µl of α-amylase (0.05 U/ µl) was pre mixed with 20 µl of sample and 250 µl of 2% starch solution in 0.1M sodium phosphate buffer (pH 6.9) was added as a substrate to start the reaction. The reaction was carried at 37^0 C for 10 min and terminated by the addition of 200 µl of DNS reagent (1% 3,5 Dinitro salicylic acid sand 12% sodium potassium tartarate in 0.4M NaOH). α-amylase activity was determined by measuring absorbance at 540 nm.

$IR=\{ 1- (A_1 – A_{1b})/A_o - A_{o\,B})\} X 100\%$

A_1 is the A_{540} of the sample reactive solution, A_o is control the A_{540} of control reactive solution, A_{1B} is the blank of sample and $A_{o\,B}$ is the blank of control[16].

In vitro 5-Lipoxygenase inhibition: 5-LOX enzyme inhibitory activity of *Ocimum sanctum* was measured using the method[14, 15]. The assay mixture contains 80 mM linoleic acid and 10 µl potato 5-LOX in 50 mM phosphate buffer (pH 6.3). The reaction was initiated by the addition of enzyme buffer mix to linoleic acid and the enzyme activity was monitored as increase in absorbance at 234 nm. The reaction was monitored for 120 sec and the inhibitory potential of test substances was measured by incubating various concentrations of test substances for two minutes before addition of linoleic acid. All assays were performed in triplicate. Percentage inhibition was calculated by comparing slope of test substances with that of enzyme activity.

Screening of Antimicrobial activity: The antimicrobial screening was evaluated against *Bacillus subtilus*. The antimicrobial assay was performed by agar disc diffusion

Figure 3: α-Amylase activity of O-E, O-EA, O-M, O-C extracts of *Ocimum sanctum*

O-E O-EA O-M O-C STANDARD (Acarbose)

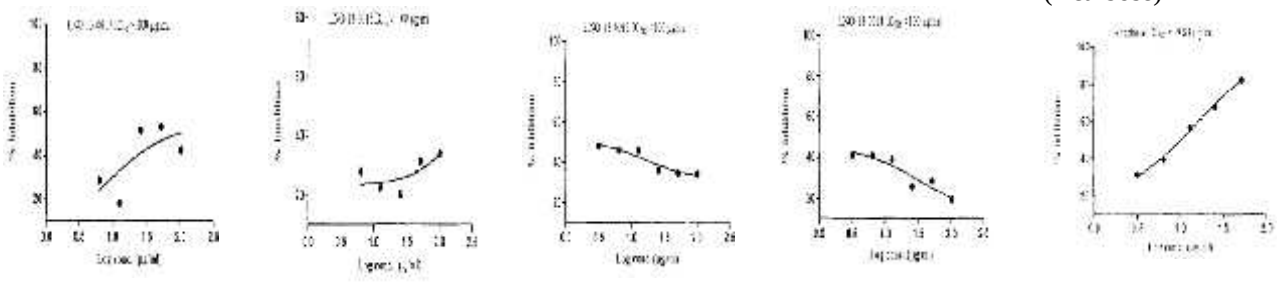


Table 1: Cytotoxicity bioassay of O-E, O-EA, O-M, O-C extracts of *Ocimum sanctum*

Plant extract	ED_{50}	Degree(s)of freedom	UCL	LCL
O-E	468.40	0.041	19.08	205.73
O-EA	116.25	0.066	160.55	60.93
O-M	198.04	0.005	287.35	121.46
O-C	157.24	0.576	244.21	57.46
Standard	2.50	0.013	3.11	1.64

method[17]. The molten nutrient agar was inoculated with 100 µl of the inoculum (1 x 10^8 cfu/ml) and poured into the Petri plate, the disc (0.7cm) (Hi-Media), was saturated with 100 µl of the test compound, allowed to dry and was introduced on the upper layer of the seeded agar plate. The plates were incubated over 37^0 C and microbial growth was determined by measuring the diameter of zone of inhibition. Pure solvents were used as control.

Cytotoxicity bioassay: Brine shrimp lethality bioassay was carried out to investigate the cytotoxicity of extracts of medicinal plants of India. Brine shrimps(*Artemia salina*) were hatched using brine shrimp eggs in a conical shaped vessel (1L)filled with sterile artificial seawater (prepared using sea salt 38 g/L and adjusted to pH 8.5 using 1N NaOH) under constant aeration for48 h. After hatching, active nauplii free from egg shells were collected from brighter portion of the hatching chamber and used for the assay. Ten nauplii were drawn through a glass capillary and placed in each vial containing4.5 ml of brine solution. In each experiment, 0.5 ml. of the plant extract was added to 4.5ml of brine solution and maintained at room temperature for 24 h under the light and surviving larvae were counted. Experiments were conducted along with control (vehicle treated), different concentrations (1-5000 tubes per dose).

Lethality concentration determination: The percentage lethality was determined by comparing the mean surviving larvae of the test and control tubes. LC50 values were obtained from the best-fit line plotted concentration verses percentage lethality. Podophyllotoxin was used as a positive control in the bioassay. The percentage lethality was calculated from the mean survival larvae of extracts treated tubes and control. LC_{50} values and ED_{50} values were obtained by best-fit line method.

RESULT AND DISCUSSION

Among the plants known for medicinal value, the plants of genus *Ocimum* are very important for their therapeutic potentials. Although Tulsi is known as a general vitalizer and increases physical endurance, it contains no caffeine or other stimulants. The stem and leaves of holy basil contain a variety of constituents that may have biological activity, including saponins, flavonoids, triterpenoids, and tannins. Nowadays Antioxidants have gained more importance on account of their positive effects, as health promoters in the treatment of cardiovascular problems, atherosclerosis, many forms of cancer, the ageing process, etc. many antioxidant compounds which are naturally occurring in plant sources have been identified as free radical scavengers[17]. In present study, in vitro antioxidant activity (FRSA) of ethanolic extract (O-E),ethyl acetate extract,(O-EA) methanolic extracts (O-M) and chloroform extract(O-C) of show potential free radical scavenging activities expressed in IC_{50}42.39µg/ml,58.45µg/ml,42.99 µg/ml,76.56(µg/ml) respectively. This result is compared to a standard, Ascorbic acid with IC_{50}3.15 (µg/ml) [Fig 1].

Results revealed that O-C has greater antioxidant property when compared to O-EA, O-E and O-M. These results suggesting that the antioxidant capacity of chloroform extracts of *Ocimum sanctum* is due to the presence of phenolic compounds to a great extent and indicates that phenolic compounds, likely to contribute to the free radical scavenging activity[18].

Anti-inflammatory activities of the ethanolic extract, ethyl acetate methanolic extracts and chloroform of *Ocimum sanctum* were studied by 5-Lox activity. O-E, O-EA, O-M and O-C are showing IC_{50} values 15.99 µg/ml, 16.73 µg/ml, 30.76 µg/ml, 14.11 µg/ml respectively. This result is compared to a standard, Curcumin with IC_{50}9.38 (µg/ml) [Fig 2]. All the extracts of *Ocimum sanctum* proved to be possessing anti inflammatory activity.

Many natural resources have been investigated with respect to suppression of glucose production from carbohydrates. α- amylase activity inhibition is considered to be effective to control diabetes and obesity. Therefore effective and nontoxic inhibitors of alpha amylase have been sought. In this study anti-obesity potential of *Ocimum sanctum* has also been investigated. The results clearly established that α- amylase activity of all the extracts of

Ocimum sanctum (>100µg/ml). This result is compared to a standard, Acarbose with IC_{50} 9.88 µg/ml [Fig 3]. However it is clearly shown that the ethanolic extract, ethyl acetate, methanolic extracts and chloroform of *Ocimum sanctum*, have anti obesity potential.

The brine shrimp lethality assay represents a rapid, inexpensive and simple bioassay for testing plant extracts bioactivity which in most cases correlates reasonably well with cytotoxic and anti-tumor properties[19]. In the present study the brine shrimp lethality of extracts of *Ocimum sanctum* was determined using the procedure of Meyer et al[20]. O-E, O-EA, O-M and O-C are showing ED_{50} values 468.40 µg/ml,116.25µg/ml,198.04µg/ml,157.24µg/ml respectively. This result is compared to a standard, Podophyllotoxin with ED_{50} 2.50 µg/ml [Table 1]. It is clearly evidenced that extracts of *Ocimum sanctum* possess anti tumor potential.

Chemical compounds produced biosynthetically that could destroy or usefully suppress the metabolism of pathogenic microbes are referred as antibiotics which are extensively studied in various higher plants in recent times. However to our knowledge a little is known about antimicrobial activity of ethanolic extract, ethyl acetate, methanolic extracts and chloroform extract of *Ocimum sanctum*.

Therefore the ethanolic extract, ethyl acetate methanolic extracts and chloroform extract of *Ocimum sanctum* show a significant FRSA, inhibition of 5- Lox, α- amylase, antimicrobial activities and also cytotoxicity and suggesting that the plant contain potential bioactive compounds. Further bioactive studies and the identification of these compounds in *Ocimum* sanctum are more useful in studies of immunostimulating effects**.**

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