

**INVITRO ASSESSMENT OF ANTIINFLAMMATORY ACTIVITY OF
OCIMUM SANCTUM (KARUNTHULASI LEAVES)****P. SANGEETHA * AND J. JULIET POORNAMATHY***Department of Biochemistry, Asan Memorial College of Arts and Science, Jaladampet, Chennai***ABSTRACT**

The present study evaluate the anti-inflammatory activity of petroleum ether extract, ethanolic extract and aqueous extract of *ocimum sanctum* Linn (karunthulasi leaves). Extract was carried out using soxhlet apparatus. These three extract were subject to phytochemical analysis in order to find out the constituents present in the sample. Secondly, HRBC membrane stabilization was selected for further anti-inflammatory studies. Standard drug such as Diclofenac sodium was used. Phytochemical constituents such as alkaloids, flavanoids, tannin, steroids, saponin, and proteins were found to be present in all the three type of extract. Result shown that anti-inflammatory activity of *ocimum sanctum* Linn, extract has more potent than the standard drug.

KEYWORDS: Ocimum Sanctum Linn, Diclofenac sodium, phytochemical analysis, Anti-inflammatory activity

**P.SANGEETHA**Department of Biochemistry, Asan Memorial College of Arts and Science,
Jaladampet, Chennai.

*Corresponding author

INTRODUCTION

The inflammatory responses are known to involve a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, and tissue break down and repair which are aimed at host defense and usually activated in most disease conditions [1]. It also helps body to protect it-self against infection, burns, toxic chemicals, allergens or other noxious stimuli, the complex events and mediators involved in the inflammatory reaction can induce, maintain or aggravate many diseases [2]. The commonly used drugs for the treatment of inflammatory conditions are non-steroidal anti-inflammatory drugs (NSAIDs), which have several adverse effects, especially gastric irritation leading to formation of gastric ulcers. Herbal drugs constitute a major part in all the traditional system of medicine. Herbal medicine is a triumph of popular therapeutic diversity. In India, the factors responsible for the continued and extensive use of herbal remedies are their effectiveness, easy availability, low cost, comparative less toxic effects and shortage of practitioners of modern medicine in rural areas. Number of synthetic medicines has been derived from medicinal herbs.[3] The major advantages of herbal medicine seem to be their perceived efficacy, low incidence of serious adverse effects and low cost [4]. *Ocimum sanctum* (Tulsi or Holy Basil) belongs to Family *Labiaceae* and is cultivated abundantly throughout tropical and semitropical region of India and other Asian countries. Different parts of this plant are traditionally utilized in the Ayurveda and Siddha systems for treatment of several ailments [5]. It is an erect, many branched subshrub, 30–60 cm tall with hairy stems and simple opposite green or purple leaves that are strongly scented. Leaves have petioles and are ovate, up to 5 cm long, usually slightly toothed. The flowers are purplish in elongate racemes in close whorls [6]. It is commonly used for curing skin diseases, hepatic disorders, cold, cough, malarial fever and so on. Several recent investigations using the extracts of *Ocimum sanctum* have indicated that they possess significant anti-stress 3 and anti-carcinogenic properties [7]. *Ocimum sanctum* Leaves have

been reported to have anti-inflammatory and analgesic activity [8].

MATERIALS AND METHODS

COLLECTION OF PLANT

The leaves of *Ocimum sanctum* L., was collected from local market and authenticated by National Institute of Siddha (certificate no: NISMB1552015). The plants are collected for Dec 2014. They were washed and air dried in the shade. They were powdered using grinder and sieved.

DRUGS AND CHEMICALS

Diclofenac sodium was obtained from sigma-aldrich, India. Double distilled water was used throughout the study.

EXTRACTION USING SOXHLET APPARATUS

A) Preparation of petroleum ether extract

Extraction were prepare and according to their increasing order of polarity. 20g of sample were weighed and wrapped in whatman filter paper which is inserted into thimbles. 200ml of petroleum ether was taken in the round bottom flask. The extraction was carried out for 5 hours at 40-60°C. Finally, 100ml of extract was obtained and collected in clean bottle which stored at 15°C for further studies.

B) Preparation of ethanol extract

Sample used for petroleum ether extraction was air dried and again wrapped in another whatman filter paper, which is inserted into thimbles. 200ml of ethanol was taken in the round bottom flask. The extraction was carried out for 5 hours at 40-60°C. Finally, 110ml of extract was collected in clean bottle and stored at 15°C.

C) Preparation of aqueous extract

For aqueous extract the sample used for ethanol extraction was air dried and again wrapped in whatman filter paper which is inserted into thimbles. 200ml of distilled water was taken in the round bottom flask. The extraction was carried out for 5 hours at 80°C. Finally, 130ml of extract was collected in clean bottle and stored at 15°C.

PHYTOCHEMICAL ANALYSIS

Phytochemical analysis was done to investigate the phytochemical constituents present in *ocimum sanctum*, which is carried out by the method described by [9,13].

ANTI INFLAMMATORY ACTIVITY BY HRBC METHOD

In-vitro anti-inflammatory activity of extracts of *Ocimum sanctum L.*, was assessed by Human Red Blood Corpuscles (HRBC) membrane stabilizing method [10,11] with slight modifications. The blood was collected from healthy human volunteer who had not taken any anti-inflammatory drugs for 2 weeks prior to the experiment and transferred to the heparinised centrifuge tubes and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and a 10% suspension in

normal saline was made. Diclofenac sodium (50 mcg/ml) was used as standard. The reaction mixture (4-5 ml) consisted 2 ml of hypotonic saline (0.25% w/v NaCl), 1 ml of 0.15 M phosphate buffer (pH 7.4), 1 ml of test solution (1000 mcg/ml) in normal saline and 0.5 ml of 10% HRBC in normal saline. For control, 1 ml of isotonic saline was used instead of test solution. The mixtures were incubated at 56°C for 30 min. and cooled at running tap water, centrifuge at 3000 rpm for 20 min. The absorbance of supernatant was read at 560 nm using visible Spectrophotometer. The experiment was performed in triplicates. The control represents 100% lyses. The results were depicted in Table.3 The Percentage membrane stabilization was calculated [12] using the following formula:

$$\% \text{ Inhibition of haemolysis} = 100 \times \frac{[\text{Absorbance of control} - \text{Absorbance of test}]}{\text{Absorbance of control}}$$

STATISTICAL ANALYSIS

The values were represented as mean \pm S.E.M. and the data obtained from this study was subjected to one-way analysis of variance (ANOVA) followed students "t" test. The values of $aap < 0.001$, $aap < 0.01$, were considered to indicate the significant levels.

RESULTS

TABLE 1
COLOUR, CONSISTENCY AND % YIELD OF THE VARIOUS EXTRACTS OF OCIMUM SANCTUM EXTRACTS

Extraction	Colour	Consistency	%Yield
Petroleum ether	Green	Liquid	45%
Ethanol	Green	Liquid	55%
Aqueous	Green	Liquid	65%

The colour, consistency and percentage yield values of the aqueous, ethanol and petroleum ether extracts of *ocimum sanctum* were noted (Table 1). Aqueous extract of *ocimum sanctum* had the highest percentage yield followed by ethanol extract and petroleum ether extract (Table 1). This might be due to the presence of high content of secondary metabolites which may be soluble in high polarity solvents.

TABLE 2
PHYTOCHEMICAL ANALYSIS OF OCIMUM SANCTUM EXTRACT

PHYTOCHEMICAL CONSTITUENTS	PETROLEUM ETHER EXTRACT	ETHANOLIC EXTRACT	AQUEOUS EXTRACT
ALKALOIDS	+	+	+
FLAVANOIDS	-	+	+
SAPONINS	-	+	+
CARBOHYDRATES	-	+	+
PROTEINS	-	+	+
PHENOLS	-	+	+
STEROIDS	+	+	+
TANNINS	+	+	+

+ indicates present, - indicates absent

The preliminary Phytochemical screening of leaves of *ocimum sanctum* extract result shown in the table 2. Revealed that alkaloid, steroids & tannins are present in extract, and flavanoids, proteins & phenols are absent in petroleum ether extract

TABLE 3
IN-VITRO ANTI-INFLAMMATORY ACTIVITY OF VARIOUS EXTRACTS OF OCIMUM SANCTUM

TREATMENT	ABSORBANCE	%INHIBITION
CONTROL	0.69 ± 0.06	-
PETROLEUM ETHER	0.57 ± 0.09^a	17.39
ETHANOL	0.19 ± 0.02^{aa}	72.46
AQUEOUS	0.38 ± 0.10^{aa}	44.92
DICLOFENAC SODIUM	0.14 ± 0.02^{aa}	79.7

Mean \pm S.E.M = Mean values \pm Standard error of means of three experiments. $aaP < 0.001$, $aP < 0.01$ compared to control group. All the data (Mean \pm SEM) were evaluated by Student's "t" test and the probability was determined for all the extracts. Anti inflammatory effects of *ocimum sanctum* was studied by using HRBC methods (Table 3). All the tested extracts of *ocimum sanctum* at the concentration of 1000 mcg/ml exhibited varying degree of anti-inflammatory activity as compare to that of Diclofenac sodium.

DISCUSSION

The incredible development in the field of synthetic drugs during present era is accompanied by numerous undesirable side effects. Whereas plants still hold their own unique place, with lesser side effects. For the preliminary study, HRBC membrane stabilization methods were selected. The method is well established model for screening the anti-inflammatory activity. *Ocimum Sanctum* has been reported with the presence of flavanoids which has a remarkable anti-inflammatory activity. Therefore different extracts have been taken in assessing the in vitro anti-inflammatory activity. Since the membranes of RBC are structurally similar to the lysosomal membrane, the effect of any substance on stabilization of RBC membrane may be extrapolated to the stabilization of lysosomal membrane.

CONCLUSION

From the results it can be concluded that the leaves of *Ocimum Sanctum Linn* posses anti-inflammatory action in HRBC membrane stabilization method. This study gives an idea that this compound of the plant *Ocimum sanctum* can be used as lead compound for designing a potent anti-inflammatory drug which can be used for treatment of various diseases such as neurological disorder, inflammation and pain.

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