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COMPARATIVE ANALYSIS OF THE ANTIOXIDANT AND IMMUNOMODULATORY POTENTIAL OF THE FRACTIONS FROM LEAVES OF *OCIMUM SPP.* (*O. SANCTUM, O. BASILICUM, O. GRATISSIMUM*)

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ABSTRACT

Ocimum spp. (family Lamiaceae) has a documented history of pharmacological properties which can be attributed to its rich reservoir of phytochemicalsthathave been extensively used in the Ayurveda for treatment of various ailments. Herein, we analyzed the antioxidant and immunomodulatory properties of extract/fractions prepared from *Ocimum spp*. (*O. sanctum, O. basilicum and O.gratissimum*) leaves using liquid-liquid extraction method.Phytochemical tests revealed the presence of different phytoconstituents like alkaloids, flavonoids, terpenoids, tannins and carbohydrates in the extracts and fractions of *Ocimum spp*. leaves. ABTS assay was performed to analyze the antioxidant/free radical scavenging properties of *Ocimum spp*. Among the selected species, free radical scavenging activity was seen in following order; *O. gratissimum* (aqueous fraction IC_{50}15.57±0.04)>*O. sanctum* (butanol fraction IC_{50} 19.40±0.04)>*O. gratissimum* (butanol fraction IC_{50}20.36±0.07)>*O. basilicum* (butanol fraction IC_{50} 21.78±0.05)and the results were comparable with that of the standard antioxidant compound ascorbic acid (IC_{50} 12.56±0.1). The human peripheral blood mononuclear cells (PBMC) were used as a model system to evaluate the comparative immunomodulatory effects of extracts/fractions of *Ocimum spp*. The cytostimulation assay demonstrated that among the three species used, the ethyl acetate fraction of *O. basilicum* exhibited highest immune enhancing

effect on the human PBMC (EC_{50}21.69±0.6) followed by butanol fraction of *O. sanctum* (EC_{50}26.55±0.1). The ethyl acetate fraction of *O. gratissimum* also exhibited stimulating effect on human PBMC (EC_{50}43.92±0.1) whereas the chloroform fractions of *O. gratissimum* and *O. sanctum* havecytotoxic effects on human immune cells. The IL-2 production was found to be8 fold and 15 fold enhanced, after incubating PBMC with ethyl acetate fraction of *O. basilicum* for 24 and 48 h, respectively. Taken together, these results suggest that the phytoconstituents present in the extracts and fractions of *Ocimum spp.* display remarkable antioxidant and immunomodulating potential.

Keywords: *O. basilicum*, *O. gratissimum*, *O. sanctum*, PBMC, IL-2, Phytochemicals.

1. INTRODUCTION:

In recent years, extensive research on free radicals has shown their critical role in a variety of normal regulatory pathways. Free radicals; reactive oxygen species (ROS) and reactive nitrogen species are continuously generated as result of various biochemical systems, like duringmetal catalyzed reactions, by neutrophils or eosinophils and by macrophages during inflammatory cell activation [1]. Thus a balance between free radicals and antioxidants is highly required for healthy cell functioning to maintainintegrity of the cell membrane, cellular proteins and nucleic acids. The human body has multifaceted antioxidant defense system to counteract free radicals, by sufficient dietary intake of antioxidants, as well as the endogenous production of antioxidative enzymessuch as glutathione peroxidase, catalase and superoxide dismutase etc. [2-3]. Immune cells also use ROS in order to maintain their functions and hence suitablelevels of antioxidant defenses are required in order to avoid the detrimental effect of the excessive production of ROS. In fact, theimmune cells have comparatively higher concentrations of antioxidants than normal cells [4]. Therefore,modulation of immune system via stimulation or suppression is an important factor in maintaining a disease free environment [5].A wide range of agents have been reported to normalize or modulate pathophysiological processes and therefore called immunomodulatory agents [6]. Immunomodulators are either natural or synthetic constituents, which potentially alter the immune responses thereby display significant therapeutic advantages.

Among large number of herbs mentioned in Ayurveda, Tulsi (*Ocimum sanctum*) is leading herb and scientific research is now

confirming its advantageous effects. Different parts of Tulsi plant have revealed notable properties such as anti-diabetic, hepato-protective, anti-inflammatory, antimicrobial, adaptogenic, anti-carcinogenic, radio-protective, neuro-protective, cardio-protective and larvicidal/mosquito repellent activity [7]. Tulsi leaves have been reported to contain high concentration of eugenol which acts as a COX-2 inhibitor, like several contemporary pain killers[8]. Tulsi also aids to avert cancers caused by noxious compounds by reducing DNA damage [9]. Essential oil of leaf and fixed oil of *O. sanctum* seeds have been shown to modulate humoral and cell mediated immune responses in non-stressed and stressed animals [10-11]. The aqueous extract of *O. sanctum* at the oral doses of 100 and 200 mg/kg/day in rats enhances the production of RBC, WBC and haemoglobin and also enhanced the production of antibodies without affecting the biochemical parameters [12].

In vitro studies have established that compounds in basil leaves (*O. basilicum*) have potent antioxidant, antifungal, antimicro bial and anticancer properties, [13-15]. The volatile oilof*O. gratissimum* L. has been credited with antimicrobial, antihelminthic and insect-repellant properties [16] while the essential oil exhibited sedative and anxiolytic activities [17].Till date there are no reports that establish comparative immunomodulatory and antioxidant potential of the extracts and fractions from *Ocimum spp.*leaves. Therefore, the current work demonstrates the fractionation of the *Ocimum spp*. leaves, their phytochemical analysis and the comparative analysis of the immunomodulatory and antioxidant potential of these fractions.

2. MATERIAL AND METHODS

2.1 Materials

ABTS (2-2'-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt, MTT (3- (4, 5-dimethyl thiazol-2-yl)- 2, 5-diphenyltetrazolium bromide), ascorbic acid was purchased from sigma-aldrich. Hisep, phosphate buffer saline (PBS), RPMI-1640 media, fetal bovine serum (FBS), penicillin and streptomycin were brought from HiMedia Laboratories Pvt. Ltd. Dimethyl sulphoxide, sodium biocarbonate, potassium persulfate, lead acetate, ferric chloride, Mayer's reagent, benedict's solution, acetic anhydride, glacial acetic acid, sodium hydroxide, hydrochloric acid, sulphuric acid were purchased from Loba Chemie. All organic solvents(methanol, ethyl acetate, chloroform, and butanol)were of HPLC grade.

2.2 Preparation of fractions from *Ocimum spp*.

Leaves were collected at the time of flowering as the phytochemical content is relatively high at this time. Botanical identification of species was done by renowned taxonomist of YS Parmar University Nauni (H.P., India),identified as *O. sanctum, O. gratissimum* and *O. basilicum* and linked to UHF-Herbarium with Field book No. 13533, 13534 and 13535, respectively.Dried leaves were powdered and extracted with methanol with a ratio of 1:5 (g/ml) for 24 hours. The extracts were filtered using Whatman filter paper and then concentrated in vacuo at 40°C using a rotary evaporator and then freeze-dried in vacuum. The methanolic extract was dissolved in water and using liquid-liquid fractionation method, the extracts were partitioned with chloroform, ethyl acetate, butanol and aqueous successively [18].

2.3 Preliminary Phytochemical Screening

The crude extracts and fractions of following plants were subjected to various chemical tests for the detection of different phytoconstituents using standard procedures [19-22].

Alkaloids: Few drops of Mayer's reagent were added to the extracts. Formation of white or pale yellow colour indicates the presence of alkaloids.

Carbohydrates: Few drops of Benedict's reagent were added to the extracts and then the extracts were boiled in water bath for 2 minutes. Formation of yellow, green or brick red colour indicates the presence of carbohydrates.

Terpenoids: Addition of chloroform to the dried extracts of all the species were followed by filtration. Then few drops of sulphuric acid were added followed by shaking for proper mixing. Formation of red colour indicates the presence of steroids and reddish brown colour indicates the presence of terpenoids.

Flavonoids: Extracts were treated with few drops of lead acetate solution. Formation of a yellow colour precipitate indicates the presence of flavonoids.

Tannins: Formation of blue-green colour with few drops of $FeCl_3$ solution to the extracts indicates the presence of tannins.

2.3 Antioxidant assay:

ABTS solution was prepared by dissolving light green colored ABTS in autoclaved distilled water to obtain final concentration of 7.4Mm:2.46mM potassium persulfate solution was also prepared. Then $ABTS^{\cdot+}$ stock solution was prepared by mixing the two solutions properly in ratio 1:1 and the solution was kept in dark for 24 hours. The working solution of $ABTS^{\cdot+}$ was prepared by

diluting the solution in methanol in the ratio of 1:25[23]. Ascorbic acid was taken as standard drug as it has known antioxidant potential and methanol was used as blank whereas DMSO was used as negative control. To 3ml of $ABTS^{+}$ solution, sample extracts containing antioxidant were added at the concentrations 20, 40, 60 and 80µg/ml and mixed properly for 30 seconds. Absorbance was taken at wavelength of 734 nm. Assay was performed three times and percentage inhibition was calculated. IC_{50} value was used for the interpretation of the outcomesfrom ABTS method [24]. The percentage inhibition of $ABTS^{+}$ can be calculated using the formula:

$$I_{734} = \left(1 - \frac{A_f}{A_o}\right) \times 100$$

Formula for free radical inhibition
Where, A_o is the absorbance of the radical cation before addition of the sample extracts and A_f is the absorbance after addition of sample. The inhibitory radical scavenging concentration of samples was calculated using regression analysis by Graphpad prism 5.0.2.

2.4 Preparation and cultivation of PBMC

Blood was collected from healthy human donors. Informed consent was acquired from all donors and this work was carried out in compliance with the ethical committee guidelines(Ref No. SUBMS/IEC/14/21)of Shoolini University, Solan. The blood samples were diluted withthe equal volume of PBS (1:1). After that, the diluted blood sample was carefully layered on lymphocytes separation media (HiMedia Laboratories Pvt. Ltd). The mixture was centrifuged at 400×*g* for 40 min. at 18-20°C. The undisturbed lymphocyte layer was carefully transferred out. The lymphocytes were washed and pelleted down with three volumes of PBStwice and resuspended in RPMI-1640 media supplemented with 10% Fetal bovine serum (FBS), 2mM L-glutamine and antibiotics (100 µg/ml penicillin and streptomycin). The cell number was counted with a hemocytometer and cell viability was tested by trypan blue dye exclusion assay [25].

2.5 Cell viability and Cytotoxicity Assay:

PBMC were seeded (5 x 10^4 cells/well) in 10% RPMI-1640 medium in a 96-well plate. After overnight incubation, the medium was replaced with fresh 10% RPMI containing fractions prepared from *Ocimum spp*. Concanavalin A (Con A, 10 µg/ml) treated PBMC and untreated PBMC or DMSO (10%) treated cells were used as positive and negative controls, respectively. All plates were incubated in 37°C, 5% CO_2 for selected time period. For MTT assay, 10µl MTT (5mg/ml) was added into each well to generate formazan crystals, and then cells were incubated in humidified atmosphere with 5% CO_2 at 37°C for 4 h. After removing

the supernatant, 100µl DMSO was added to dissolve the purple crystal with 10 min. The optical density of each well was measured at 595 nm by a microplate reader. Assay was done in triplicate for three times. The percentage of proliferation was calculated by the following formula:

%Proliferation = (ODsample − ODcontrol) / ODcontrol × 100

2.6 Cytokine Analysis

The secretion of cytokine (IL-2) was analyzed according to the procedures recommended in the Enzyme Link Immunosorbent assay kit (BD Biosciences). In brief, the isolated PBMC were treated with ethyl acetate fraction of *O. basilicum* and incubated further for 24 hours and 48 hours. The PBMC culture supernatants of various control and treatment groups were subjected to ELISA for determination of extra-cellular cytokines. The supernatant from each group was collected, added into the pre-coated plates and incubated for 3 hours. After that, these samples were washed and immediately 3,3′,5,5″-tetramethylbenzidine (TMB) Peroxidase substrate was added followed by 1 M Phosphoric acid stop solution. The ELISA plate was read at absorbance of 450 nm wavelengths using an ELISA Reader [26].

2.7 Statistical Analysis

Results were expressed as mean ± standard error. The results were the means from three replicates from three independent experiments.The significance of differences among mean values were determined by one-way ANOVA and Student's t-test by the graphpad prism software for Windows. A *p* value ≤0.05 was considered to be significant.

3. RESULTS AND DISCUSSION

3.1Fractionation and Phytochemical analysis

Using liquid-liquid fractionation process, crude methanolic extracts of *O. basilicum*,*O. gratissimum*and*O. sanctum* were partitioned with chloroform, ethyl acetate, butanol and aqueous successively. The phytochemicals present in the extracts and fractions were analyzed using various qualitative assays which revealed that *Ocimum spp.*have rich variety of secondary metabolites. The extracts and fractions of *O. basilicum*,*O. gratissimum*and*O. Sanctum*werefound to be rich in flavonoids, terpenoids, tannins and carbohydrates while alkaloids were only obtained in methanolic extract (Table 1).Earlier, it has been reported that *O. basilicum*and *O. gratissimum*contains alkaloids, flavonoids, terpenoids, carbohydrates, saponins, and tannins in methanolic extracts[27, 28].Similarly, themethanolic extracts of *O. sanctum* leaves

have been found to contain alkaloids, glycosides, flavonoids, terpenoids, saponins, tannins and carbohydrates [29].

3.2 *In vitro* antioxidant assay

The free radical scavenging activity was analyzed by the ABTS assay. The discoloration of the samples (% scavenging activity)were plotted against the sample concentration to calculate the IC_{50} value.Ascorbic acid (IC_{50} 12.56±0.1 µg/ml) was taken as standard drug as it has well-known antioxidant potential (Fig. 1). The free radical scavenging activity of extracts and fractions of *Ocimum spp*. was analyzed at a concentration range of 20 µg/ml to 80µg/ml.

Table 1: Phytochemical assessment in different extracts/fractions of *Ocimum spp.* (+ indicates presence and – indicates absence of a phytochemical).

	Phytochemicals	Methanolic extract	Chloroform fraction	Ethyl acetate fraction	Butanol fraction	Aqueous fraction
Ocimum basilicum	Alkaloids	+	-	-	-	-
	Carbohydrates	+	+	+	+	+
	Flavonoids	+	+	+	+	+
	Tannins	+	+	+	+	+
	Terpenoids	+	+	+	+	+
Ocimum gratissimum						
	Alkaloids	+	-	-	-	-
	Carbohydrates	+	+	+	+	+
	Flavonoids	+	+	+	+	+
	Tannins	+	+	+	+	+
	Terpenoids	+	+	+	+	-
Ocimum sanctum						
	Alkaloids	+	-	-	-	-
	Carbohydrates	+	+	+	+	+
	Flavonoids	+	+	+	+	+
	Tannins	+	+	+	+	+
	Terpenoids	+	+	+	+	-

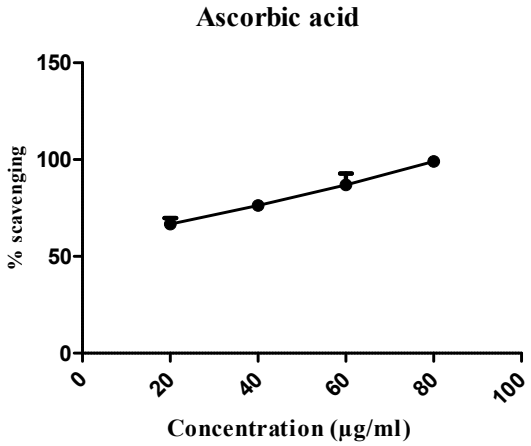

Fig. 1: Antioxidant activity of standard ascorbic acid; IC_{50} value of 12.56±0.1µg/ml. The experiment was done in triplicate and data was significant as *p < 0.05.

3.2.1 Antioxidant activity of *O. basilicum*

The ABTS assay revealed that the butanol fraction of *O. basilicum* leaves exhibited highest antioxidant activity as suggested by IC_{50} value (21.78±0.05 µg/ml). The ethyl acetate and aqueous fractions also showed appreciable antioxidant activities (Fig. 2, Table 2). As reported by Keita *et.al*,the antioxidant activity of *O. basilicum* and its extracts have been accredited primarily to the presence of phenolic acids especially rosmarinic acid and cichoric acid that were found in considerable concentrations [30]. Therefore our results are in consent with earlier reported data which suggest that the presence of flavonoids, terpenoids and tannins in the various fractions of *O. basilicum* could be attributed towards the potential antioxidant activity depicted by these fractions.

3.2.2 Antioxidant activity of *O. gratissimum*

O. gratissimum aqueous fraction exhibited maximum antioxidant activity as evident by IC_{50} value of 15.57±0.04which suggests presence of antioxidant compounds in this fraction. All other fractions like chloroform, ethyl acetate and butanol fractions also exhibited significant free radical scavenging activity (Fig. 2, Table 2). As revealed by phytochemical screening these fractions possesses polyphenols like flavonoids and tannins which could to be responsible for the antioxidant activity. Previously, it has been shown that the aqueous extractfrom the leaves of *O. gratissimum* possess antioxidant potential [31].

3.2.3 Antioxidant activity of *O. sanctum*

The ABTS assay revealed that the butanol fraction (IC_{50} 19.40±0.04) exhibited maximum antioxidant activity followed by methanolic extract (IC_{50} 24.14±0.03) and chloroform fraction (IC_{50} 30.04±0.04) (Fig. 2, Table 2).This experiment suggests that the polyphenols present in the *O. sanctum* fractions (as revealed by phytochemical analysis) are able to scavenge free radicals under *in vitro* conditions. Earlier, it has been reported that (phenols) flavonoids especially orientin and vicenin, isolated from aqueous extract of *O. sanctum*have exhibited strong radical scavenging effect *in vitro* [32].

Based on these results, the free radical scavenging activity among the three *Ocimum spp.* can be arranged in the following order; aqueous fraction of*O. gratissimum*> butanol fraction of*O. sanctum* >butanol fraction of*O. gratissimum*>butanol fraction of*O. basilicum*.The reductive potentials of *Ocimum spp*. and ascorbic acid were dose-dependent. As discussed above, the high amount of phenols and flavonoids in extracts

demonstrate their high antioxidant value [33].The antioxidant prospective of phenols is due to the presence of their hydroxyl group, which is bonded directly to an aromatic hydrocarbon (phenyl) ring which allowthem to donate electrons effortlessly to electron-seeking free radicals.Our results are in confirmation with the earlier studies which suggest that the alcoholic extracts of *Ocimum spp.* plants possess antioxidant activity [34]. The broad range of antioxidant activity of this *Ocimum spp.* indicates the potential of these plants as a source of natural antioxidants with possible application to reduce oxidative stress and consequent health benefits.

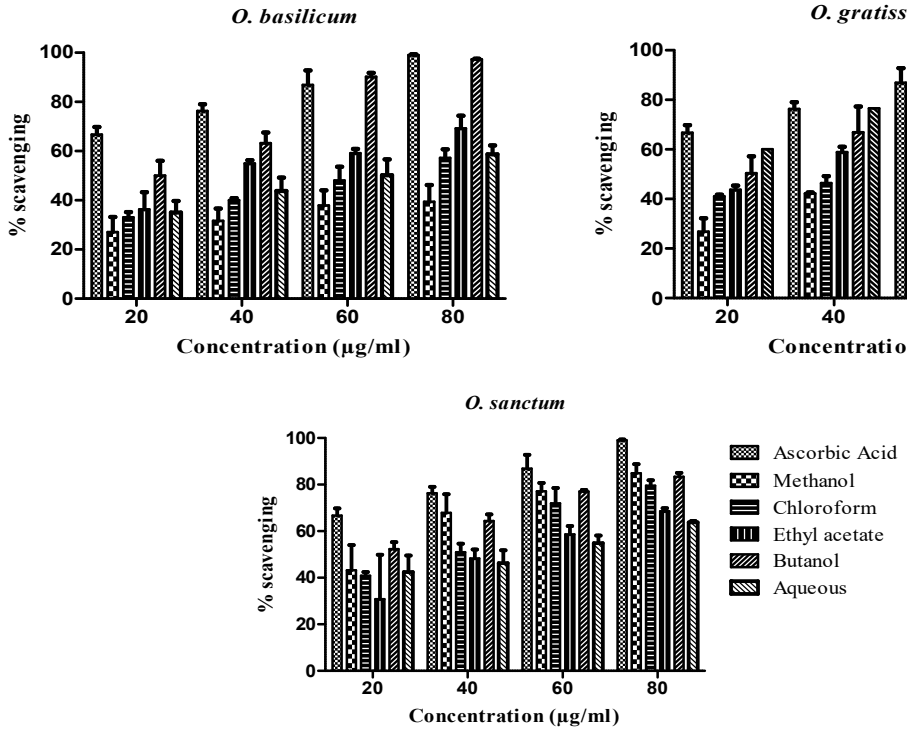

Fig. 2: Antioxidant activity of extracts/fractions of *O. sanctum*, *O. basilicum*, *O. gratissimum.* The experiment was done twice (in triplicate) using two different samples and data was significant as *p < 0.05.

Table 2:*In vitro* 50% inhibition concentration (IC_{50}) of extracts/fractions of *O. sanctum*, *O. basilicum*, *O. gratissimum.* Each value represents mean ± S.E.M (*n* = 3).

	IC_{50} value (µg/ml)				
Plant species	**Methanol extract**	**Chloroform fraction**	**Ethyl acetate fraction**	**Butanol fraction**	**Aqueous fraction**
Ocimum basilicum	**210.8±0.3**	**61.24±0.04**	**35.61±0.04**	**21.78±0.05**	**53.64±0.06**
Ocimum gratissimum	**57.32±0.03**	**43.20±0.06**	**26.38±0.04**	**20.36±0.07**	**15.57±0.04**
Ocimum sanctum	**24.14±0.03**	**30.04±0.04**	**42.07±0.06**	**19.40±0.04**	**39.38±0.07**

3.3 *In vitro* immunomodulatory activity of the extracts/fractions of *Ocimum spp.*:

The immunomodulatory activity of various fractions was evaluated on human peripheral blood lymphocytes via proliferation assay for 48 hrs. The data revealed that the ethyl acetate fraction of *O. basilicum* showed highest immunostimulatory activity with EC_{50} value of21.69±0.6followed by aqueous and methanol fractions having EC_{50} values of 26.58±0.05 and 32.92±0.05, respectively (Table 3, Fig. 3). With an increase in concentration, there was increase in human lymphocyte proliferation in the presence of ethyl acetate fraction of *O. basilicum.*

In case of *O. gratissimum,* the ethyl acetate fraction of showed highestmitogenic activity on human PBMC having EC_{50} value of43.92±0.1followed by butanol fraction having EC_{50} value of119.2±0.1 (Table 3). However, the methanol and chloroform fractions of *O. gratissimum* showed cytotoxicity against human PBMC at all concentrations (Fig.3). The aqueous fraction of *O. gratissimum* showed immunostimulatory only at higher concentrations (100 and 200µg/ml).The butanol fraction (EC_{50}26.55±0.1) of *O. sanctum*also showed promisingimmunomodulatory activity followed by aqueous fraction with EC_{50} value 39.53±0.06and ethyl acetate fraction having EC_{50} value 33.42±0.08 (Table 3 and Fig. 3). The chloroform fraction of*O. sanctum* showed cytotoxic activity towards human PBMC.

These results suggested that secondary metabolites present in *Ocimum spp.* fractions display both immune enhancing and immunosuppressing capabilities. Previously, the immunomodulatory effects of ethanolic extract of *O. sanctum* leaves on healthy volunteers showed a significant increase in the T helper cells and NKcells after 4 weeks of intervention [35]. Similarly studies have shown that the aqueous extract of *O. basilicum* stimulate antibody production in rats and enhances the production of RBC and haemoglobin [36]. Additionally, it has been reported that the methanolic and aqueous extract of *O. basilicum* enhanced mouse lymphocyte proliferation under *in vitro* conditions [37].According to these results, the immunomodulatory activity among the three *Ocimum spp.* can be organised in the following order; ethyl acetate fraction of*O. basilicum* >butanol fraction of*O. sanctum* >ethyl acetate fraction of*O. gratissimum.*Taken together, our data and earlier studies suggest that the phytocomponents present in the fractions of

these plants are responsible for their immunomodulatory properties.This is the first study demonstrating the comparative *in vitro* immunomodulatory potential of*Ocimum spp.* extracts and fractions onhumanPBMC.

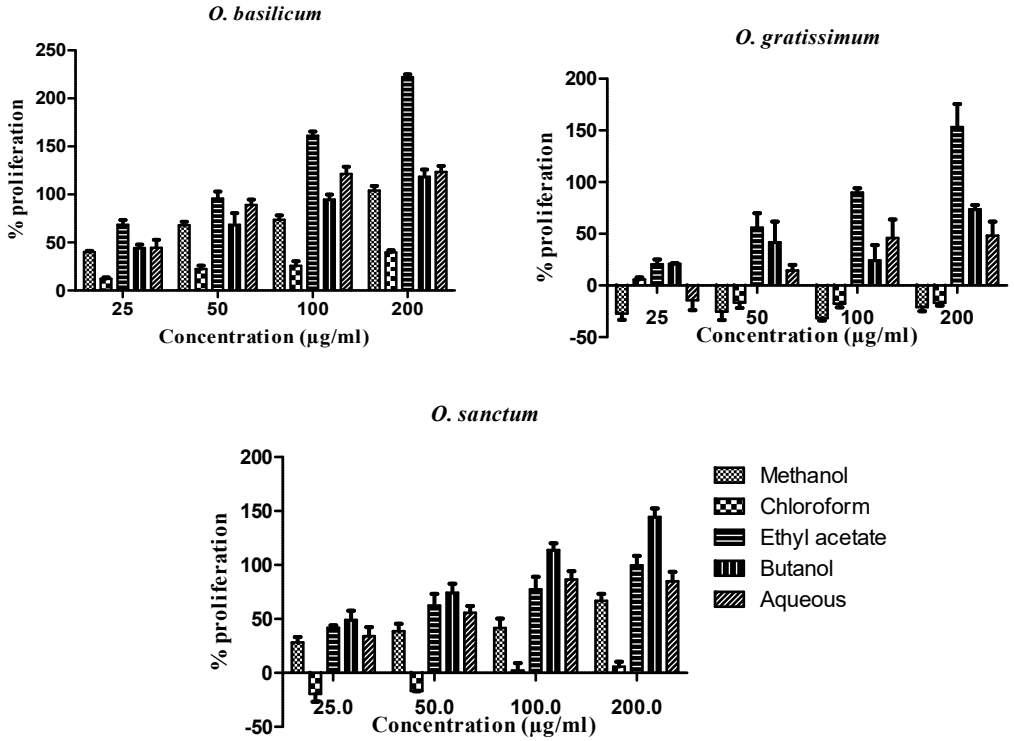

Fig. 3: Immunostimulatory and Cytotoxic activity of *O. basilicum*, *O. gratissimum* and *O. sanctum* fractions on human lymphocytes. The experiment was performed in triplicate. *p value< 0.05 as analyzed by one way ANOVA test.

Table 3: EC_{50} value of different fractions of *Ocimum spp.* (*p-value <0.05). Each value represents as mean ± S.E.M (*n* = 3)

	EC_{50} value (µg/ml)				
Plant species	**Methanol extract**	**Chloroform fraction**	**Ethyl acetate fraction**	**Butanol fraction**	**Aqueous fraction**
Ocimum basilicum	**32.92±0.05**	**386.5±0.1**	**21.69±0.6**	**29.65±0.06**	**26.58±0.05**
Ocimum gratissimum	**Not converged**	**Not converged**	**43.92±0.1**	**119.2±0.1**	**167.4±0.1**
Ocimum sanctum	**103.5±0.09**	**385.9±2.88**	**33.42±0.08**	**26.55±0.1**	**39.53±0.06**

3.5 Cytokine analysis

As discovered by proliferation assay, the ethyl acetate fraction of *O. basilicum* displayed highest immunostimulatory activity (EC_{50} 21.69±0.69µg/ml) as compared to the all other fractions of *Ocimumspp.* Thereforethe interleukine-2 (IL-2) production was analyzed after treating human PBMC with ethyl acetate fraction of *O. basilicum* at 21 µg/ml for 24 and 48 hrs. Con A and DMSO were used as positive and negative controls respectively. The cytokine

production analysis revealed that ethyl acetate fractionof *O. basilicum* significantly enhance the production of IL-2 by PBMC (8 and 15 fold after 24 and 48 hrs respectively) as compared to DMSO treated PBMC (Fig. 4).IL-2 has been shown to influence various lymphocyte subsets during differentiation, immune responses and homeostasis. For $CD8^+$ T cells, IL-2 signals both effector T cell generation and differentiation into memory cells [38].Theincreased levels of IL-2 indicate thatethyl acetate fractionof *O. basilicum*might be stimulating human immune cells via IL-2 secretion.Earlier, it had been reported that the aqueous extract of *O. basilicum*and its constituents (caffeic acid and p-coumaric acid) exhibited an immunomodulating effect on human PBMC with significant release of cytokines IL-2, IFN-γ, IL-5, IL-10 and TNF-β [39].

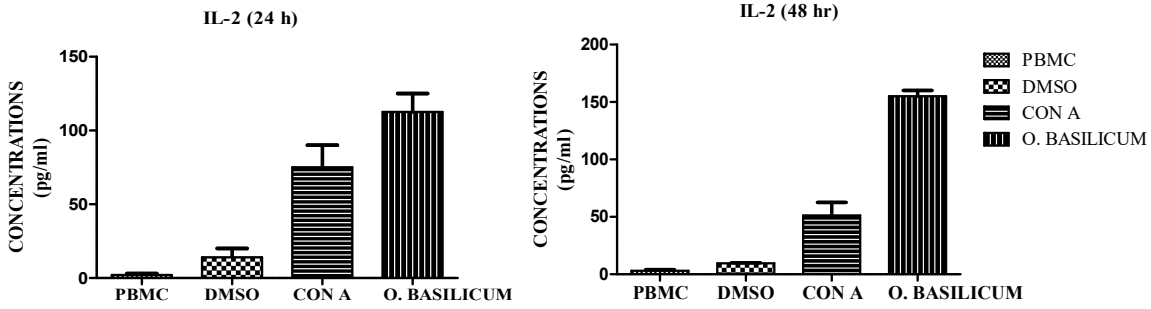

Fig. 4: Cytokine IL-2 expression of ethyl acetate fractions of *O. basilicum* after 24 h and 48 hrs. The experiment was performed in duplicate. *p value< 0.05 as analyzed by one way ANOVA test

4.CONCLUSION

In the present study, we investigated the dose dependent radical scavenging activity and immunomodulatory activity of the *Ocimum spp*(*O. sanctum, O. basilicum, O.gratissimum*). The broad range antioxidant activity of these*Ocimum spp.* leaves indicates potential of theseplant as asource of natural antioxidants that could improve current antioxidant therapies. Theethylacetate fraction*O. basilicum*was found to be a potent immunostimulant which enhanced IL-2 production by PBMC thereby endorsingimmune enhancing potential of the phytoconstituents present in this fraction. In conclusion, the data provide a rational basis for the beneficial usage of *Ocimum spp*. as conventional medicine since antiquity. Further studies need to be done in order to identify bioactive compounds responsible for the above mentioned activities.

6. ACKNOWLEDGMENT

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the infrastructure and facilities to conduct this research.

5. CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

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