

## FREE RADICAL SCAVENGING AND *IN VITRO* IMMUNOMODULATORY ACTIVITIES OF ENDOPHYTIC FUNGI OF *OCIMUM SANCTUM* LINN.

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### Abstract

In the present investigation, we aimed to isolate root fungal endophytic fractions (TRF) from Tulsi (*Ocimum sanctum* Linn.). Endophytic fungi (TRF-3 and TRF-6) from *Ocimum sanctum* Linn. roots were isolated and cultured aseptically in potato dextrose agar (PDA) media. The full fledged grown fungus cultures were fractionated using ethyl acetate. Crude fungal fractions were subjected to *in-vitro* free radical scavenging (2,2-diphenyl-1-picrylhydrazyl, (DPPH) assay, hydroxyl radical assay and for their reducing power) and immunomodulatory activities *in vitro* on the functions of human polymorphonuclear (PMN) cells such as phagocytosis, intracellular killing activity of *Candida albicans*, chemotaxis and reduction of nitroblue tetrazolium (NBT) dye. The IC<sub>50</sub> values for TRF-3 and TRF-6 were found to 271.74 µg/mL and 140.54 µg/mL respectively for DPPH assay and 298.61 µg/mL, and 361.76 µg/mL for hydroxyl assay respectively. A dose dependent decrease in the neutrophil was observed with maximum reduction of 89.78% for TRF-3 and 74.75% for TRF-6 respectively in the NBT assay. The mean particle number of phagocytosis of killed *Candida albicans* was found to be 7-8 for both TRF-3 and TRF-6 respectively at 100 µg/mL as compared to standard. In chemotaxis assay, treatment with TRF-3 and TRF-6 showed maximum number of neutrophils at 100 µg/mL which was comparable with the standard. These results confirmed the anti-free radical potentiality and immunostimulatory effects of *Ocimum sanctum* endophytic fungi. Thus plant endophytes can be explored and used as an alternative source for secondary metabolites and for therapeutic utility.

### Rezumat

În acest studiu s-a realizat izolarea fracțiunilor fungice din rădăcinile speciei *Ocimum sanctum*.

Fracțiunile au fost realizate prin izolarea ciupercilor endofite și cultivarea lor în mediu aseptice și fracționarea culturilor obținute, cu acetat de etil.

Fracțiunile fungice au fost supuse unor teste *in vitro* de evaluare a capacității de neutralizare a radicalilor liberi și de determinare a activității imunomodulatoare asupra celulelor polimorfonucleare.

Rezultatele obținute au confirmat capacitatea de scavenger de radicali liberi și efectele imunostimulatoare ale ciupercilor endofite din *Ocimum sanctum*, relevând astfel posibila lor utilizare în terapeutică.

**Keywords:** *Ocimum sanctum*, endophytes, fungi, immunomodulatory, free radical scavenging.

### Introduction

The modulation of immune response with the aid of various bioactives in order to alleviate certain diseases is an active area of interest. In order to adapt to environmental insults, plants produce a vast number of natural products that have immunomodulating potential. A number of plant products are being examined for their immunomodulating activity [37]. Some of the plants found to be potential immunomodulatory agents are *Panax ginseng* [31], *Viscum album* [16], *Tinospora cordifolia* [20], *Boehervia diffusa* [29], *Withania somnifera* [6], *Ocimum sanctum* [22] etc. A plethora of plant-derived materials like proteins, lectins, polysaccharides, flavanoid glycosides, sesquiterpenes, verbascoside have been shown to stimulate the immune system [38,1,2].

In the last decade, screening of microbial natural products as sources of novel lead candidates for the development of new drugs has been washing from industrial laboratories to find place in academic and small biotechnology research centers [34]. Endophytes have been found virtually in every plant studied, where they colonize the internal tissues of their host plant and can form a range of different relationships including symbiotic, mutualistic, communalistic and trophobiotic. Endophytes provide a wide variety of structurally unique bioactive natural products, such as alkaloids, benzopyranones, quinones, flavonoids, phenolic acids, quinines that could be harnessed for potential use in medicine, agriculture or industry [10, 44]. Further, these are also recognized as potential source of novel secondary metabolites for exploitation in medicine industry [30]. Endophytic, bacterial and fungal diversity has been extensively screened for antibacterial [23], cytotoxic, antioxidant, antidiabetic and anti-immunosuppressive compounds [19]. Because they are relatively unstudied, much attention is now being paid to endophytic biodiversity for their chemical composition and bioactivity. In view of their widespread application in plant, human health and environment, concerted efforts at endophytic diversity searches coupled with exploitation are necessary in the country on account of the varied and rich plant diversity.

*Ocimum sanctum* Linn. (*Tulsi*) has been used for thousands of years in Ayurveda for its diverse healing properties. Marked by its strong aroma

and astringent taste, it is regarded in Ayurveda as a kind of “elixir of life” and it is believed to promote longevity [26]. *Tulsi* has been claimed in classical texts to possess antioxidant, antiasthmatic and antitussive antimalarial, antipyretic, anti-inflammatory, antidiabetic, antiasthmatic, nematocidal effect, and is a good immunomodulatory agent with other numerous properties [37]. Recent studies proved the efficacy of the plant for antidiabetic [20], hypoglycemic and antioxidant [32, 7], hepatoprotective [27], cardioprotective [25], antistress [14] wound healing [9], antianxiety [4] activities. Some of the important secondary metabolites reported from *Tulsi* are Oleanolic acid, Urosolic acid, Rosmarinic acid, Eugenol, Carvacrol, Linalol and  $\beta$ -caryophyllene, Ocimumosides A&B and Ocimarin [16,11]. Hence based on the previous literature that endophytes of medicinal plants are the potential source for secondary metabolites and possess the same biological activity, it was thought worthwhile to isolate fungal endophytes from *Ocimum sanctum* Linn. roots and assess the crude fractions for *in vitro* immunomodulatory and free radical scavenging activity.

## **Materials and Methods**

### **Chemicals and Reagents**

2,2-diphenyl-1-picrylhydrazyl (DPPH) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Deoxy D-ribose, mannitol, thiourea, ascorbic acid, potato-dextrose-agar were obtained from Himedia Laboratories, Mumbai, India. All other chemicals were of analytical grade and used as received.

### **Plant material**

The roots of *Ocimum sanctum* Linn. were collected from in and around Dharwad district, Karnataka, India and were authenticated by Dr. G R Hegde, Karnatak University, Dharwad (India). A voucher specimen has been kept in the herbarium of the department of Pharmacognosy (SETCPD/Ph.cog/herb/19/07/2010).

### **Isolation of fungal endophytes [42]**

*Tulsi* (*Ocimum sanctum* Linn.) seedlings from different locations of Dharwad were uprooted without causing damage to roots. Samples were cleaned under running water to remove soil adhering to roots and then air-dried. Leaves stem and roots were partitioned and before sterilization, cleaned roots were cut in to pieces of 5 cm long. Roots were surface sterilized by 4% sodium hypochlorite for 5 min, 70% ethanol for 1 min and sterile distilled water for 1 min 2-3 times. The surface sterilized roots pieces were transferred to an alcohol sterilized mortar and macerated separately into suspension using distilled water and serial dilutions were made. The

diluted aliquots were transferred on sterile potato-dextrose-agar (PDA) plates. After incubation at 30 °C for 7-14 days, predominant isolates of fungi were picked up and purified. Culture purity was determined from colony morphology.

#### **Fermentation and Extraction [35, 36]**

Endophytic fungal isolates were grown on PDA plates at 30°C for 7-14 days depending on growth rate. Pure fungal isolates of TRF-3 and TRF-6 (TRF: root fungal endophytic fractions) respectively were used for fermentation and extraction. Purified isolates of each fungus were inoculated and fermented separately into a 3000 mL Erlenmeyer flask containing 600 mL of potato-dextrose broth (potato infusion from 200 g potatoes + 20 g of dextrose, pH 5.1±0.2, 24 g/L Hi-media). After incubation at 23°C for 21 days under stationary condition, each fungal culture was filtered and homogenized at 4000 rpm to separate the mycelia from broth. The filtrate was then extracted with 300 mL of ethyl acetate three times. The organic phase was separated to dryness under reduced pressure using a rotary evaporator and weighed to constitute the crude extract.

#### ***In vitro* free radical scavenging activity [40]**

##### **Reaction with DPPH (2,2-diphenyl-1-picrylhydrazyl) radical**

The scavenging effect of TRF-3 and TRF-6 (20-200 µg/mL) against DPPH stable radical was determined using ascorbic acid (ASC, 1-5 µg/mL) as standard. Plotting the percentage DPPH scavenging against ASC concentration gave the standard curve.

##### **Reaction with Hydroxyl radical**

Steady state hydroxyl radical scavenging activity of TRF-3 and TRF-6 (0.5-450 µg/mL) was measured by the degradation of deoxy-D-ribose method. Mannitol was used as standard for comparison. Different concentrations (0.5-5.0 mg/mL) of mannitol were mixed as explained above. Plotting the percentage inhibition of \*OH scavenging against mannitol concentration gave the standard curve.

##### **Determination of reducing power**

The reducing power of TRF-3 and TRF-6 (25–500 µg/mL) was determined according to the method previously reported [24]. In this method, the antioxidant compound forms a colored complex with potassium ferricyanide, trichloro acetic acid and ferric chloride, which is measured at 700 nm. The increase in absorbance of the reaction mixture indicates the reducing power of the samples. Ascorbic acid was used as the standard. Phosphate buffer (pH 6.6) was used as blank solution. The absorbance of the final reaction mixture of two parallel experiments was taken and is expressed as mean ± S.E.M.

***In vitro* immunomodulatory activity****Nitroblue Terazolium (NBT) test [43]**

A suspension of leucocytes ( $5 \times 10^6$  /mL) was prepared in 0.5 mL of PBS (phosphate buffer saline) activated plasma. The standard was added to the 1<sup>st</sup> tube and to the other tubes there were added 0.1 mL of different concentrations (5, 10, 25, 50 and 100  $\mu$ g/mL) of the test samples; 0.2 mL of freshly prepared 0.15% NBT solution were added to each tube and incubated at 37°C for 20 min. Further they were centrifuged at 400 grams for 3-4 min to discard the supernatant. The cells were resuspended in a small volume of PBS solution. A thin film was made with the drop on the slide, dried, fixed by heating, counterstained with dilute carbol-fuchsin for 15 sec. The slide was washed under tap water, dried and focused under 100X oil immersion objective; 200 neutrophils were counted for the % of NBT positive cells containing blue granules /lumps.

**Phagocytosis of killed *Candida albicans* [43]****Preparation of *Candida albicans* suspension**

The *Candida albicans* culture was incubated in Sabouraud broth overnight and then centrifuged to form a cell bottom and the supernatant was discarded. The cell button was washed with sterile Hank's Balanced Salt Solution (HBSS) and centrifuged again. This was repeated 3-4 times. The final cell button was mixed with a mixture of sterile HBSS and human serum in proportion of 4:1. The final cell suspension of concentration  $1 \times 10^8$  was used for the experiment.

**Slide preparation**

Human blood (0.2 mL) was obtained by finger prick method on sterile glass slide and incubated at 37 °C for 25 min to allow clotting. The blood clot was removed very gently and slide was drained slowly with sterile normal saline, taking care not to wash the adhered neutrophils (invisible). The slide consisting of polymorphonuclear neutrophils (PMNs) was flood with concentration of test sample and incubated at 37 °C for 15 min. The PMNs were covered with *Candida albicans* suspension and incubated at 37 °C for 1hr. The slide was drained, fixed with methanol and stained with Giemsa stain.

**Phagocytosis evaluation**

The mean number of *Candida* cells phagocytosed by PMNs on the slide was determined microscopically for 100 granulocytes using morphological criteria. This number was taken as phagocytic Index (PI) and was compared with basal PI of control. This procedure was repeated for different concentrations (5, 10, 25, 50 and 100  $\mu$ g/mL) of test sample. Immunostimulation in % was calculated by using the following equation.

Stimulation (%) =  $\frac{\text{PI (test)} - \text{PI (control)}}{\text{PI (control)}} \times 100$

#### **Candidacidal Assay**

A suspension of leucocytes ( $7 \times 10^6/\text{mL}$ ) was prepared in 0.25 mL of Hank's solution in 7 tubes. A volume of 0.25 mL Hank's solution (control) and 0.25 mL of pooled serum (standard) was added to the 1<sup>st</sup> and 2<sup>nd</sup> tube respectively, and to the other 5 tubes added 0.25 mL of different concentrations (5, 10, 20, 40, 50, 100  $\mu\text{g}/\text{mL}$ ) of test samples. A volume of 0.25 mL of *Candida albicans* suspension was added to each tube and incubated at 37 °C in water bath for 60 min with shaking every 15 min. After 30 min, 0.1 mL solution was taken on glass slides from each tube to make thin films. Slides were stained with Giemsa stain and observed at 100X lens. These should show that the majority of the *Candida* organisms have been ingested by the leucocytes. At the end of one-hour incubation, 0.25 mL of 2.5% sodium deoxycholate was added to each tube and mixed. The deoxycholate lyses the leucocytes but does not damage the *Candida* cells. A volume of 4 mL 0.01% methylene blue was added to each tube and mixed, centrifuged at 1500 rpm at 4°C for 10 min. The supernatant containing methylene blue was carefully removed with a Pasteur pipette leaving about 0.5 mL to resuspend the organisms. The suspension was put in an ice bath until ready for counting. About 300 *Candida* cells were counted using an improved Neubauer counting chamber. The proportions of dead cells, i.e. those which have taken up methylene blue were determined.

#### **Neutrophil locomotion and chemotaxis test [43]**

Neutrophil cell suspension was prepared in phosphate buffer saline solution (PBS) at about  $10^6$  cells/mL. The lower compartment of chemotactic chamber to a pH of 7.2 e.g. chamber 1-PBS solution (control); chamber 2-casin 1 mg/L (standard); and chamber 3, 4, 5, 6, 7 with different concentrations (5, 10, 25, 50 and 100  $\mu\text{g}/\text{mL}$ ) of test sample. The upper compartment (1mL syringe) was filled with neutrophil cell suspension and the wet filter (Millipore) 3mm pore size was fixed at the bottom of the upper compartment. The upper compartment was placed into the lower compartment and incubated at 37 °C for 180 min. The upper compartment was removed and inverted to empty the fluid. The lower surface of the filter was fixed with 70% ethanol for 2 min and then stained with Haematoxylin dye for 5 min. The fixed filters were observed under microscope using 100 X lenses and the number of neutrophil cells that reached the lower surface was counted.

#### **Results and Discussion**

*Ocimum santum* Linn. roots were found to harbor various fungi. Four pure fungal isolates were obtained from the roots of *Ocimum santum*

Linn. and designated TRF-1, TRF-2 TRF-3 and TRF-6. The yield of ethyl acetate fungal crude extracts of TRF-3 and TRF-6 was found to be 85 mg and 75 mg per liter of fermented medium.

#### ***In vitro* immunomodulatory activity**

In the case of Nitroblue tetrazolium assay, TRF-3 and TRF-6 had significantly increased the intercellular reduction of NBT dye to formazan by the neutrophil which is dependent on metabolic thrust generated through the hexose monophosphate shunt activation, which is necessary for microbicidal activity. A dose dependent decrease in the neutrophil was observed with maximum reduction of 89.78% for TRF-3 and 74.75% for TRF-6 respectively in NBT assay (Table I).

**Table I**

Percentage of reduced neutrophils after the treatment with TRF-3 and TRF-6 of *Ocimum Sanctum* Linn. by Nitroblue Tetrazolium Test (NBT)

Concentration	5 µg/mL	10 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL
Control (Phosphate buffer Saline)	26.85±0.55	26.83±0.42	28.32±0.46	28.37±0.26	29±0.25
Standard (Endotoxin-activated plasma)	31±0.99	37.86±1.60	78.28±2.48	79.28±1.61	82.24±4.09
TRF-3	26.8±1.34	35.08±1.30 <sup>C</sup>	75.02±3.94 <sup>C</sup>	79.78±1.16 <sup>C</sup>	89.78±1.08 <sup>C</sup>
TRF-6	60.225±0.52 <sup>C</sup>	60.1±0.31 <sup>C</sup>	74.65±0.34 <sup>C</sup>	74.52±0.49 <sup>C</sup>	74.75±0.95 <sup>C</sup>

The data are expressed as mean percentage reduced neutrophils ± SEM (n=4). One way ANOVA followed by Tukey's 't' test. <sup>a</sup>P < 0.01, <sup>b</sup>P < 0.001, <sup>c</sup>P < 0.0001 as compared to control.

The endophytic fractions TRF-3 and TRF-6 had stimulated the phagocytosis of killed *Candida albicans*. Both fungal fractions stimulated the phagocytosis at 10, 25, 50 and 100 µg/ mL respectively. The mean particle numbers (MPN) were found to be 4, 4-5, and 7-8 for TRF-3 at a concentration of 25 µg/mL, 50 µg/mL and 100 µg/mL, where as for TRF-6 MPN was found to be 4, 4-5, 5 and 7-8 for at a concentration of 10 µg/ mL, 25 µg/ mL 50 µg/ mL and 100 µg/mL respectively when compared to the standard (pooled serum- 4, 4-5, 4, 5-6, 6) at the studied concentrations. The results are shown in Table II.

**Table II**

Mean particle number of killed *Candida albicans* after the treatment with TRF-3 and TRF-6 of *Ocimum Sanctum* Linn.

Concentration	5 µg/mL	10 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL
Control	2	2-3	2-3	3	3
Standard ( Pooled serum)	4	4-5	4	5-6	6
TRF-3	2-3	3-4	4	4-5	7-8
TRF-6	3	4	4-5	5	7-8

The data are expressed as mean particle number of *Candida albicans* phagocytosed (n=4)

In the case of Candidacidal assay, TRF-3 and TRF-6 had significantly shown an increase in the dead cells, which, when treated with methylene blue, absorb the blue colour hence responsible for immunostimulating activity. The endophytic fractions TRF-3 and TRF-6 had shown significant candidacidal activity to the extent of 38.25% and 43.00.00% at 100 µg/mL respectively compared to standard (31.5%) and normal control (18.05%) at 100 µg/mL (Table III).

**Table III**  
Percentage of killed *Candida* after the treatment TRF-3 and TRF-6 of *Ocimum sanctum* Linn.

Concentration	5 µg/mL	10 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL
Control (Phosphate buffer saline)	18.25±0.30	17.9±0.52	17.67±0.29	17.03±0.5	18.05±0.3
Standard (Pooled serum)	31±0.66	31.4±0.36	31.27±0.29	31.05±0.58	31.5±0.21
TRF-3	20.02±0.55 <sup>b</sup>	22.2±1.00 <sup>c</sup>	30.6±1.59 <sup>c</sup>	37.5±0.88 <sup>c</sup>	38.25±0.41 <sup>c</sup>
TRF-6	21.77±0.61 <sup>c</sup>	31.2±0.58 <sup>c</sup>	32.65±0.17 <sup>c</sup>	40.45±0.75 <sup>c</sup>	43.00±0.67 <sup>c</sup>

The data are expressed as mean percentage of killed *Candida* ± SEM (n=4). One way ANOVA followed by Tukey's 't' test. <sup>a</sup>P < 0.01, <sup>b</sup>P < 0.001, <sup>c</sup>P < 0.0001 as compared to control.

In the case of neutrophil and chemotaxis assay, the endophytic fractions TRF-3 and TRF-6 had shown very significant chemotactic activity at all concentrations. The mean number of neutrophils per field for TRF-3 and TRF-6 at 100 µg/mL was found to be 2.57 and 2.35 respectively when compared to Casein (2.35). The results are shown in Table IV.

**Table IV**  
Number of neutrophils per field after the treatment with TRF-3 and TRF-6 of *Ocimum Sanctum* Linn. by Chemotaxis test

Concentration	5 µg/mL	10 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL
Control (Phosphate buffer saline)	0.825±0.30	0.675±0.09	0.65±0.09	0.5±0.40	0.5±0.25
Standard (Casein)	2.5±0.30	2.2±0.41	2.12±0.41	2.15±0.37	2.35±0.34
TRF-3	0.975±0.38 <sup>a</sup>	1.125±0.16 <sup>b</sup>	1.65±0.16 <sup>c</sup>	2.32±0.44 <sup>c</sup>	2.57±0.34 <sup>c</sup>
TRF-6	1.075±0.52	1.35±0.31 <sup>a</sup>	1.57±0.34 <sup>c</sup>	2.25±0.49 <sup>c</sup>	2.35±0.95 <sup>c</sup>

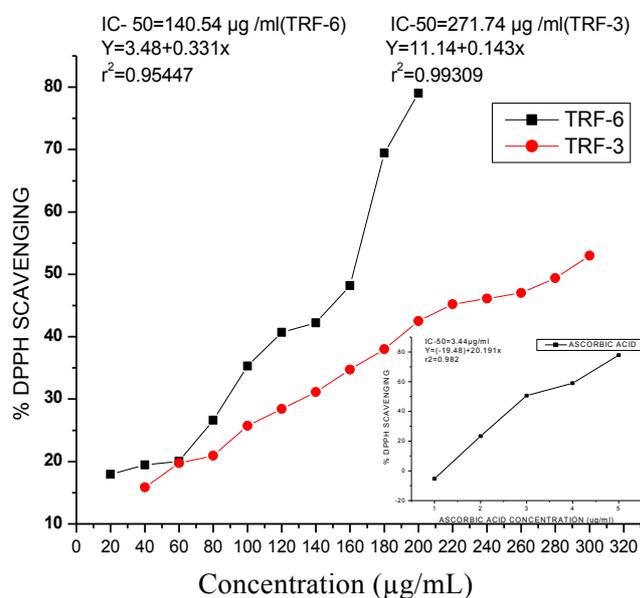
Mean number of neutrophils per field ± SEM (n=4). One way ANOVA followed by Tukey's 't' test. <sup>a</sup>P < 0.01, <sup>b</sup>P < 0.001, <sup>c</sup>P < 0.0001 as compared to control.

### Free radical scavenging activity of TRF-3 and TRF-6

#### (a) DPPH assay

DPPH assay method is based on the reduction of methanolic solution of colored free radical DPPH by free radical scavenger. The procedure involves the measurement of decrease in absorbance of DPPH at its absorption maxima of 516 nm, which is proportional to concentration of

free radical scavenger added to DPPH reagent solution. Reaction with DPPH radicals of TRF-3 and TRF-6 showed scavenging activity. The  $IC_{50}$  value for TRF-3 and TRF-6 were found to be 271.74  $\mu\text{g/mL}$  and 140.54  $\mu\text{g/mL}$  respectively where as  $IC_{50}$  value for ascorbic acid was found to be 3.44  $\mu\text{g/mL}$ . A linear correlation coefficient ( $r^2=0.982$ ) was obtained (Figure 1).

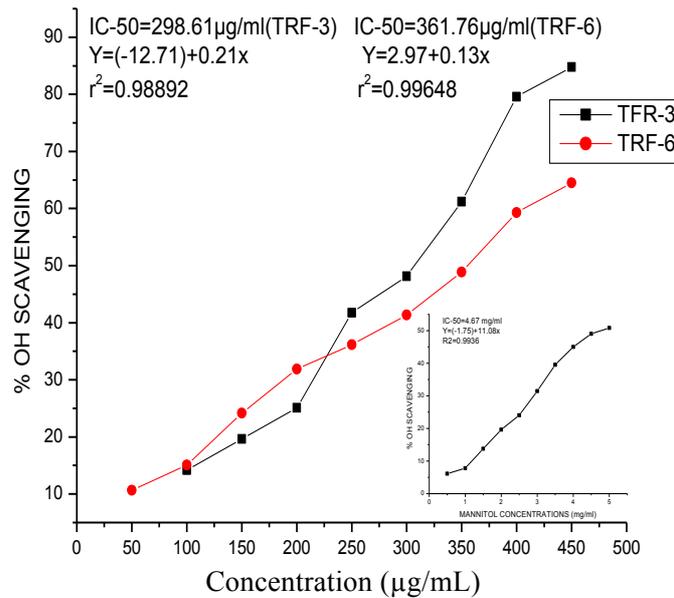


**Figure 1**

DPPH scavenging activity of TRF-3 and TRF-6 of *Ocimum sanctum* Linn.

**(b) \*OH Radical Scavenging assay**

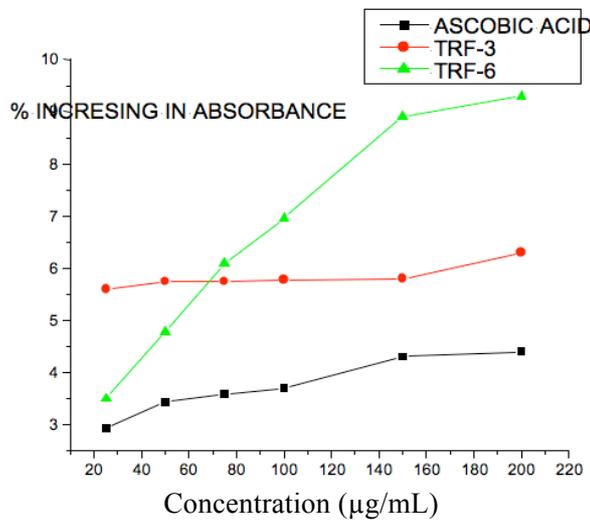
Hydroxyl radical scavenging capacity of an extract is directly related to its antioxidant activity. This method involves *in-vitro* generation of hydroxyl radicals using  $\text{Fe}^{3+}$ /ascorbate/EDTA/ $\text{H}_2\text{O}_2$  system using Fenton reaction. The intensity of yellow color formed is measured at 412 nm spectrophotometrically against reagent blank. Results presented indicate that phenyl hydrazine in solution generates \*OH radicals as measured by 2-Deoxyribose degradation assay. It was found that the activity of ethyl acetate endophytic fractions from *Ocimum sanctum* Linn. have \*OH radicals scavenging activity same as mannitol scavenges the \*OH radicals and inhibited the production of TBA (thiobarbituric acid) reactive species significantly over a period of 1 hr.  $IC_{50}$  values were found to be 298.61  $\mu\text{g/mL}$ , and 361.76  $\mu\text{g/mL}$  for TRF-3 and TRF-6 respectively where as  $IC_{50}$  value for mannitol was found to be 4.67  $\mu\text{g/mL}$ . A linear correlation coefficient ( $r^2=0.993$ ) was obtained (Figure 2).



**Figure 2**  
Hydroxyl radical scavenging activity of TRF-3 and TRF-6 of *Ocimum sanctum* Linn.

**c) Reducing power**

Results showed that as the concentration of TRF-3 and TRF-6 (25–500 µg/mL) was increased, the absorbance increased for each fungal fraction. This depicts that fractions have reducing power activity (Figure 3).



**Figure 3**  
Reducing power assay of TRF-3 and TRF-6 of *Ocimum sanctum* Linn.

The immunomodulating activity refers to biological or pharmacological effects of compounds on humoral or cellular aspects of immune response. The human immune response is a highly complex and extraordinarily sophisticated system involving both innate and adaptive mechanisms [28]. Modulation of the immune response through stimulation or suppression may help in maintaining a disease-free state. Agents that activate host defense mechanisms in the presence of an impaired immune responsiveness can provide supportive therapy to conventional chemotherapy [41].

Endophytic fungi are a rich source of novel organic compounds with interesting biological activities and a high level of biodiversity. They represent a relatively unexplored ecological source, and their secondary metabolism is particularly active because of their metabolic interactions with their hosts [15]. An attempt has been made in the present study to evaluate the free radical scavenging and the *in vitro* immunomodulatory activity of endophytic fungal fractions (TRF-3 and TRF-6) from the roots of *Ocimum sanctum* Linn.

Preliminary phytochemical investigation of TRF-3 and TRF-6 revealed the presence of glycosides, flavonoids, tannins as important secondary metabolites. In the present study, the fractions of TRF-3 and TRF-6 significantly increased the phagocytic function of human neutrophils, when compared to control indicating the possible immunostimulating effect. The engulfment of microorganisms by leukocytes called phagocytosis and which is one of the main defense mechanisms of an organism [5]. The fractions of TRF-3 and TRF-6 have significantly increased the neutrophil chemotactic movement as indicated by the increase in number of cells reached the lower surface.

Many studies reported novel secondary metabolites from endophytic community. Secondary metabolites like Pthalides, isocoumarins and steroids have been isolated from the EtOAc (ethylacetate) extract of an endophytic fungus from the plant *Melilotus dentatus*. Antibacterial, antialgal, and antifungal activities of these compounds against pathogens is also reported [12] (Hidayat et al. 2009). Many structurally unique metabolites like 1-*O*-(2,4-dihydroxy-6-methylbenzoyl)-glycerol, *N*-acetyltryptophan, 1-(2,4-dihydroxy-3,5-dimethylphenyl) ethanone, 2-(2,5-dihydroxyphenyl) acetic acid were isolated from endophytic fungal strain *P. commune* that was isolated from the semi-mangrove plant *Hibiscus tiliaceus* [13] Xyloketal, cyclic peptides, sphingolipids, xanthenes and anthraquinones, steroids, esters and lactones were isolated from the endophytes of *Avicennia marina*

[8]. Most of the compounds isolated from the fungus exhibited antibacterial and antitumour activities.

Immunostimulation in a drug-induced immunosuppression and immunosuppression in an experimental hyper-reactivity model by the same preparation can be said to be true immunomodulation [3]. The presence of immunostimulant compounds in higher plants has been extensively reviewed but only a limited amount of immunosuppressive products of plant origin have been reported. Such products, if well tolerated by the patient, may be developed into alternative adjuvants in the treatment of disorders caused by an exaggerated or unwanted immune response, such as in autoimmune diseases, allergies, glomerulonephritis, chronic hepatitis, etc [29].

Although many medicinal plants and their secondary metabolites have been screened for free radical scavenging and immunomodulatory activities, this is the first report on the free radical scavenging and immunomodulatory activities of fungal endophytes of *Ocimum sanctum* Linn. This may be attributed the secondary metabolites of TRF-3 and TRF-6 which are yet to be explored. The present investigation has also opened avenues for identification, characterization of potential plant endophytes as an alternative source for novel metabolites which can be used in biomedicines for therapeutic utility.

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