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Research Article

# *IN VITRO* ASSESSMENT OF FREE RADICAL SCAVENGING ACTIVITY OF *JASMINUM GRANDIFLORUM* (L.) FLOWER

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**ABSTRACT**

**Objectives:** The aim of the present study was to evaluate the *in vitro* free radical scavenging activity of *Jasminum grandiflorum* flower.

**Methods:** The free radical potential of ethanoic extract *J. grandiflorum* was assessed against 2, 2-diphenyl-1-picrylhydrazyl (DPPH), radical, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^+$) radical, hydrogen, hydrogen peroxide radical, and ferric reducing antioxidant power assay.

**Results:** At (20-100 µg/ml) concentration, ethanolic extract of *J. grandiflorum* flower exhibited free radical quenching capacity against various antioxidant assays when compared to the standard Vitamin C with increasing percentage of inhibition in a dose-dependent manner. The maximum percentage of inhibition was shown at the concentration 100 µg/ml.

**Conclusion:** Hence, the ethanolic extract of *J. grandiflorum* flower may be a potent source of natural antioxidant and it may be used in the management of diseases associated with oxidative stress.

**Keywords:** *Jasminum grandiflorum,* 2, 2-diphenyl-1-picrylhydrazyl, Antioxidant power assay, Vitamin C, Free radical, Natural antioxidant.

## INTRODUCTION

Free radicals are formed in our body due to biological oxidation and over production of oxidative stress which leads to the excessive damage of biomolecules such as proteins, DNA, and lipid which is associated with the various ailments including cancer, coronary artery diseases, hypertension, and diabetes, etc [1]. The most common free radicals include hydrogen peroxide ($H_2O_2$), superoxide anion ($O_2$–), peroxyl ($ROO^-$) radicals, and reactive hydroxyl radicals ($OH^-$). The nitrogen derived free radicals are nitric oxide ($NO_2$) and peroxynitrate anion ($ONOO^-$). All these free radicals are known as reactive oxygen species (ROS), which can able to react with those biomolecules resulting in cellular damage [2].

Antioxidants are compounds which are potential quenchers of free radicals or ROS. Recent investigations have also revealed plant products as potential antioxidants against various diseases, induced by free radicals due to the presence of secondary metabolites [3]. The most commonly used antioxidants are butylated hydroxyanisole, butylated hydroxytoluene, propyl gallate, and tert-butylhydroquinone [4]. However, they have been suspected of being responsible for liver damage and carcinogenesis in laboratory animals. Therefore, the development and use of more effective antioxidants are desired [5].

*Jasminum grandiflorum* Linn. (Oleaceae) is commonly known as Jasmine. It is a well-known glabrous twining shrub widely grown in gardens throughout India. The flower is acrid, bitter with a sharp taste. The leaves of *J. grandiflorum* are used in the treatment of odontalgia, fixing loose teeth, ulcerative stomatitis, leprosy, skin diseases, otorrhea, otalgia, strangury, dysmenorrhea, ulcers, wounds, and corns [6]. The *J. grandiflorum* flowers and leaves are largely used in folk medicine to prevent and treat breast cancer and stopping uterine bleeding [7]. It is widely used in the ayurveda, as an antileprotic, skin diseases, and wound healing [8].

No scientific studies have been carried out concerning *in vitro* anti-inflammatory of *J. grandiflorum*. Hence, the study was undertaken to evaluate the antioxidant, anti-bacterial, and anti-inflammatory activity from the ethanolic extract of *J. grandifloruim* flower.

## METHODS

### Plant material

The flowers of *J. grandiflorum* were collected from Coimbatore District. The plant was authentified by plant taxonomist in Kongunadu Arts and Science College, Coimbatore.

### Preparation of extract

*J. grandiflorum* flowers were picked and washed under running tap water, air-dried, powdered, and stored in airtight bottles. 30 g of dried powder was extracted in 300 ml of ethanol for 72 hrs using a soxhlet apparatus. The extracts were filtered through Whatman No.1 filter paper. The filtered sample was concentrated and dried. The plant extract was stored at 0-4°C further studies.

### Antioxidant activity

#### *2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity*

The radical scavenging activity of the ethanolic extract was evaluated using DPPH as an indicator [9]. Various concentrations of samples and Vitamin C were taken in different test tubes. The volume was adjusted to 500 µl by adding methanol. 5 ml of 0.1 mM methanolic solution of DPPH was added to these test tubes and vortexed. The tubes were allowed to stand at room temperature for 20 minutes. The control was prepared as above without any extract and methanol was used for the baseline correction. Changes in the absorbance of the samples were measured at 517 nm. Vitamin C was taken as the reference standard. The percentage inhibition versus concentration was plotted, and the concentration required for 50% inhibition of radicals was expressed as $IC_{50}$ value.

#### 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^+$) radical scavenging activity

The radical scavenging activity of the ethanolic extract was evaluated using $ABTS^+$ as an indicator [10]. Samples were diluted to produce 0.2-1.0 mg/ml. The reaction was initiated by the addition of 1.0 ml of diluted ABTSto10 µl of different concentration of ethanolic extract of

the sample or 10 µl of ethanol as a control. The absorbance was read at 734 nm, and the percentage inhibition was calculated. Vitamin C was taken as the reference standard. The percentage inhibition versus concentration was plotted, and the concentration required for 50% inhibition of radicals was expressed as $IC_{50}$ value.

**Hydroxyl radical scavenging activity**

The ability of the samples on hydroxyl radical was determined by Klein *et al*., [11]. The mixture contains 1 ml of iron-ethylenediaminetetraacetic acid solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5 ml of EDTA solution (0.018%), and 1 ml of DMSO (0.85% v/v in 0.1 M phosphate buffer, pH 7.4). The reaction was initiated by adding 0.5 ml of ascorbic acid (0.22%) and incubated at 80-90°C for 15 minutes in a water bath. After incubation, the reaction was terminated by the addition of 1 ml of ice-cold TCA (17.5% w/v). 3 ml of NASH reagent was added and left at room temperature for 15 minutes. The reaction mixture without sample was used as a control. The intensity of the color formed was measured spectrometrically at 412 nm against reagent blank.

**Hydrogen peroxide radical scavenging activity**

The activity of reducing the power of crude extract was depicted by Ruch *et al*., [12]. A solution of $H_2O_2$ was prepared in phosphate buffer. $H_2O_2$ concentration was determined spectrophotometrically from its absorption at 230 nm. Various concentrations of plant extract were added to $H_2O_2$ and incubated for 10 minutes. The absorbance at 230 nm was determined against a blank containing phosphate buffer without $H_2O_2$. The percentage of scavenging of $H_2O_2$ and standard compounds was calculated using the formula:

$$\text{Percentage of radical scavenging activity} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

**Ferric reducing antioxidant power (FRAP) assay**

FRAP assay were determined according to Benzie and Strain, [13]. The stock solution of 10 mM 2, 4, 6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl, 20 mm $FeCl_3$, $6H_2O$, and 0.3M acetate buffer (pH 3.6) were prepared. The FRAP reagent contained 2.5 ml TPTZ solution, 2.5 ml ferric chloride solution, and 25 ml acetate buffer. It were freshly prepared and warmed to 37°C. 900 µl FRAP reagent were mixed with 90 µl water and 30 µl test sample/methanol/distilled water/standard antioxidant solution. The reaction mixture was then incubated at 37°C for 30 minutes, and the absorbance was recorded at 595 nm. An intense blue color complex were formed when ferric TPTZ ($Fe^{3+}$-TPTZ) complex were reduced to ferrous ($Fe^{2+}$) form. The absorption at 540 nm was recorded. The calibration curve was plotted with absorbance at 595 nm versus concentration of ferrous sulfate in the range 0.1 mM both aqueous and methanol solutions. The concentrations of $FeSO_4$ were in turn plotted against the concentration of standard antioxidants.

## RESULTS AND DISCUSSION

In this present study, the antioxidant activity of the ethanolic extract of the *J. grandiflorum* flower was investigated by DPPH scavenging assay, $ABTS^+$ radical scavenging assay, hydroxyl radical scavenging assay, hydrogen peroxide radical assay, and FRAP assay. All the five methods have proven the effectiveness of the ethanolic extract of the flower compared to the reference standard antioxidant ascorbic acid (Vitamin C).

**DPPH radical scavenging assay**

DPPH is used for the evaluation of antioxidant capacity in a short time and frequently applied for testing food products. The reduction capability of the DPPH radical is determined by its decreased absorbance at 517 nm as induced by natural antioxidants [14]. It has been reported that oxidative stress occurs when free radical formation exceeds the body's ability to protect or scavenge them, forms the pathological basis of several chronic disease conditions [15].

The results are depicted in Fig. 1 illustrates the DPPH radical scavenging ability of *J. grandiflroum* and standard (Vitamin C). Free radical scavenging activity also increased with increasing concentrations of the extract in the range of 20-100 µg/ml. The percentage of extract from plant showed 76%, and its $IC_{50}$ value is 75 µg/ml when compared to 80% with $IC_{50}$ value 71 µg/ml for Vitamin C. Based on the results of this study, it is clearly indicate that plant extract has powerful *in vitro* free radical scavenging properties against DPPH model in a concentration-dependent manner. Our results are in tune agreement with earlier investigations of Nomaani *et al.,* [16].

**$ABTS^+$ radical scavenging assay**

The reduction of the $ABTS^+$ has been widely used to measure the antioxidant capacity of natural extracts [17,18]. The reductions of $ABTS^+$ with free radical scavengers present in the tested sample occur rapidly and can be assessed by following the decrease in the sample absorbance at 734 nm. Generation of the ABTS radical cation forms the basis of one of the spectrophotometric methods that have been applied to the measurement of the total antioxidant activity of various substances. The experiment carried out using a decolorization assay which involves the generation of the ABTS chromophore by the oxidation of ABTS with potassium per sulfate [19].

Fig. 2 indicates the effect of ethanolic extract of *J. grandiflorum* on $ABTS^+$ radical assay. The percentage of extract from the plant showed 80% at 100 µg/ml concentration which was compared to the standard Vitamin C. The extract showed better activity in quenching ABTS radical with an $IC_{50}$ value of 55 µg/ml comparable to reference standard Vitamin C 50 µg/ml.

The highest concentration of the extract was more effective in quenching free radicals in the system. The result obtained clearly implies that the ethanolic extract *J. grandiflorum* scavenge the radical in a concentration-dependent manner. Inhibiting and scavenging properties of antioxidants toward $ABTS^+$ radicals have been reported earlier by De Omenaa *et al.,* [20] who worked on the *in vitro* antioxidant activities of in ethanolic peel extracts of *Genipa americana*, *Spondia tuberose,* and *Spondia purpurea*.

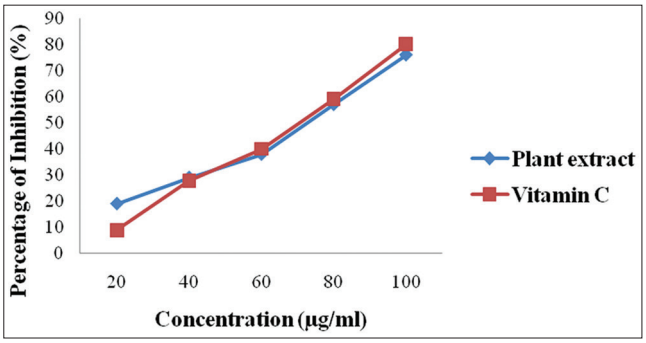


**Fig. 1: 2, 2-diphenyl-1-picrylhydrazyl radical scavenging activity**

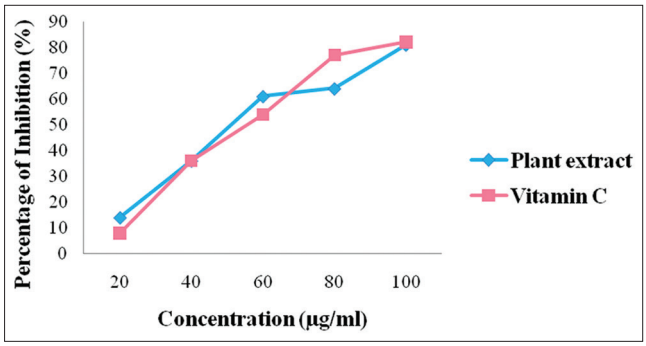


**Fig. 2: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity**

**Hydroxyl radical scavenging assay**

The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cell. This radical has the capacity to join nucleotide in DNA and cause strand breakage which contributes to carcinogenesis, mutagenesis, and cytotoxicity. In addition, this species is considered to be one of the quick initiators of lipid peroxidation process, abstracting a hydrogen atom from the unsaturated fatty acids [21-23].

The hydroxyl radical scavenging activity of plant extract from *J. grandiflorum* was shown in Fig. 3. The extract exhibits hydroxyl radical scavenging activity in a concentration-dependent manner. At the concentration of 100 µg/ml, the ethanolic extract exhibited 83% inhibition, whereas with standard antioxidant Vitamin C showed 85% inhibition, respectively. The $IC_{50}$ value of extract was found to be 65 µg/ml comparable to reference standard 55 µg/ml. The radical scavenging power of the extract can be accounted by the presence of secondary metabolites or neutralize free radicals or by their chelating ability due to their high nucleophilic character of the aromatic ring [24]. The above result were coincides with the study of Manjamalai and Berlin Grace, [25] who reported the *in vitro* studies on free radical scavenging activities of *Wedelia chinensis* exhibited more than 60% inhibition.

**Hydrogen peroxide radical scavenging activity**

Hydrogen peroxide itself is not particularly reactive with most biologically important molecules, but it is an intracellular precursor of hydroxyl radicals which is very toxic to the cell [26]. Although hydrogen peroxide itself is not very reactive, it can sometimes cause cytotoxicity by giving rise to hydroxyl radicals in the cell. Thus, removed of $H_2O_2$ is very important throughout the food systems [27,28].

The hydrogen peroxide radical scavenging activity of the ethanolic flower extract of *J. grandiflorum* was shown in Fig. 4. At concentration 100 µg/ml, *J. grandiflorum* and the standard antioxidant Vitamin C exhibited 72% and 75% inhibition, respectively, and the $IC_{50}$ value of the extract was found to be 64 µg/ml comparable to reference standard Vitamin C 60 µg/ml. The increase in the percentage of scavenging might be due to the presence of higher concentration of bioactive components [29].

**FRAP assay**

In FRAP non-enzymatic antioxidant reacts with prooxidants and inactive them. In this context assay, an easily reducible oxidant Fe (III) TPTZ complex by antioxidant to formed Fe (II) TPTZ [30]. Antioxidant power assay of the ethanolic extract *J. grandiflorum* was found to be increased in a concentration-dependent manner. Fig. 5 shows the FRAP activity of the ethanolic extract of *J. grandiflorum* was 0.76 µg/ml when compared with the standard Vitamin C 0.80 µg/ml at 100 µg/ml concentration.

According to Oktay *et al*., [31], positive relationship between total phenols and antioxidant activity appears to be the trend in many plant species. Recently, Li *et al*., [32] reported that the presences of phytochemical in several plants significantly correlated with high FRAP values. Since, the extract has the ability to scavenge free radicals, thereby preventing lipid oxidation via a chain breaking reaction; they could serve as potential nutraceuticals when ingested along with nutrients. In this study, the ethanolic extract possesses the better hydrogen donation capacity which suppresses the free radicals. The above result was similar to that of Arazo *et al*., [33] who reported the antioxidant potential of *Garcinia tinctoria*.

## CONCLUSION

On the basis of our results of the present study, it is concluded that the ethanolic extract of *J. grandiflorum* has potent antioxidant activity. Hence, *J. grandiflorum* can be considered for the preparation of neutraceuticals which may be suitable for the prevention of various ailments.

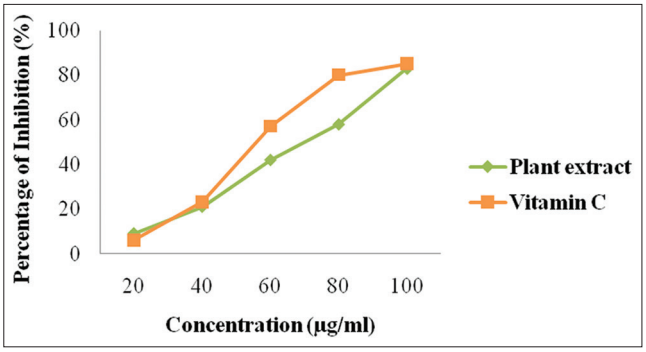


**Fig. 3: Hydroxyl radical scavenging activity**

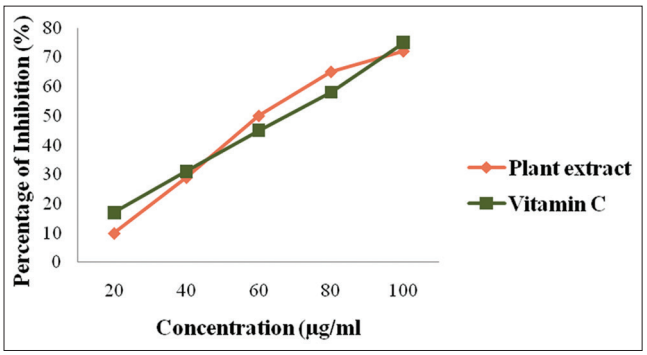


**Fig. 4: Hydrogen peroxide radical scavenging activity**

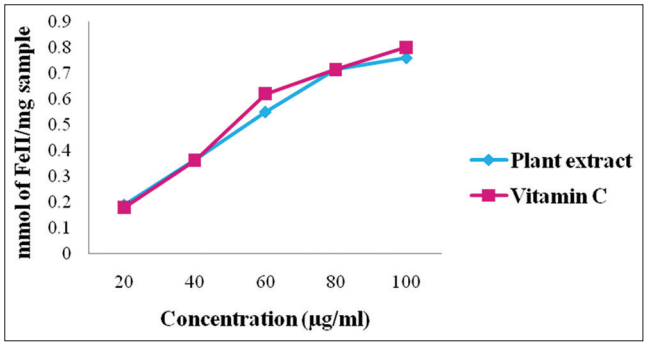


**Fig. 5: Ferric reducing antioxidant power assay**

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