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PHYTOCHEMICAL AND BIOCHEMICAL STUDIES OF *JASMINUM OFFICINALE* LEAVES

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ABSTRACT: Medicinal Plants are endowed with phytochemicals that are vital to counter various metabolic disorders like Oxidative damage in cells causing various degenerative diseases. Hence, the present study deals with the assessment of antioxidant activity and phytochemical screening of the aqueous extract of *Jasminum officinale* leaves. Antioxidant potential was evaluated *in vitro* using free radical scavenging assays for DPPH, NO, superoxide and ABTS radicals in addition to Reducing Power assessment. The total phenolic, flavonoid and flavonol contents of the extract were also assessed using standard procedures. The phytochemical analysis of the extract revealed the presence of tannins, flavonoids, terpenoids, alkaloids, steroids and its glycosides in addition to saponins and coumarins. A positive correlation between the inhibition of free radicals and concentration of the extract suggests significant antioxidant potential of the extract. The IC₅₀ values for DPPH, NO, superoxide and ABTS radicals were 41.16, 30.29, 20.19, and 29.48 µg/ml respectively as compared to the standard, ascorbic acid, having 42.79, 36.74, 38.22, and 45.57 µg/ml, for the same radicals. It is interesting to note that the extract has shown higher reducing ability as compared to the standard ascorbic acid. Thus, findings confirm its use in folkloric medicines and hence, it could be explored further for the development of a novel antioxidant agent due to its significant antioxidant potential.

INTRODUCTION: Medicinal plants act as an indigenous source of new phytochemicals possessing therapeutic value which can be used in drug development. Certain phytochemicals being non-nutritive are not essential for life sustenance but due to their medicinal properties they offer protection against diseases¹.

80% of the population in developing countries depends upon plant based traditional medicines, being less toxic and cost effective, for their primary health care needs, as estimated by WHO². Phytochemicals like polyphenolics can also act as natural antioxidant to protect cells against cellular injury caused by reactive oxygen species (ROS).

Reactive oxygen species (ROS) are produced as a result of normal cellular metabolism, but at high concentrations, they produce adverse modifications to cell components^{3, 4}. Imbalance between ROS production and oxidative defence system is termed as "oxidative stress". Cell damage caused by

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oxidative stress appears to be a major contributor to various diseases such as diabetes, cancer, neurodegenerative disorders and inflammation etc⁵. Since the process of biological ageing also have a free radical basis⁶ therefore, ingestion of natural antioxidants has been associated with antiaging^{7,8}. An antioxidant is a substance that blocks the process of oxidation by scavenging these free radicals and other oxidants. In doing so antioxidant molecules themselves oxidized into a harmless product. Hence, there has been a worldwide trend towards the use of the natural phytochemicals present in plants^{9,10,11}.

Jasminum officinale, family: Oleaceae, is a shrub cultivated throughout India. It is 'a proven plant' in the homoeopathic sense. Its leaves are used for headache. Since leaves of the other species of jasmine are considered as one of the highest accumulator parts of the plant containing bioactive compounds which are synthesized as secondary metabolites¹², therefore the present study deals with the evaluation of bioactive potential of *J. officinale* leaves with special reference of antioxidant potential in addition to their phytochemical analysis.

MATERIALS AND METHODS:

Plant material:

Fresh leaves of *Jasminum officinale* (500g) were collected from the local area of Allahabad-U.P. (India) and authenticated by Professor Satya Narayan, Taxonomist Department of Botany, University of Allahabad, India. The authenticated

fresh leaves were washed and dried under shade. The shade dried leaves (180g) were mechanically crushed, powdered and preserved until further use.

Chemicals:

1,1-Diphenyl - 2 - picrylhydrazyl (DPPH), 2, 2'-azinobis(3-ethylbenzothiazoline)-6-sulphonic acid diammonium salt (ABTS), Trichloroacetic acid (TCA), Nitro blue Tetrazolium (NBT), and Quercetin were purchased from Alfa Aesar Pvt. Ltd. Nicotinamide adenine dinucleotide (NADH), Phenazonium Methosulphate (PMS), Folin-Ciocalteu reagent were purchased from Merck India Pvt Ltd. Sodium phosphate dibasic (Na₂HPO₄), Sodium phosphate monobasic (NaH₂PO₄) were purchased from Hi Media Laboratories and Potassium ferricyanide, Ascorbic acid, Sodium acetate, AlCl₃, FeCl₃ were purchased from Sisco Research Laboratories Pvt. Ltd, India. All other chemicals used including the solvents were of analytical grade.

Preparation of Extract:

The dried leaf powder (180g) was refluxed repeatedly thrice with double distilled water, for 2 hour each time and filtrates were collected, combined and concentrated in rotatory evaporator at 45°C ± 5°C under reduced pressure, to obtain semisolid material, which was then lyophilized to get dark brown powder (yield ~15.5% w/w).

Phytochemical screening

Screenings for Phytochemicals was carried out based on the following chemical tests.¹³⁻¹⁷

TABLE 1: PHYTOCHEMICAL SCREENING

Phytoconstituents	Colour Tests	Observation
Alkaloids (Hager's Test)	2ml extract + few drops of Hager's reagent	Yellow precipitate
(Wagner's test)	2 ml extract+ 2 drops HCl (1.5%) + 3 drops Wagner's reagent	Brown precipitate
(Mayer's test)	2 ml extract(EtOH) + few drops Mayer's reagent	Yellow precipitate
Anthraquinones (Borntrager's Test)	3ml extract + 3ml Benzene + 5ml NH ₃ (10%)	Pink, Violate or Red colouration
Anthocyanins	2ml extract + 2ml HCl (2N) + NH ₃	Pinkish Red to Bluish Violet colouration
Coumarins	2ml extract + 3ml NaOH (10%)	Yellow colouration
Carbohydrate (Molisch's Test)	2ml extract(EtOH) + 10ml H ₂ O + 2 drops ethanolic α-naphthol (20%) + 2 ml conc.H ₂ SO ₄	Reddish Violet ring at the junction
(Fehling's test)	2 ml extract + 1 ml of Fehling's solution A and B + heat	Red precipitate
Emodins	2ml extract + 2ml NH ₄ OH + 3ml Benzene	Red colouration
Flavonoids	1ml extract + 1ml Pb(OAc) ₄ (10%)	Yellow precipitate

Glycosides (Liebermann's Test) (Salkowski's Test)	2ml extract + 2ml CHCl ₃ + 2ml CH ₃ COOH 2ml extract + 2ml CHCl ₃ + 2ml conc. H ₂ SO ₄	Violet to Blue to Green colouration Reddish Brown ring at the Junction
Leucoanthocyanins	5ml extract + 5ml Isoamyl alcohol	Organic layer turns into Red
Phlobatannins (Precipitate Test)	2ml extract + 2ml HCl (1%) + boil	Red precipitate
Proteins (Xanthoproteic test)	1ml extract + 1ml conc. H ₂ SO ₄	White precipitate turned Yellow on boiling
(Biuret's test)	1 ml extract + 5-6 drops w/v NaOH + 2 drops CuSO ₄ (30% w/v)	Violet Red colouration
Saponins (Foam Test)	5ml extract + 5ml H ₂ O + heat	Froth appearance
(Emulsion test)	5ml extract + Olive oil (few drops)	Emulsion formation
Steroids (Salkowski's Test)	2ml extract + 2ml CHCl ₃ + 2ml conc. H ₂ SO ₄	Reddish Brown colouration at interface
Tannins (Braymer's Test)	2ml extract + 2ml H ₂ O + few drops of FeCl ₃ (5%)	Green colouration
Terpenoids	2ml extract + EtOH+2ml CHCl ₃ + Δ(2 mint.) 3 drops conc. H ₂ SO ₄	Deep red colouration

Experimental Design:

Antioxidant potential of *JOLAE* (*J.officinale* leaves aqueous extract) was evaluated using different *in vitro* assays such as estimation of total phenolics, total flavonoids, reducing power, and total antioxidant power by measuring the scavenging activity of DPPH, NO[•] superoxide and ABTS radicals. All the assays were carried out in triplicates and their average values were taken into consideration.

Antioxidant Assay- *in vitro*:

Estimation of Total Phenolics:

Total phenolic contents were estimated spectrophotometrically using Folin–Ciocalteu reagent¹⁸. The sample (*JOLAE*) and standard (Gallic acid), were prepared in 60:40 acidified methanol/water (0.3% HCl). 2.0 mL of 2% Na₂CO₃ was added to 100 µl of each test solution of sample and standard. After 2 minute of incubation, 2.5 ml of Folin-Ciocalteu reagent (diluted with water 1:1 v/v) was added and allowed to stand at room temperature for 30 minutes. Absorbance of sample, standard and blank was measured at 750 nm. The standard of Gallic acid was prepared in varied concentrations of 25µg/mL to 400µg/mL. The phenolic contents of the sample were estimated by comparing with the standard calibration curve. Total content of phenolics was expressed as mg/g of gallic acid equivalent.

Estimation of Total Flavonoids:

Total flavonoid content was estimated by using the method of Ordon et.al.¹⁹. 0.5 ml of 2% AlCl₃ in 95% ethanol was added to 0.5 mL of each test

solution of sample (*JOLAE*) and standard (Quercetin). A yellow colour indicated the presence of flavonoids after 1 hour of incubation at room temperature. The absorbance of sample, standard and blank was measured at 420 nm. The standard of Quercetin (qr) was prepared in varied concentrations of 5µg/mL to 25µg/mL. The flavonoid content of the sample was estimated by comparing with standard calibration curve. The total flavonoid content was expressed as mg/g quercetin equivalent.

Estimation of Total Flavonols:

Total content of flavonol was estimated by the reported method of Oyaizu²⁰. 2ml of each test solution of sample (*JOLAE*) and standard (Quercetin) was mixed with 2ml (20 mg/ml) of 95% ethanolic AlCl₃ and 6 ml of sodium acetate (50 mg/ml) in methanol. After 2.5 hours of incubation at 20°C the absorbance of sample, standard and blank was measured at 440 nm. The standard of Quercetin (qr) was prepared in varied concentrations of 1µg/mL to 25µg/mL. The flavonol content of the sample was estimated by comparing with standard calibration curve. The total flavonol content was expressed as mg/g quercetin equivalent.

Estimation of Reducing Power:

Reducing power of *JOLAE* was determined by the method described by Oyaizu²¹. An aliquot of extract (1.0 ml) at various concentrations ranging from 10-1000 µg/ml was mixed with phosphate buffer

(0.2 M, pH 6.6, 2.5 ml) and 1% potassium ferricyanide (2.5 ml, 1%). The mixture was incubated at 50 °C for 20 min. After adding 10% trichloroacetic acid (2.5 ml, 10%), the mixture was centrifuged at 6500 rpm for 10 min. The supernatant (2.5 ml) was mixed with distilled water (2.5 ml) and 0.1% iron (III) chloride (0.5 ml) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic Acid was used as standard.

Free radical scavenging assays:

DPPH Radical Scavenging Assay:

Ability of *J. officinale* leaves to scavenge the stable DPPH (2,2'-diphenyl-1-picrylhydrazyl) radicals was assessed by using the method of Mensor²². 0.3 mmol/L solution of DPPH in methanol was prepared, and 1 mL of this solution was added to different concentrations of the leaves extract. The mixture was shaken vigorously and allowed to stand for 30 min at room temperature in dark and finally absorbance of each concentration was measured at 517 nm. A lower absorbance value indicates the higher radical scavenging activity. Results were compared with the standard ascorbic acid. The ability of DPPH radical (DPPH[•]) scavenging activity was calculated by using the following formula:

DPPH radical scavenging activity (%) =

$$[(A_0 - A_1)/A_0] \times 100$$

where A_0 is the absorbance of the control, and A_1 is the absorbance of the extract and standard. IC_{50} value was the effective concentration at which DPPH[•] were scavenged by 50% and was obtained by interpolation from linear regression analysis. A lower IC_{50} value indicated a greater antioxidant activity.

Nitric Oxide Radical Scavenging Assay:

Scavenging of Nitrosyl radical was determined by incubating 5 mM SNP in PBS (Phosphate Buffered Saline), with different concentrations (20-1000 µg/ml) of the plant samples/standard at 25°C. After 120 min, 0.5 ml of incubation solution was withdrawn and mixed with 0.5 ml of Griess reagent²³. The absorbance was measured at 550 nm against a control. Ascorbic acid was used as

standard and treated in the same way with Griess reagent and the absorbance was measured. The decrease in absorbance of the mixtures indicates an increasing Nitrosyl radical scavenging activity. The amount of nitrite was calculated from standard curve constructed by sodium nitrite.

Nitrosyl radical scavenging activity (%) =

$$[(A_0 - A_1)/A_0 \times 100],$$

A_0, A_1

where A_0 is the absorbance of the control, and A_1 is the absorbance of the extract and standard.

IC_{50} values were calculated from linear regression analysis of the graph.

Superoxide Anion Scavenging Assay:

Superoxide radicals were generated by the PMS/NADH system according to the method of Kakkar et al.²⁴. The reaction mixture was composed of 1 ml of NBT (156 µM NBT in 100 mM phosphate buffer, pH 7.4), 1 ml NADH (468 µM in 100 mM phosphate buffer, pH 7.4) and 100 µl plant sample/standard compounds. The reaction was started by addition of 100 µl of PMS (60 µM PMS in 100 mM phosphate buffer, pH 7.4) to the mixture. After 5 min incubation at 25°C, absorbance was measured at 560 nm against an appropriate blank to determine the quantity of formazan generated. Ascorbic Acid was used as a standard. The decrease in absorbance of the mixture indicates an increasing superoxide radical scavenging activity. The ability to scavenge superoxide radical was calculated by the following equation.

Superoxide radical scavenging activity (%) =

$$[(A_0 - A_1)/A_0 \times 100]$$

where, A_0 is the absorbance of control and A_1 is the absorbance of the extract and standard.

IC_{50} values were calculated from linear regression analysis of the graph.

ABTS Radical Scavenging Assay:

The ability of *J. officinale* leaves extract, to scavenge ABTS radical cation was determined by

Re et al.²⁵. The stock solutions included 7 mM ABTS solution and 2.4 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12–16 h at room temperature in the dark. The solution was then diluted by mixing 1 mL ABTS•+ solution with 60 mL methanol to obtain an absorbance of 0.706 units at 734 nm. Plant extract with different concentrations (1 mL of each) were allowed to react with 1 mL of the ABTS•+ solution and the absorbance was taken at 734 nm after 7 min. The ABTS•+ scavenging capacity of the extracts were compared with different concentrations of Ascorbic Acid and percentage inhibition calculated as ABTS radical scavenging activity (%) =

$$[(A0-A1)/A0 \times 100]$$

where, A0 is the absorbance of ABTS radical + methanol and A1 is the absorbance of ABTS radical + sample extract/standard.

IC₅₀ values were calculated from linear regression analysis of the graph.

RESULTS AND DISCUSSION:

Phytochemical Analysis:

Table 1, clearly reveals the phytochemical analysis performed on aqueous extract of *Jasminum officinale* leaves and indicates the presence of high amount of alkaloids, coumarins, flavonoids and tannins while terpenoids, glycosides and saponins were also present but in lesser extent. On the contrary phytochemical screening also revealed the complete absence of anthocyanins, anthraquinones, emodins, leucoanthocyanins, phlobatanins, proteins and steroids. Thus the medicinal values of the plant leaves may be due to these specific groups of phytochemicals present in it. Phenolic compounds are well known as antioxidant and scavenging agents for free radicals associated with oxidative damage²⁶.

Tannins are known to be useful in the treatment of inflamed or ulcerated tissues and they have remarkable anticancer property. Terpenoids influence the human immune function and gap-junctional communication. It has been confirmed that pharmacological effect of flavonoids is also correlating with their antioxidant activities²⁷. Thus,

the JOLAE contain a variety of phytochemical compounds having therapeutic efficacy with special reference of antioxidant and hence, can effectively protect from oxidative damage caused by free radicals.

TABLE 1: PHYTOCHEMICAL ANALYSIS AQUEOUS EXTRACT OF *J. OFFICINALE* LEAVES

Phytoconstituents tested	Results observed
Alkaloids	(Wagner's Test) + (Mayer's Test) +
Anthraquinones	(Borntrager's Test) -
Anthocyanins	-
Carbohydrate	(Molisch's Test) + (Fehling's Test) -
Coumarins	+
Emodins	-
Flavonoids	+
Glycosides	(Liebermann's Test) - (Salkowski's Test) + (Molish's Test) +
Leucoanthocyanins	-
Phlobatanins	(Precipitate Test) -
Protein	(Xanthoproteic Test) - (Biuret's test) -
Saponins	(Foam Test) + (Emulsion Test) -
Steroids	(Salkowski's Test) -
Tanins	(Braymer's Test) +
Terpenoids	+

Antioxidant assay - *in vitro*:

Total Phenolic contents:

Table 2, shows the results of total phenolic content of JOLAE expressed as Gallic acid Equivalents (GAE). Standard curve equation of Gallic acid was calculated with $y = 0.0093x + 0.004$, $R^2 = 0.9958$. Total phenolic content was estimated 104.02 mg/g gallic acid equivalent. Thus *J. Officinale* leaves were found to be a potential source of phenolics and hence could be explored as natural antioxidant. Their redox properties permit them to act as reducing agents, hydrogen donors, singlet oxygen quenchers, as well as their metal chelaters.

Total Flavonoid content

Table 2, also shows the results of total flavonoid content of JOLAE, expressed as Quercetin Equivalent (QE). Standard curve equation of Quercetin was calculated with $y = 0.0428x - 0.0196$, $R^2 = 0.9956$. Total flavonoid content was estimated 10.76 mg/g quercetin equivalent.

Flavonoids are also important secondary metabolites of plant like phenolics which modulates lipid peroxidation involved in atherogenesis, thrombosis and carcinogenesis. It is well established that pharmacological effect of flavonoids is associated with their antioxidant activities²⁸.

Total Flavonol content:

In addition to phenolics and flavonoids, **Table 2**, also shows the results of total flavonol content of JOLAE. The results are expressed as Quercetin

Equivalent (QE). Standard curve equation of Quercetin was calculated with and $y=0.0031x + 0.00406$, $R^2= 0.9678$. Total flavonol content was estimated 5.65 mg/g quercetin equivalent. Flavonols are specific group of compounds, possess an ideal structural chemistry for free radical scavenging activity²⁹. The antioxidant activity of the natural extracts may be attributed to the phytochemicals present in them specially polyphenolic compounds which act as primary antioxidants or free radical scavengers³⁰.

TABLE 2: QUANTITATIVE ESTIMATION OF TOTAL PHENOLICS, FLAVONOIDS AND FLAVONOLS OF JOLAE

Total Phenolic Content (mg/g gallic acid equivalent)	Total Flavonoids Content (mg/g quercetin equivalent)	Total Flavonols content (mg/g quercetin equivalent)
104.02 ± 1.28	10.76 ± 0.83	5.65 ± 0.45

Estimation of Reducing Power:

Fig.1, depicts the results of Reducing Power of varied concentrations of JOLAE and of standard, Ascorbic Acid (AA). The reducing power of both; the sample and the standard were found to be concentration-dependent as the absorbance recorded has increased with increase in concentration. Moreover, the reducing power of the *J. Officinale* was found to be significantly greater than that of the AA, at the highest evaluated concentration of 800 µg/ml, confirming thereby, antioxidant potential of its leaves.

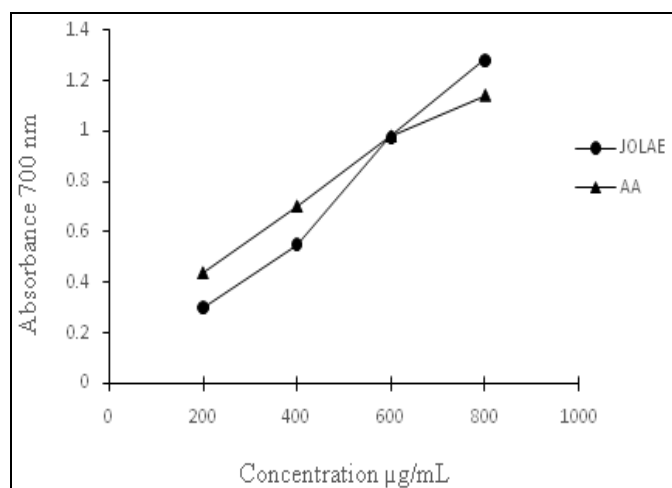


FIG. 1: REDUCING POWER OF JOLAE AND STANDARD EACH VALUE REPRESENTS A MEAN ± SD (N=3)

Reducing power of an extract is defined as the ability to reduce the free radicals and it depends upon the quantity and type of reductones i.e. antioxidants present in the extract.

Free Radical Scavenging Assay:

Fig. 2, describes the results of free radicals scavenging potential of JOLAE and the standard AA in terms of IC₅₀ against DPPH, NO, Superoxide, and ABTS radicals. IC₅₀ is a measure of inhibitory activity of free radicals by 50% at particular concentration and is used as a parameter for antioxidant activity assessment.

DPPH Radical Scavenging:

Fig. 2(a): It clearly reveals the results of IC₅₀ values for DPPH of JOLAE and AA as 41.16µg/mL and 42.79µg/mL respectively. The data validates the primary antioxidant nature of *J. officinale* leaves which could be due to hydrogen donating ability of its phenolic constituents.

Nitric oxide Radical Scavenging:

Fig. 2(b): It shows the results of IC₅₀ values for NO of JOLAE and AA as 30.29µg/mL and 36.74 µg/mL respectively. The data is confirming JOLAE has higher NO scavenging activity than that of the standard, AA and thus, *J. officinale* leaves could be considered as better antioxidant due to their NO scavenging potential, which is widely used as a measure of antioxidant activity of plant extract.

Superoxide radical scavenging:

Fig. 2(c): It depicts the results of IC₅₀ values for O₂⁻ radical of JOLAE and AA as 20.19 µg/mL and 38.22 µg/mL respectively. This data clearly indicates that the *J. officinale* leaves exhibits strong scavenging effect against superoxide radicals.

Since, superoxide anion radical ($O_2^{\cdot-}$), the product of a one-electron reduction of oxygen, is known to be a very harmful species to cellular components as a precursor of most ROS and a mediator in oxidative chain reactions³¹. Thus $O_2^{\cdot-}$ is one of the main culprit free radicals in the body responsible for damaging biomolecules resulting into chronic diseases³². Flavonoids are well known effective antioxidants mainly because they scavenge superoxide anions. Robak and Glyglewski³³.

ABTS Radical Scavenging:

Fig. 2(d): It clearly reveals the results of IC_{50} values for $ABTS^{\cdot+}$ of JOLAE and AA as

29.48 μ g/mL and 45.57 μ g/mL respectively. The data shows appreciably higher scavenging activity of JOLAE against $ABTS^{\cdot+}$ radical. This implies that the extract may be useful for treating radical related pathological damage.

Proton radical scavenging is an important attribute of antioxidants. It is often used for screening complex antioxidant mixtures such as plant extracts, beverages and biological fluids³⁴. The stability of $ABTS^{\cdot+}$ for more than 2 days over a wide pH range raised the interest in the use of $ABTS^{\cdot+}$ for the estimation of antioxidant activity.

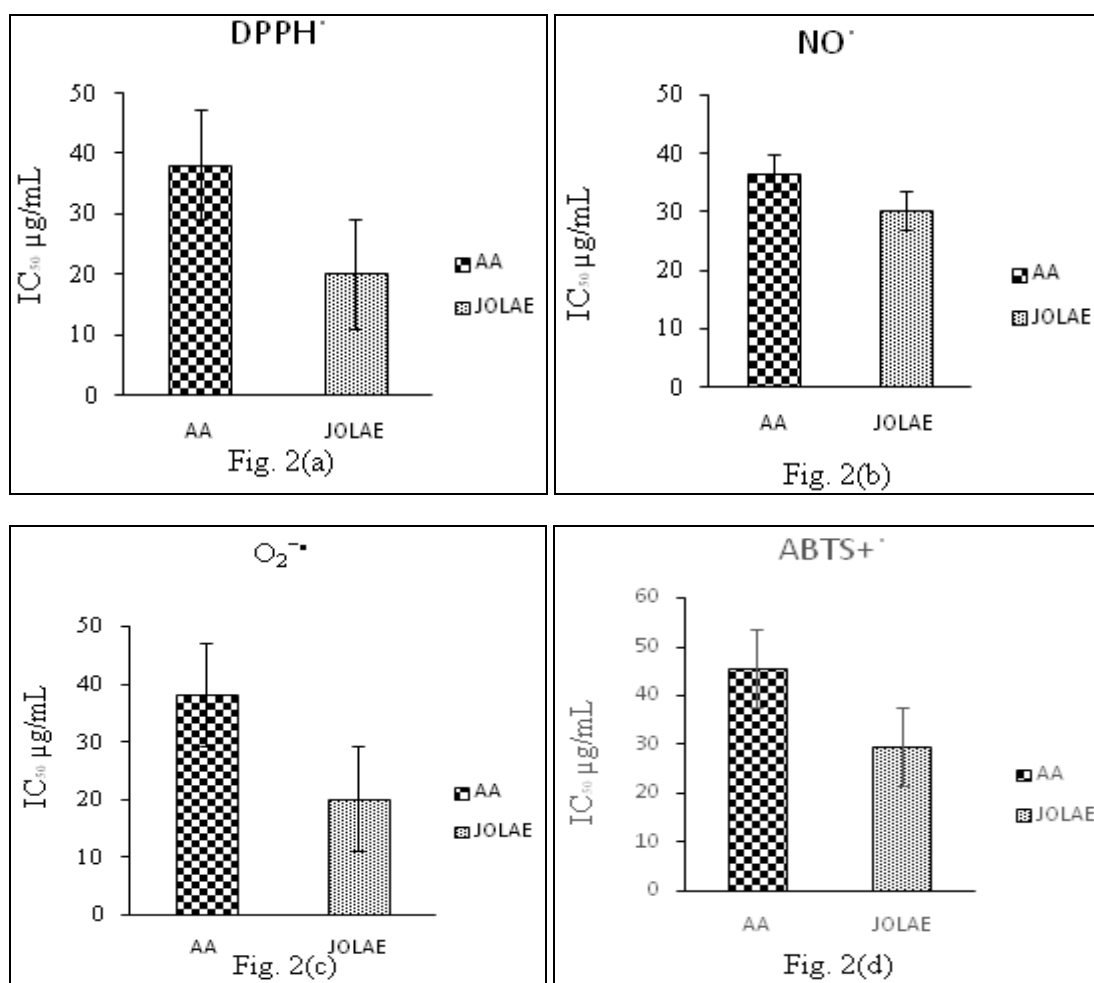


FIG. 2: FREE RADICALS SCAVENGING POTENTIAL OF JOLAE AND AA IN TERMS OF IC_{50} AGAINST DPPH $^{\cdot}$, NO $^{\cdot}$, SUPEROXIDE, AND ABTS RADICALS - each value represents a mean \pm sd (n=3)

CONCLUSION: Phytochemical screening of aqueous extract of *Jasminum officinale* leaves (JOLAE) reveals the presence of polyphenolics in high concentration which must be contributing synergistically to its significant antioxidant potency. The results of various free radicals

scavenging assays also validate its antioxidant potential. The IC_{50} values obtained for all free radicals were much lower as compared to that of the standard, confirming thereby once again significant antioxidant efficacy of *J. officinale* leaves. Thus the leaf extract of *J. officinale* could

be explored further for management and treatment of various radical related diseases like diabetes etc.

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