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CHEMICAL CONSTITUENTS FROM DIFFERENT EXTRACTS OF LEAVES OF *JASMINUM SAMBAC* FOR THEIR ANTIOXIDANT ACTIVITY

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Abstract

Many diseases are associated with oxidative stress caused by free radicals. Current research is directed towards finding naturally-occurring antioxidants of plant origin. The aim of the present study was to evaluate the *in vitro* antioxidant activities of *Jasminum sambac* leaves extract. Petroleum ether, Ethyl acetate and methanol extract of *Jasminum sambac* leaves was studied *in vitro* for DPPH free radical scavenging, nitric oxide, hydrogen peroxide, phenolic and flavonoid contents. Methanol extract of *Jasminum sambac* leaves exhibited potent and concentration dependant free radical scavenging activity in all the tested parameters. Reducing ability was found to increase with increase in Methanol extract of *Jasminum sambac* leaves concentration. Total phenol and flavonoidal content determination showed that the extract is rich in phenols and flavonoids. All the results of the in-vitro antioxidant assays reveal potent antioxidants and free radical scavenging activity of the *Jasminum sambac* leaves equivalent to that of standard ascorbic acid and rutin. This potent antioxidant activity may be attributed to its high phenolic and flavonoidal contents.

***Keywords*:** Methanol extract; *Jasminum sambac* leaves; DPPH; Hydrogen peroxide; Nitric oxide.

Introduction

Herbal medicine, also known as herbalism, is a medical practice based on the use of plants or plant extracts that may be taken orally or applied to the skin. Since ancient times, herbal medicine has been used by many different cultures throughout the world to treat illness and to assist bodily functions (Aruoma & Halliwell, 1987). Nearly all cultures from ancient times to the present day have used plants as a source of medicines. As a result, different remedies tended to develop in different parts of the world. Throughout humankind evolution, the importance of natural products for medicine and health has been enormous. Earliest ancestors chewed certain herbs to relieve pain, or wrapped leaves around wounds to improve healing. Natural products were the sole means to treat diseases and injuries (Bailly *et al.,* 2000; Beckman *et al.,* 1994; Bibitha *et al.,* 2002; Braca *et al.,*2002).

The modern tools of chemistry and biology have currently allowed scientists to detail the exact nature of the biological effects of natural compounds on the human body, as well as to uncover possible synergy, which holds much promise for the development of new therapies against many devastating diseases, including dementia and cancer (Chakraborty & Tripathy, 1992; Elizabeth & Rao, 1990). Modern chemistry has ushered in a new era for the study and use of natural products. Analytical and structural chemistry has provided the tools to purify various compounds and to determine their structures, which, in turn, has given insights into their action on the human body (Floriano *et al.,* 2006; Fontana *et al.,* 2001; Garratt, 1964). In 1805, a German pharmacist Friedrich Wilhelm Sertürner (1783–1841) isolated morphine from opium, and it became both the first pure naturally derived medicine and the first to be commercialized, by Merck in 1826. Medicinal plants, either as extracts, pure compounds or as derivatives, offer unlimited opportunities for the discovery of new drugs (Ghosal & Thakur, 1981; Leksomboon *et al.,* 2001).

Jasminum sambac is an evergreen vine or shrub reaching up to 0.5 to 3 m (1.6 to 9.8 ft) tall The species is highly variable, possibly a result of spontaneous mutation, natural hybridization, and auto polyploidy. Cultivated *Jasminum sambac* generally do not bear seeds and the plant is reproduced solely by cuttings, layering, marcotting, and other methods of asexual propagation. The leaves are ovate, 4 to 12.5 cm (1.6 to 4.9 in) long and 2 to 7.5 cm (0.79 to 2.95 in) wide. The phyllotaxy is opposite or in whorls of three, simple (not pinnate, like most other jasmines). They are smoothing (glabrous) except for a few hairs at the venation on the base of the leaf (Long *et al.,* 1999; Mahanta *et al.,* 2006; Maxwell, 1995).

The flowers bloom all right through the year and are produced in clusters of 3 to 12 together at the ends of branches. They are muscularly scented, with a white corolla 2 to 3 cm (0.79 to 1.18 in) in diameter with 5 to 9 lobes. The flowers open at night, and close in the morning, a span of 12 to 20 hours. The fruit is a purple to black berry 1 cm (0.39 in) in diameter. The sweet, heady fragrance of *Jasminum sambac* is its distinct feature. It is widely grown throughout the tropics from the Arabian peninsula to Southeast Asia and the Pacific Islands as an ornamental plant and for its strongly scented flowers. Numerous cultivars currently exist (Nikki *et al.,* 1994; Pedraza *et al.,* 2004; Re *et al.,* 1999).

Typically, the flowers are harvested as buds during early morning. The flower buds are harvested on basis of color, as firmness and size are variable depending on the weather. The buds have to be white, as green ones may not emit the characteristic fragrance they are known for. Open flowers are generally not harvested as a larger amount of

them is needed to extract oils and they lose their fragrance sooner (Tandon & Rastogi 1976; Yerra *et al.,* 2005).

Material and Method

Selection and Collection of Plant

The plant material was selected on the basis of Ethano botanical survey. Leaves of *Jasminum sambac*are collected from the Pinnacle Biomedical Research Institute, Bhopal Campus.

Authentication of Plant

The identification and authentication of plant was done by Dr. Saba Naaz, Botanist, from the Department of Botany, Saifia College of Science, Bhopal. A voucher specimen number was kept in Department of Botany, Saifia College of Science, Bhopal for future reference.

Solvent Extraction

Hot Soxhlet Extraction Method

In this method, the leaves of *Jasminum sambac* were collected, washed and rinsed properly. They were dried in shade and powdered mechanically. The whole or coarsely powdered plant material of *Jasminum sambac* were successively extracted by solvent like petroleum ether, ethyl acetate and methanol in increasing polarity order for different period of time (6h, 8h, and 10h). The powder was placed "thimble" in chamber of the Soxhlet apparatus. The extracting solvent in flasks was heated, and its vapours condense in condenser. The condensed extract and drips into the thimble containing the powder, and extracts it by contact. When the level of liquid in chamber rises to the top of siphon tube, the liquid contents of chamber siphon drop into flask. This process was continuous and was carried out until a drop of solvent from the siphon tube does not leave residue when evaporated. The extract thus obtained were filtered and concentrated to dryness, weighed and stored for further use (**Handa *et al*., 2008**). The yield of the extract is calculated by using the following formula.

$$\text{Yield } (\%) = \frac{\text{Weight of the residue obtained}}{\text{Weight of the plant material taken}} \times 100$$

Phytochemical Investigation – Qualitative Test

Detailed qualitative phytochemical analysis was performed to identify presence or absence of different phytoconstituents. The color intensity or the precipitate formation was used as analytical responses to these tests. Following standard procedures were used **(Kokate *et al*., 2006)**

Test for Carbohydrates

Molisch's Test: To 1 ml of aqueous solution of the extract mixed with few drops of Molish reagent (α naphthol) and conc. H_2SO_4 (sulphuric acid) was added along the wall of the tube. Formation of purple colored ring at junction indicated the presence of carbohydrates.

Fehling's Test: Equal volume of Fehling A and Fehling B solution were mixed (1ml each) and 2ml of aqueous solution of extract was added followed by boiling for 5-10 minutes on water bath. Formation of reddish brown colored precipitate due to formation of cuprous oxide indicated presence of reducing sugar.

Benedict's Test: Equal volume of Benedict's reagent and extract were mixed in a test tube and heated in the water bath for 5-10 minutes. Solution appears green, yellow or red depending on the amount of reducing sugar present in the test solution which indicated the presences of reducing sugar.

Barfoed's Test: To the aqueous solution of extract, 1 ml of Benedict solution was added and heated almost to boiling. Red colour due to formation of cupric oxide indicates the presence of monosaccharides.

Tests for Alkaloids

Dragendorff's Test: To 1 ml of extract dissolved in alcohol was shaken well with a few drops of acetic acid and Dragendroff's reagent. An orange red precipitate formed indicated the presence of alkaloids.

Wagner's Test: To 1 ml of extract dissolved in acetic acid, a few drops of Wagner's reagent were added. A reddish-brown precipitate formed indicated the presence of alkaloids.

Mayer's Test: To 1 ml of extract dissolved in acetic acid, a few drops of Mayer's reagent were added. A dull white precipitate formed indicated the presence of alkaloids.

Hager's Test: To 1-2 ml of extract dissolved in acetic acid, 3 mL of Hager's reagent was added; the formation of yellow precipitate indicated the presence of alkaloids.

Test for Saponins

Froth Test: To 1ml of extract, distilled water was added and shaken. Stable froth formation indicated the presence of saponin.

Test for Triterpenoids and Steroids

Libermann-Burchard Test: The extract was dissolved in chloroform, 1 mL of acetic acid and 1 mL of acetic anhydride were added, then heated on a water bath and cooled. Few drops of concentrated sulphuric acid were added along the sides of the test tube. Appearance of bluish green colour indicated the presence of steroids.

Salkowski Test: The extract was dissolved in chloroform and equal volume of concentrated sulphuric acid was added. Formation of bluish red to cherry red colour in chloroform layer and green fluorescence in the acid layer indicated the presence of steroids.

Test for Tannin and Phenolic Compounds

Ferric Chloride Test: Some amount of extract was dissolved in distilled water, a few drops of dilute solution of ferric chloride was added, formation of dark blue colour showed the presence of tannins.

Gelatin Test: Some quantity of extract was dissolved in distilled water. Add 2ml of 1% gelatin solution containing 10% sodium chloride was added. Development of white precipitate indicates presences of phenolic compounds.

Lead Acetate Test: Some amount of extract dissolved in distilled water, few drops of lead acetate solution were added. Formation of white precipitate indicates presences of phenolic compounds.

Test for Flavonoids

Shinoda's Test: To the 1 ml of extract in alcohol, a few magnesium turnings and few drops of concentrated hydrochloric acid were added and heated on a water bath. Formation of red to pink colour indicated the presence of flavonoids.

Test for Glycosides

Borntragers Test: To 3 ml of test solution, dilute sulphuric acid was added, boiled for 5 minutes and filtered. To the cold filtrate, equal volume of benezene or chloroform was added and shake it welled. The organic solvent layer was separated and ammonia was added to it. Formation of pink to red color in ammonical layer indicates presence of anthraquinone glycosides.

Keller Killiani Test: To 2 ml of test solution, 3 ml of glacial acetic acid and 1 drop of 5%ferric chloride were added in a test tube. Add carefully 0.5 ml of concentrated sulphuric acid by the side of the test tube. Formation of blue color in the acetic acid layer indicates the presences of Cardiac glycosides.

Quantitative Phytochemical Assay

Total Phenolic Content Estimation (TPC)

Procedure - The amount of total phenolic in extracts was determined with the Folin Ciocalteu reagent. Gallic acid was used as a standard and the total phenolic were expressed as mg/g gallic acid equivalent (GAE). Concentration of 0.01, 0.02, 0.03, 0.04 and 0.05 mg/ml of gallic acid were prepared in methanol. Concentration of 0.1and 1mg/ml of plant extract were also prepared in methanol and 0.5ml of each sample were introduced in to test and mixed with 2.5ml of a 10 fold dilute folin Ciocalteu reagent and 2ml of 7.5% sodium carbonate. The tubes were covered with parafilm and allowed to stand for 30 minutes at room temperature before the absorbance was at read at 760 nm spectrometrically. All determination was performed in triplicate. The folin-Ciocalteu reagent is sensitive to reducing compounds including polyphenols. They produce a blue colour upon reaction. This blue colour was measured spectrophotometrically. Line of regression from Gallic acid was used for estimation of unknown phenol content. From standard curve of gallic acid line of regression was found to be

$$y = 0.005x + 2.569 \text{ and } R^2 = 0.991$$

Thus the goodness of fit was found to be good for selected standard curve. By putting the absorbance of test sample (y = absorbance) in line of regression of above mentioned GA.

Total Flavonoid Content Estimation (TFC)

Procedure

The amount of total flavonoids was measured by a colorimetric assay according to Dewanto *et al*. An aliquot of diluted sample or standard solution of rutin was added to a 75 µl of $NaNO_2$ solution, and mixed for 6 min, before adding 0.15 mL $AlCl_3$ (100 g/L). After 5 min, 0.5 mL of NaOH was added. The final volume was adjusted to 2.5 ml with distilled water and thoroughly mixed. Absorbance of the mixture was determined at 510 nm against the same mixture, without the sample, as a blank. Total flavonoid content was expressed as mg rutin/g dry weight (mg rutine/g DW), through the calibration curve of Rutin. All samples were analysed in three replications (Zhishen *et al*., 1999).

Line of regression from rutin was used for estimation of unknown flavonoid content. From standard curve of rutin, line of regression was found to be

$$y = 0.001x - 0.020 \text{ and } R2 = 0.994$$

Thus the goodness of fit was found to be good for selected standard curve. By putting the absorbance of test sample (y = absorbance) in line of regression of above mentioned rutin.

***In Vitro* Antioxidant Activity**

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

Antioxidants reacts with DPPH, which is stable free radical and is reduced to DPPHH and as consequence the absorbance's decreased from the DPPH radical to the DPPH-H form. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in term of hydrogen donating ability. To assess the scavenging ability on DPPH, each extract (5-20mg/ml) in water and ethanol was mixed with 1 ml of methanolic solution containing DPPH radicals (0.2mM).The mixture was shaken vigorously and left to stand for 30 mins in the dark before measuring the absorbance at 517nm against a blank. Then the scavenging ability was calculated using the following equation:

$$I\% = 100\times (A \text{ blank} - A \text{ sample}/ A \text{ blank})$$

Where, I (%) is the inhibition percent, A blank is the absorbance of the control reaction (containing all reagents except the test compound) and A sample is the absorbance of the test compound.

Nitric oxide assay

Sodium nitroprusside (5 mM) in phosphate buffered saline was mixed with different concentrations of the extract dissolved in methanol and incubated at 25°C for 30 min. A control without the test compound but with an equivalent amount of methanol was taken. After 30 min, 1.5 ml of the incubated solution was removed and diluted with 1.5 ml of Griess reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% N-1-naphthylethylenediamine dihydrochloride). The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with N-1-naphthylethylene diamine dihydrochloride was measured at 546 nm and percentage scavenging activity was measured with reference to standard.

Hydrogen peroxide scavenging activity

A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4). 2 ml (20-100 µg/ml) extract in phosphate buffer were added to 1 ml H_2O_2 (40 mM). Absorbance was determined at 230 nm after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of H_2O_2 scavenging of extract and ascorbic acid (standard compound) was calculated as: % Inhibition= [(A control – A sample)/A control] x 100

Result & Discussion

Preliminary Phytochemical Analyses

Table 1: Qualitative Phytochemical Analyses

S. No.	Experiment	Result		
		Pet Ether Extract	Ethyl acetate Extract	Methanolic Extract
Test for Carbohydrates				
1.	Molisch's Test	-ve	+ve	+ve
2.	Fehling's Test	-ve	+ve	+ve
3.	Benedict's Test	-ve	+ve	+ve
4.	Barfoed's Test	-ve	+ve	+ve
Test for Alkaloids				
1.	Dragendorff's Test	-ve	-ve	-ve
2.	Wagner's Test	-ve	-ve	-ve
3.	Mayer's Test	-ve	-ve	-ve
4.	Hager's Test	-ve	-ve	-ve
Test for Triterpenoids and Steroids				
1.	Libermann-Burchard Test	-ve	+ve	+ve
2.	Salkowski Test:	+ve	+ve	+ve
Test for Saponins				
1.	Froth Test	-ve	+ve	+ve
Test for Tannin and Phenolic Compounds				
1.	Ferric Chloride Test	-ve	+ve	+ve
2.	Gelatin Test	-ve	+ve	+ve
3.	Lead Acetate Test	-ve	+ve	+ve
Test for Flavonoids				
1.	Shinoda's Test	-ve	+ve	+ve
Test for Glycosides				
1.	Borntragers Test	-ve	+ve	+ve
2.	Keller Killiani Test	-ve	+ve	+ve
Test for Protein				
1.	Biuret Test	-ve	-ve	-ve
2.	Ninhydrin Test	-ve	-ve	-ve
3.	Million Test	-ve	-ve	-ve
Test for Oil		+ve	-ve	-ve

Total Phenolic Content of extract of *Jasminum sambac*

Table 2: Total Phenolic Content of extract of *Jasminum sambac*

Extracts	Total Phenolic content (mg/gm equivalent of Gallic acid)
	Jasminum sambac
Ethyl acetate	20.500
Methanol	28.833

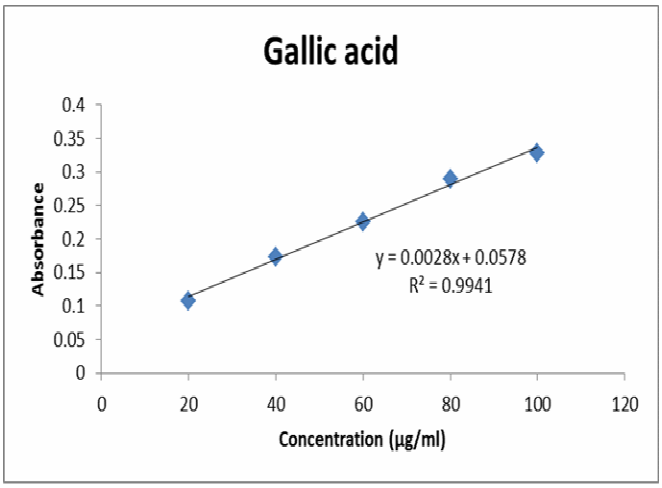

Fig. 1: Standard curve of gallic acid

Total Flavonoid Content of extract of *Jasminum sambac*

Extracts	Total Flavonoid content (mg/gm equivalent of rutin
	Jasminum sambac
Ethyl acetate	12.000
Methanol	33.667

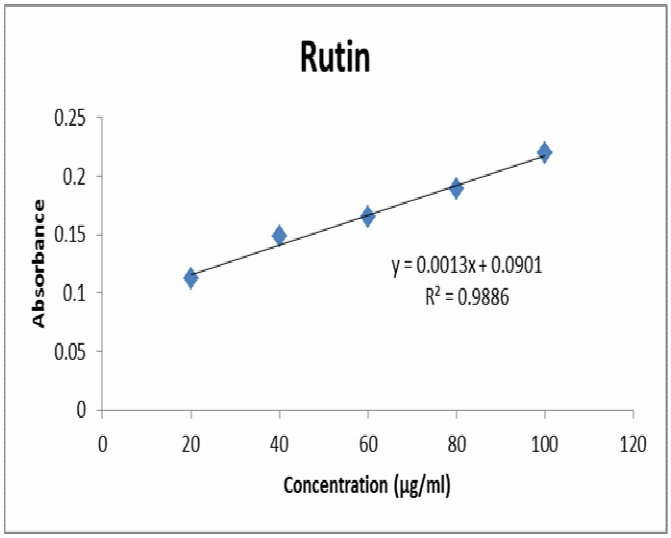

Fig. 2: Standard curve of rutin

DPPH Anioxidant Assay

Table 4: DPPH radical scavenging activity of Std. Ascorbic acid

Ascorbic acid (std.)			
S. No.	**Concentration**	**Absorbance Of Sample**	**% Inhibition**
1	20 µg/ml	0.251	54.36
2	40 µg/ml	0.228	58.55
3	60µg/ml	0.197	64.18
4	80µg/ml	0.137	75.09
5	100µg/ml	0.089	83.82
IC_{50}		**14.42µg/ml**	

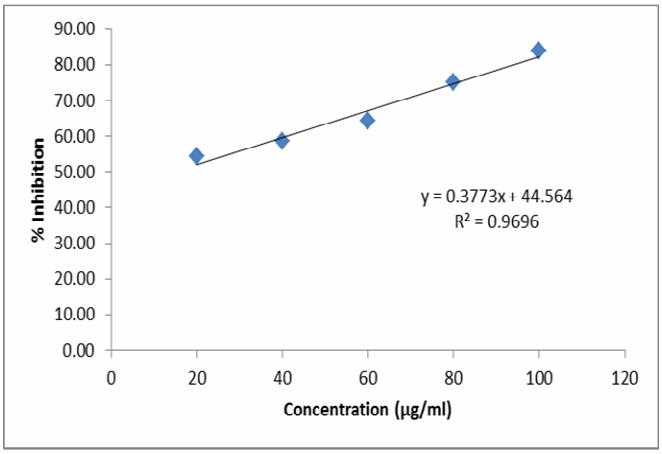

Fig. 3: DPPH radical scavenging activity of Std. Ascorbic acid

DPPH radical scavenging activity of Petroleum extract of *Jasminum sambac*

Table 5: DPPH radical scavenging activity of PEJS

PEJS			
S. No.	**Concentration**	**Absorbance of Sample**	**% Inhibition**
1	20 µg/ml	0.391	28.91
2	40 µg/ml	0.385	30.00
3	60µg/ml	0.363	34.00
4	80µg/ml	0.337	38.73
5	100µg/ml	0.316	42.55
IC_{50}		**144.27µg/ml**	

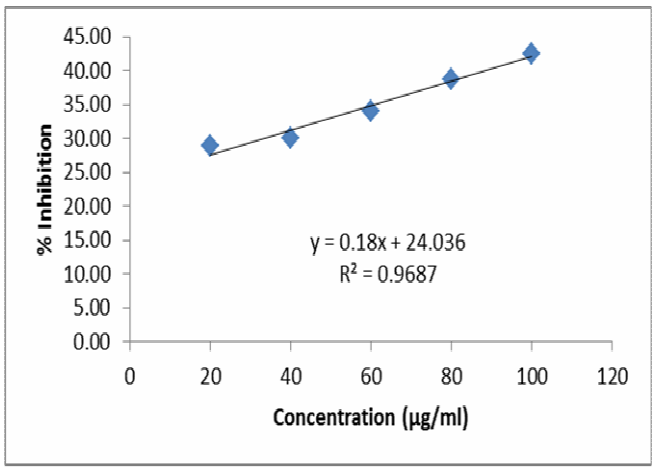

Fig. 4: DPPH radical scavenging activity of PEJS

DPPH radical scavenging activity of Ethyl acetate extract of *Jasminum sambac*

Table 6: DPPH radical scavenging activity of EAJS

EAJS			
S. No.	**Concentration**	**Absorbance of Sample**	**% Inhibition**
1	20 µg/ml	0.361	34.36
2	40 µg/ml	0.345	37.27
3	60µg/ml	0.303	44.91
4	80µg/ml	0.287	47.82
5	100µg/ml	0.266	51.64
IC_{50}		**90.35µg/ml**	

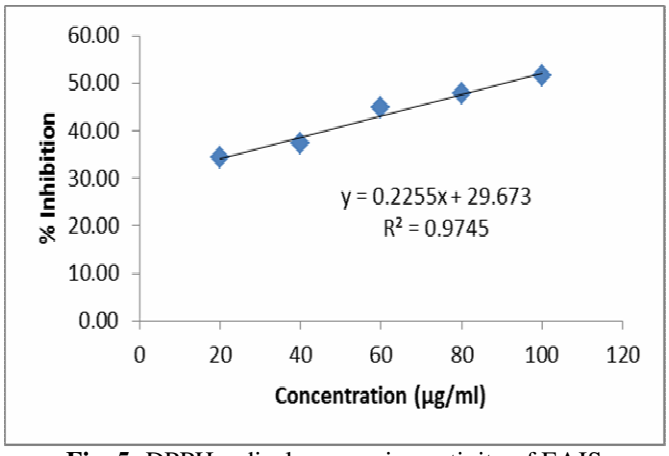

Fig. 5: DPPH radical scavenging activity of EAJS

DPPH radical scavenging activity of Methanolic Extract of *Jasminum sambac*

Table 7: DPPH radical scavenging activity of MEJS

MEJS			
S. No.	**Concentration**	**Absorbance of Sample**	**% Inhibition**
1	20 µg/ml	0.31	43.64
2	40 µg/ml	0.295	46.36
3	60µg/ml	0.274	50.18
4	80µg/ml	0.247	55.09
5	100µg/ml	0.218	60.36
IC_{50}		**54.90µg/ml**	

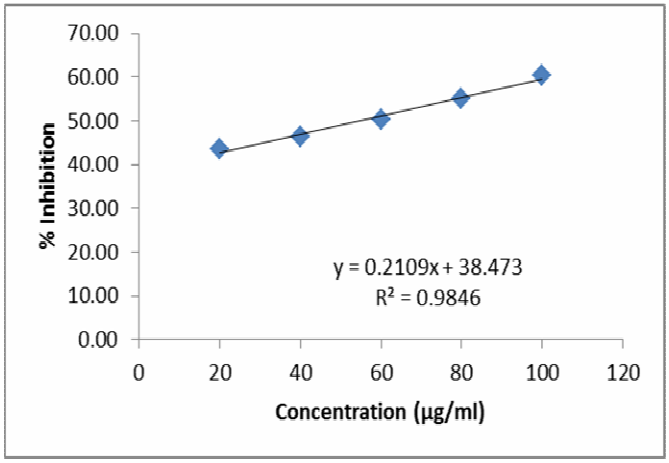

Fig. 6: DPPH radical scavenging activity of MEJS

H_2O_2 Antioxidant Assay

Table 8: H_2O_2 radical scavenging activity of Std. Ascorbic acid

Ascorbic acid (std.)			
S. No.	**Concentration**	**Absorbance of Sample**	**% Inhibition**
1	20 µg/ml	0.911	43.486
2	40 µg/ml	0.77	52.233
3	60µg/ml	0.65	59.677
4	80µg/ml	0.479	70.285
5	100µg/ml	0.38	76.426
IC_{50}		**17.59µg/ml**	

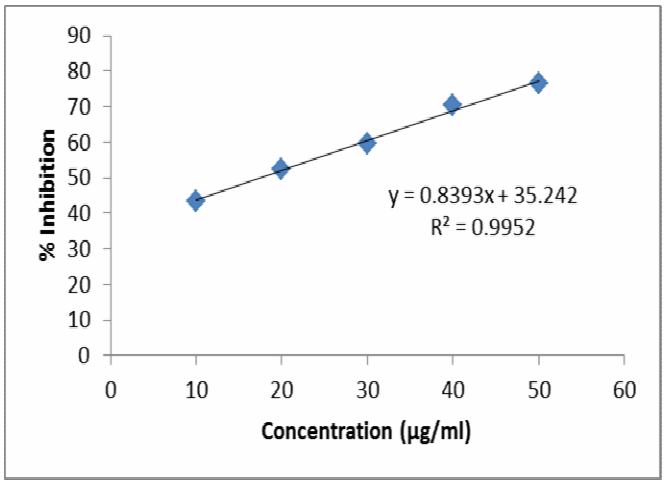

Fig. 7: H_2O_2 radical scavenging activity of Std. Ascorbic acid

H_2O_2 radical scavenging activities of Petroleum extract *Jasminum sambac*

Table 9: H_2O_2 radical scavenging activities of PEJS

PEJS			
S. No.	**Concentration**	**Absorbance of Sample**	**% Inhibition**
1	20 µg/ml	1.211	24.875
2	40 µg/ml	1.141	29.218
3	60µg/ml	1.099	31.823
4	80µg/ml	0.989	38.647
5	100µg/ml	0.863	46.464
IC_{50}		**60.03µg/ml**	

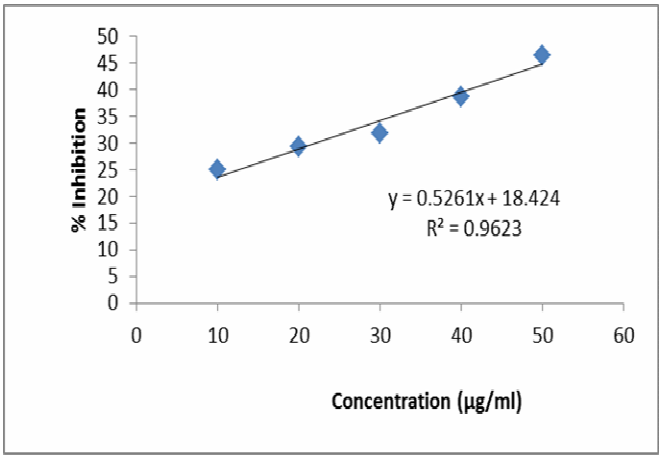

Fig. 8: H_2O_2 radical scavenging activities of PEJS

H_2O_2 radical scavenging activity of Ethyl Acetate *Jasminum sambac*

Table 10: H_2O_2 radical scavenging activity of EAJS

EAJS			
S. No.	**Concentration**	**Absorbance of Sample**	**% Inhibition**
1	20 µg/ml	1.275	12.19008
2	40 µg/ml	1.144	21.21212
3	60µg/ml	1.06	26.99725
4	80µg/ml	0.9	38.01653
5	100µg/ml	0.8	44.90358
IC_{50}		**55.97µg/ml**	

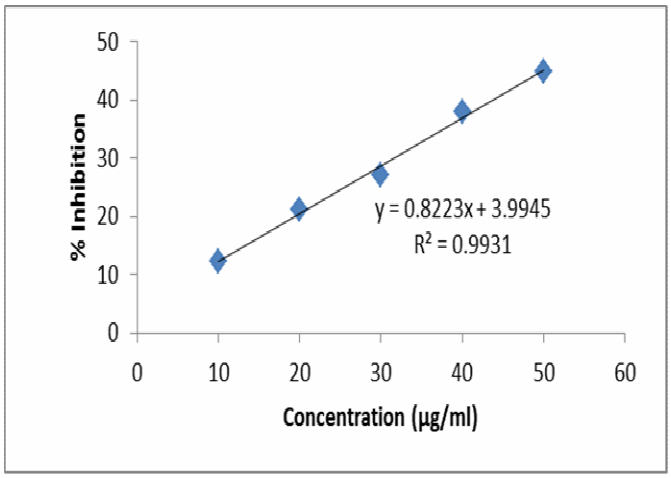

Fig. 9: H_2O_2 radical scavenging activity of EAJS

H_2O_2 radical scavenging activity of Methanolic extract of *Jasminum sambac*

Table 11: H_2O_2 radical scavenging activity of MEJS

MEJS			
S. No.	**Concentration**	**Absorbance of Sample**	**% Inhibition**
1	20 µg/ml	1.044	28.09917
2	40 µg/ml	0.933	35.7438
3	60µg/ml	0.823	43.31956
4	80µg/ml	0.634	56.33609
5	100µg/ml	0.532	63.36088
IC_{50}		**42.39µg/ml**	

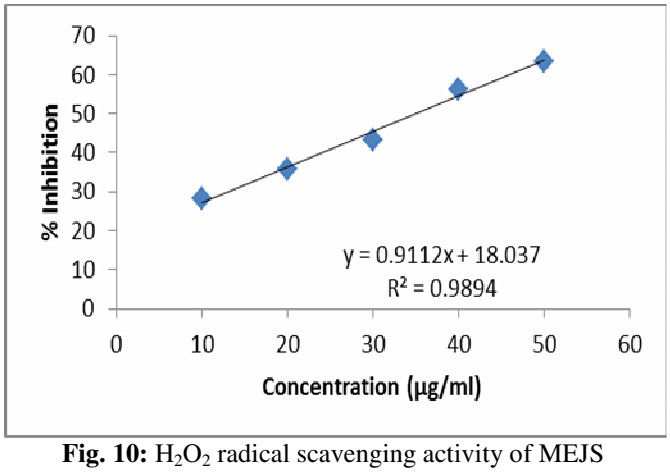

Fig. 10: H_2O_2 radical scavenging activity of MEJS

Nitric Oxide Assay

Table 12: NO radical scavenging activity of Ascorbic acid

Ascorbic acid (std.)			
S. No.	**Concentration**	**Absorbance of Sample**	**% Inhibition**
1	20 µg/ml	0.28	49.367
2	40 µg/ml	0.244	55.877
3	60µg/ml	0.225	59.312
4	80µg/ml	0.195	64.737
5	100µg/ml	0.174	68.535
IC_{50}		**9.74µg/ml**	

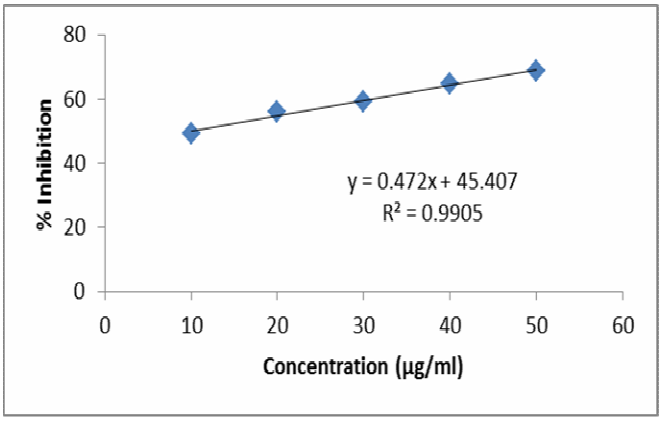

Fig. 11: NO radical scavenging activity of Ascorbic acid

NO radical scavenging activity of Petroleum extract of *Jasminum sambac*

Table 13: NO radical scavenging activity of PEJS

PEJS			
S. No.	**Concentration**	**Absorbance of Sample**	**% Inhibition**
1	20 µg/ml	0.48	13.20072
2	40 µg/ml	0.472	14.64738
3	60µg/ml	0.453	18.08318
4	80µg/ml	0.436	21.15732
5	100µg/ml	0.423	23.50814
IC_{50}		**147.67µg/ml**	

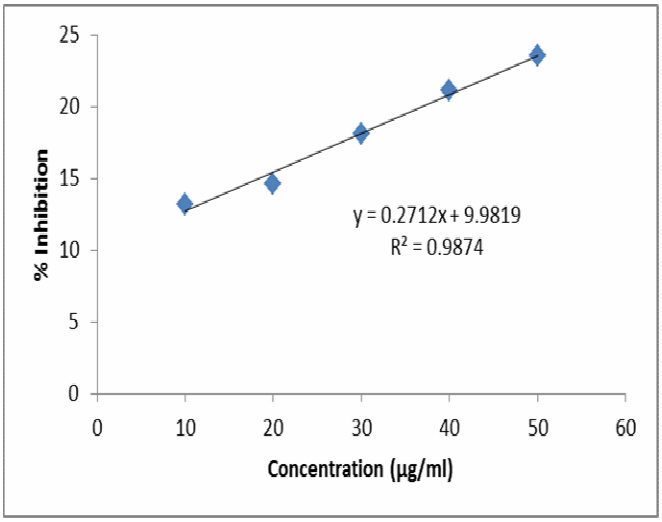

Fig. 12: NO radical scavenging activity of PEJS

NO radical scavenging activity of Ethyl Acetate extract of *Jasminum sambac*

Table 14: NO radical scavenging activity of EAJS

EAJS			
S. No.	**Concentration**	**Absorbance of Sample**	**% Inhibition**
1	20 µg/ml	0.398	28.02893
2	40 µg/ml	0.386	30.19892
3	60µg/ml	0.373	32.54973
4	80µg/ml	0.351	36.52803
5	100µg/ml	0.343	37.97468
IC_{50}		**94.69µg/ml**	

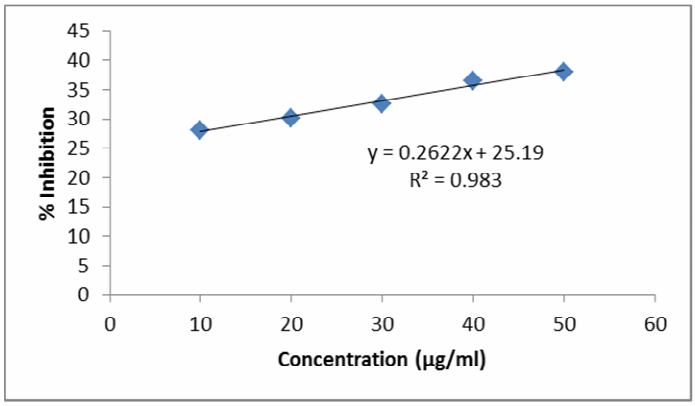

Fig. 13: NO radical scavenging activity of EAJS

NO radical scavenging activity of Methanolic Extract of *Jasminum sambac*

Table 15: NO radical scavenging activity of MEJS

MEJS			
S. No.	**Concentration**	**Absorbance of Sample**	**% Inhibition**
1	20 µg/ml	0.424	23.32731
2	40 µg/ml	0.391	29.29476
3	60µg/ml	0.355	35.8047
4	80µg/ml	0.336	39.24051
5	100µg/ml	0.323	41.59132
IC_{50}		**64.84µg/ml**	

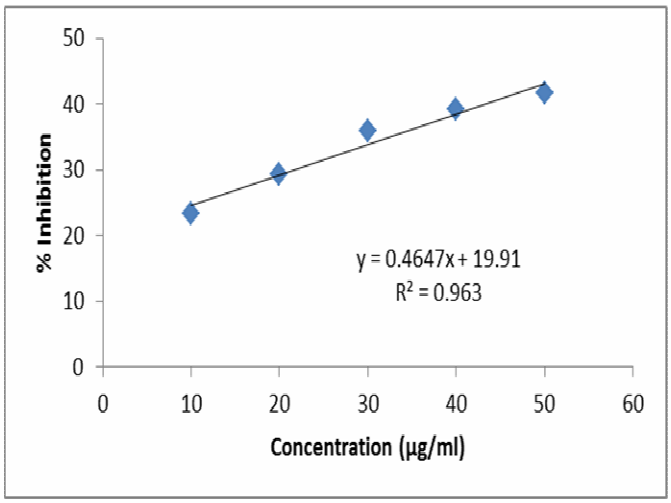

Fig. 14: NO radical scavenging activity of MEJS

The total phenolic content and total flavonoid content of the ethyl acetate and methanolic extract were found to be 20.500 and 28.833 of gallic acid equivalent/g of extract and 12.000 and 33.667 of rutin equivalent/g of extract respectively. DPPH radical scavenging activity of all the three extracts (PEJS, EAJS and MEJS) was shown in Table 4-7. The extract exhibited potent radical scavenging activity concentration dependant manner, MEJS shows the good results compared to other two extracts. The IC_{50} value of the extract was comparable to the standards used. Superoxide radical scavenging activity of all the three extracts (PEJS, EAJS and MEJS) was assessed by alkaline phosphate buffer solution method. The plant extracts strongly inhibited the superoxide radical generation. In hydrogen peroxide radical scavenging assay, the extracts were found to be equipotent with rutin but less potent when compared to ascorbic acid. The values were tabulated in table 8-11. MEJS showed a strong nitric oxide scavenging activity compared to other two extracts which was comparable to the standards ascorbic acid. The IC_{50} value of MEJS was nearly to equal of ascorbic acid. The IC_{50} value of extracts and standards were presented in table 12-15.

Conclusion

The results from various free radicals scavenging systems reveal that methanol extract of PS has significant antioxidant activity. The extracts are found to have different levels of antioxidanr activity in all methods tested. IS50 values obtained were comparable to that of the standards used as ascorbic acid and rutin. According to the study, a significant antioxidant activity was found. Total phenol and flavonoid content determination indicates the high content of phenols and flavonoids and these compounds could be najor contributors to antioxidant activity. Further studies in our laboratory are in progress for the isolation and identification of phytochemical compounds and to ensure that the medicinal values of the plant in0vivo correlate with its antioxidant activity.

References

Aruoma, O.I. and Halliwell, B. (1987). Action of hypochlorous acid on the antioxidant protective enzymes superoxide dismutase, catalase and glutathione peroxidase. *Biochem J* , 248: 973-976.

Bailly, F.; Zoete, V.; Vamecq, J.; Catteu, J.P. and Bernier, J.L. (2000). Antioxidant actions of ovothiol-derived 4-mercaptoimidazoles: glutathione peroxidase activity and protection against peroxynitrite-induced damage. *FEBS Lett.;* 486:19-22.

Beckman, J.S.; Chen, H.; Ischiropulos, H. and Crow, J.P. (1994). Oxidative chemistry of peroxynitrite. *Methods Enzymol,* 233: 229-240.

Bibitha, B.; Jisha, V.K.; Salitha, C.V.; Mohan, S. and Valsa, A.K. (2002). Antibacterial activity of different plant extracts. *Indian J Microbiol* 42(4): 361-363.

Braca, A.; Sortino, C.; Politi, M.; Morelli, I. and Mendez, J. (2002). Antioxidant activity of flavonoids from *Licania licaniaeflora*. *J Ethnopharmacol* 79: 379-381. PubMed Abstract Publisher Full Text.

Chakraborty, N. and Tripathy, B.C. (1992). Involvement of singlet oxygen in 5-aminolevulinic acid-induced photodynamic damage of cucumber (*Cucumbis sativus* L.) chloroplasts. *Plant Physiol* 98: 7-11.

Elizabeth, K. and Rao, M.N.A. (1990). Oxygen radical scavenging activity of curcumin. *Int J Pharm. Sci.* 58: 237-240.

Floriano-Sánchez, E.; Villanueva, C.; Medina-Campos, O.N.; Rocha, D.; Sánchez-González, D.J.; Cárdenas-Rodríguez, N. and Pedraza-Chaverrí, J. (2006). Nordihydroguaiaretic acid is a potent in vitro scavenger of peroxynitrite, singlet oxygen, hydroxyl radical, superoxide anion, andhypochlorous acid and prevents in vivo tyrosine nitration in lung. *Free Radic Res.;* 40: 523-533.

Fontana, M.; Mosca, L. and Rosei, M.A. (2001). Interaction of enkephalines with oxyradicals. *Biochem Pharmacol* 61: 1253-1257.

Garratt, D.C. (1964). *The quantitative analysis of Drugs. Volume 3*. Chapman and Hall ltd, Japan; 456-458.

Ghosal, P.K. and Thakur, S. (1981). Structural features of the acidic polysaccharide of *Spondias pinnata* gum exudates. *Carbohydr Res.,* 98: 75-83.

Leksomboon, C.; Thaveechai, N. and Kositratana, W. (2001). Potential of plant extracts for controlling citrus canker of lime. *Kasetsart J (Nat Sci),* 35: 392-396.

Long, L.H.; Evans, P.J. and Halliwell, B. (1999). Hydrogen peroxide in human urine: implications for antioxidant defense and redox regulation. *Biochem Biophys Res Commun* 262: 605-609.

Mahanta, R.K.; Rout, S.D. and Sahu, H.K. (2006). Ethnomedicinal plant resources of Similipal biosphere reserve, Orissa, India. *Zoos Print J,* 21(8): 2372-2374.

Maxwell, S.R. (1995). Prospects for the use of antioxidant therapies. *Drugs* 49: 345-361. PubMed Abstract

Niki, E.; Shimaski, H. and Mino, M. (1994). Antioxidantism-free radical and biological defense. *Gakkai Syuppn Center, Tokyo* 3-16.

Pedraza-Chaverrí, J.; Arriaga-Noblecía, G. and Medina-Campos, O.N. (2007). Hypochlorous acid scavenging capacity of garlic. *Phytother Res.;* 21: 884-888.

Pedraza-Chaverrí, J.; Barrera, D.; Maldonado, P.D.; Chirino, Y.I.; Macías-Ruvalcaba, N.A.; Medina-Campos, O.N.; Castro, L.; Salcedo, M.I. and Hernández-Pando, R. (2004). S-allylmercaptocysteine scavenges hydroxyl radical and singlet oxygen in vitro and attenuates gentamicininduced oxidative and nitrosative stress and renal damage *in vivo*. *BMC Clin Pharmacol,* 4:5.

Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M. and Rice-Evans, C. (1999) Antioxidant activity applying an improved ABTS radical cation decolorization asszy. *Free Rad Biol Med,* 26: 1231-1237.

Tandon, S. and Rastogi, R.P. (1976). Studies on the chemical constituents of *Spondias pinnata*. *Planta Med* 29:190-192.

Yerra, R.; Senthikumar, G.P. and Gupta, M. (2005). Studies on in vitro antioxidant activities of methonal extracts of *Mucuna pruriens* (Fabaceae) seeds. European bulletin of drug research, 1: 31-39.