

**ANTIOXIDANT POTENTIAL OF *INDIGOFERA LINNAEI* ALI. – AN *IN VITRO* STUDY.**

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### Summary

In the present study, the antioxidant activity of methanol extract of *Indigofera linnaei* (MEIL) was evaluated by different *in vitro* antioxidant assay models. The total phenol and flavonoid content was also determined in the extract. MEIL exhibited strong antioxidant and scavenging activity on ABTS radical cation, DPPH free radical, hydrogen peroxide, nitric oxide, superoxide radical and hydroxyl radical. The extract showed strong activity in Iron reducing power assay. The antioxidant and free radical scavenging activities of the extract were comparable to standard antioxidants used such as ascorbic acid and rutin. The extract had good phenol and flavonoid contents. The antioxidant activity may be due to the rich amount of phenols and flavonoids present in the extract. Therefore, the plant could be considered as a very good antioxidant source with therapeutic potential.

**Key Words:** *Indigofera linnaei*, antioxidant, free radical scavenging activity, flavonoids, total phenol content, total flavonoid content

### Introduction

Oxidation and reduction reactions are essential to many living organisms for the production of energy to biological purposes. However, oxygen free radicals and other reactive oxygen species (ROS) which are continuously produced *in vivo*, result in cell death and tissue damage. These species can react with biological substrates such as DNA and proteins, leading to several diseases including cancer, diabetes, cardiovascular diseases, aging, arthritis and atherogenesis [1, 2]. Antioxidants are vital substances which provide protection to living organisms from damage caused by uncontrolled production of ROS and the concomitant lipid peroxidation, protein damage and DNA strand breaking [3, 4]. Several anti-inflammatory, antinecrotic, neuroprotective, chemopreventive and hepatoprotective drugs have recently been shown to have antioxidant and radical scavenging mechanism as part of their activity [5, 6].

There is an increased interest in natural antioxidants present in medicinal and dietary plants, which might help to prevent oxidative damage [7]. Several members of the species of *Indigofera* like *Indigofera trita*, *Indigofera aspalathoides* etc. are used traditionally for a wide variety of ethnomedical properties such as antitumor, hepatoprotective, antioxidant, anti-inflammatory and analgesic [8-11]. Among them, *Indigofera linnaei* (Fabaceae) is a small trailing, much branched annual or biennial herb, distributed throughout India. The juice of the plant is used as antiscorbutic, diuretic and to treat burns and epilepsy. It has long been used by tribes and native medical practitioners to treat rheumatism, arthritis, inflammation, tumor and liver diseases [12]. Literature review revealed that three nitropropanoyl esters of glucose namely, 1,2,6-tri-O-(3-nitropropanoyl)- $\beta$ -D-glucopyranose, 2,3,4,6-tetra-O-(3-nitropropanoyl)- $\alpha$ -D-glucopyranose and 3,4,6-tri-O-(3-nitropropanoyl)- $\alpha$ -D-glucopyranose were isolated from the aerial parts of *Indigofera linnaei* [13]. The plant exhibit wound healing activity in rats [14]. A new isoflavone namely 7,8-methylenedioxy-4'-methoxyisoflavone was isolated from the entire plant of *Indigofera linnaei* [15]. In view of the several ethnomedical uses of *I. linnaei* described above, it was proposed to screen its methanolic extract for the antioxidant activity using various in vitro systems.

## Materials and Methods

### Chemicals

2,2'-azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt (ABTS) was obtained from Sigma Aldrich Co., St. Louis, USA. Rutin and *p*-nitroso dimethyl aniline (*p*-NDA) were obtained from Acros Organics, New Jersey, USA. Naphthyl ethylene diamine dihydrochloride (NEDD) was obtained from Roch-Light Ltd., Suffolk, UK. Ascorbic acid and nitro blue tetrazolium (NBT) were obtained from S.D. Fine Chem, Ltd., Biosar, India. 2-Deoxy-D-ribose was from Hi-Media Laboratories Ltd., Mumbai. All other chemical used were of analytical grade.

### Plant material and Extraction

Entire plants of *I. linnaei* were collected from the foothills of Yercaud in the month of November 2008. The plant was authenticated by Dr. G.V.S. Murthy, Joint Director, Botanical Survey of India, Coimbatore, Tamilnadu, India. A voucher specimen is preserved in our laboratory for future reference (Voucher No. P. Ch. IL 002). The plant material was shade dried, pulverized and extracted (500 g) with 80% methanol at room temperature for 72 h. The extract was filtered and concentrated to dryness under reduced pressure and controlled temperature (40 °C to 50 °C) in a rotary evaporator. The extract was a dark yellowish brown solid weighing 50.2 g (yield, 10.4 %) and was preserved in a vacuum desiccator until further use.

### Preparation of test and standard solutions

The methanolic extract of *I. linnaei* and the standard antioxidants (ascorbic acid and rutin) were dissolved in distilled dimethyl sulfoxide (DMSO) separately and used for the *in vitro* antioxidant assays except the hydrogen peroxide method because it interferes with the method. For hydrogen peroxide method, the extract and the standards were dissolved in distilled methanol and used. The stock solutions were serially diluted with the respective solvents to obtain the lower concentrations.

### Estimation of total phenol content

About 0.1 ml of the extract (10  $\mu$ g/ml) was mixed with 0.5 ml of Folin-Ciocalteu reagent (diluted 1:10 ratio with distilled water) and 1.5 ml of sodium carbonate. The mixture was shaken thoroughly and made up to 10 ml with double distilled water. The mixture was

allowed to stand for 2 h. the absorbance was measured at 750 nm using PerkinElmer Lambda 25 UV-Visible spectrophotometer. Using the gallic acid standard curve (2 – 10 µg/ml), the total phenol content was obtained. The total phenol content was expressed as gallic acid equivalent in mg/g of the extract [16].

#### **Estimation of total flavonoid content**

About 0.5 ml of the plant extract (100 µg/ml) was mixed with 1.5 ml of methanol (75% v/v), 0.1 ml of aluminium chloride (10 %w/v), 0.1 ml of potassium acetate (1 M) and 2.8 ml of double distilled water. The reaction mixture was allowed to incubate for 30 min at room temperature before the absorbance was taken at 435 nm. Water (0.1 ml) was used to substitute aluminium chloride for blank. Rutin was used as a standard for the calibration curve. The result was expressed as rutin equivalent mg/g of extract [17].

#### ***In vitro* antioxidant activity**

The methanol extract of *I. linnaei* was tested for its *in vitro* antioxidant activity using the standard methods. In all these methods, a particular concentration of the extract or standard solution was used which gave a final concentration of 1000-15.625 µg/ml after all the reagents were added. Absorbance was measured against a blank solution containing the extract or standards, but without the reagents. A control test was performed without the extract or standards. Percentage scavenging and IC<sub>50</sub> values ± SEM were calculated.

#### **ABTS radical scavenging activity**

In a final volume of 1 ml, the reaction mixture comprised 950 µl of ABTS<sup>+</sup> solution and 50 µl of the plant extract at various concentrations. The reaction mixture was homogenized and incubated for 20 min. absorbances of these solutions were measured spectrophotometrically at 734 nm [18].

#### **DPPH radical scavenging activity**

The DPPH assay method depends on the reduction of purple DPPH to a yellow colored diphenyl picrylhydrazine and the remaining DPPH which showed maximum absorption at 517 nm was measured. About 2 ml of various concentrations of the plant extract or standards were added to 2 ml of DPPH solution (0.1 mM, 2 ml). After 20 min of incubation at 37 °C in the dark, the absorbance was recorded at 517 nm [19].

#### **Nitric oxide radical inhibition assay**

Nitric oxide was generated from sodium nitroprusside and measured by the Greiss reaction. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitric ions that can be estimated by using Griess reagent. Scavengers of nitric oxide compete with oxygen leading to reduce production of nitric oxide. The reaction mixture (6 ml) containing sodium nitroprusside (10 mM, 4 ml), phosphate buffer saline (PBS, pH 7.4, 1 ml) and extract or standard (1ml) in DMSO at various concentrations and it was incubated at 25 °C for 150 min. After incubation, 0.5 ml of the reaction mixture containing nitrite ion was removed, sulphanilic acid reagent was added (0.33 %w/v, 1 ml), mixed well and allowed to stand for 5 min for completion of diazotisation. Then, 1 ml of NEDD was added, mixed and allowed to stand for 30 min in diffused light. A pink colored chromophore was formed. The absorbance was measured at 540 nm [20, 21].

#### **Superoxide radical scavenging activity by alkaline DMSO method**

In this method, superoxide radical is generated by the addition of sodium hydroxide to air saturated dimethyl sulfoxide. The generated superoxide remains stable in solution, which reduces nitroblue tetrazolium (NBT) into formazan dye at room temperature and that can be

measured at 560 nm. Briefly, to the reaction mixture containing 1 ml of alkaline DMSO (1 ml DMSO containing 5 mM NaOH in 0.1 ml water) and 0.3 ml of the extract in freshly distilled DMSO at various concentrations, added 0.1 ml of NBT (1 mg/ml) to give a final volume of 1.4 ml. The absorbance was measured at 560 nm [22].

### **Hydrogen peroxide radical scavenging method**

In this method, when a scavenger is incubated with hydrogen peroxide, the decay or loss of hydrogen peroxide can be measured spectrophotometrically at 230 nm. To 1 ml of various concentrations of extract or standard in methanol was added to 2 ml of hydrogen peroxide (20 mM) in phosphate buffer saline. After 10 min the absorbance was measured at 230 nm [23].

### **Hydroxyl radical scavenging activity**

#### ***p*-NDA method**

Various concentration of the extract or standards in 0.5 ml of distilled DMSO were added to a solution mixture containing 0.5 ml of ferric chloride (0.1 mM), 0.5 ml of EDTA (0.1 mM), 0.5 ml of ascorbic acid (0.1 mM), 0.5 ml of hydrogen peroxide (2 mM) and 0.5 ml of *p*-NDA (0.01 mM) in phosphate buffer (pH 7.4, 20 mM) to produce a final volume of 3 ml. Absorbance was measured spectrophotometrically at 440 nm [24].

#### **Deoxyribose method**

To the reaction mixture containing deoxyribose (0.2 ml, 3 mM), ferric chloride (0.2 ml, 0.1 mM), EDTA (0.2 ml, 0.1 mM), ascorbic acid (0.2 ml, 0.1 mM) and hydrogen peroxide (0.2 ml, 2 mM) in phosphate buffer (pH, 7.4, 20 mM), added 0.2 ml of various concentrations of extract or standard in freshly distilled DMSO to give a total volume of 1.2 ml. The solutions were then incubated for 30 min at 37 °C. After incubation, ice-cold trichloro acetic acid (0.2 ml, 15 %w/v) and thiobarbituric acid (0.2 ml, 1 %w/v), in 0.25 N HCl were added. The reaction mixture was kept in a boiling water bath for 30 min, cooled and the absorbance was measured at 532 nm [24].

#### **Iron reducing power assay**

To 1 ml of the plant extract was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1 % potassium ferricyanide. The reaction mixture was incubated at 50 °C for 20 min. after incubation period, 2.5 ml of 10% trichloroacetic acid was added and the reaction mixture was centrifuged at 1000 rpm for 10 min. The upper 2.5 ml layer was mixed with 2.5 ml of deionized water and 0.5 ml of ferric chloride and thoroughly mixed. The absorbance was measured spectrophotometrically at 700 nm. A higher absorbance indicates a higher reducing power [25].

## **Results**

### **Total Phenol Content and Total Flavonoid Content**

The content of total phenols in the extract is determined using the Folin-Ciocalteu reagent, calculated from the regression equation of calibration curve ( $y = 0.1134 x$ ,  $r^2 = 0.979$ ) and is expressed as gallic acid equivalents. Total phenol content of the extract was found to be  $392.16 \pm 11.16$  mg/g of extract. The total flavonoid content was estimated by aluminium chloride method using rutin as standard. The amount was calculated from the regression equation of calibration curve ( $y = 0.0202 x - 0.0435$ ,  $r^2 = 0.9953$ ) and is expressed as rutin equivalents. The amount of total flavonoids in the extract was found to be  $223.18 \pm 1.29$  mg/g of extract.

**ABTS Radical Scavenging Assay**

ABTS radical scavenging activity of MEIL is shown in Fig.1. The extract showed potent radical scavenging activity in concentration dependent manner. The IC<sub>50</sub> of the extract, ascorbic acid and rutin was found to be 13.18 ± 0.26, 27.61 ± 1.04 and 12.37 ± 0.24 µg/ml, respectively.

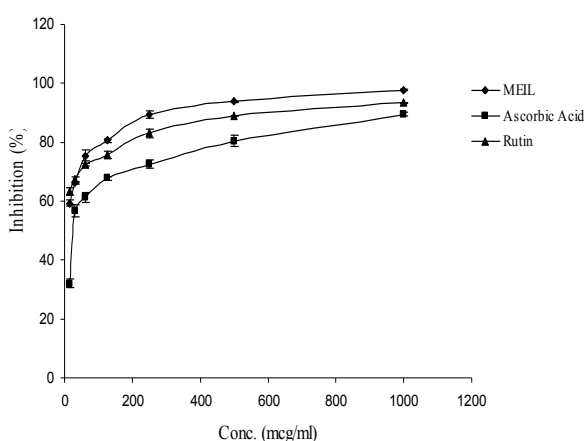


Fig.1 Effect of MEIL on ABTS Radical Scavenging Assay

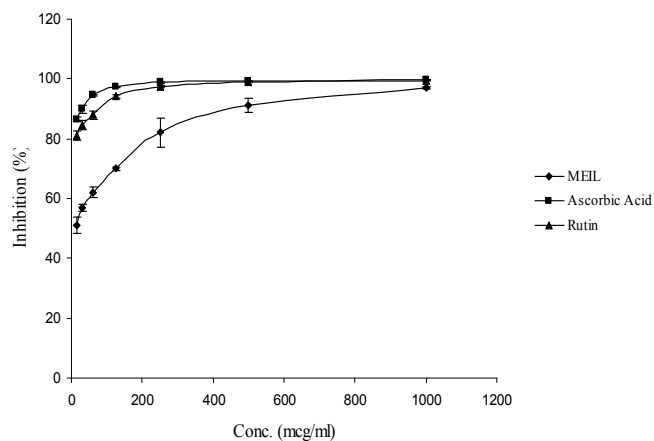


Fig.2. Effect of MEIL on DPPH Scavenging Activity

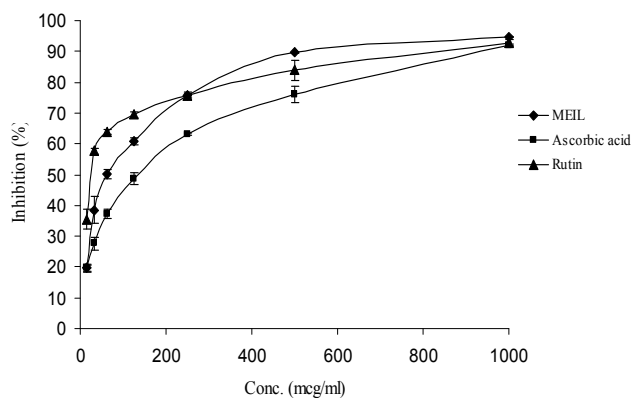


Fig.3 Effect of MEIL on Nitric oxide Radical Scavenging Activity

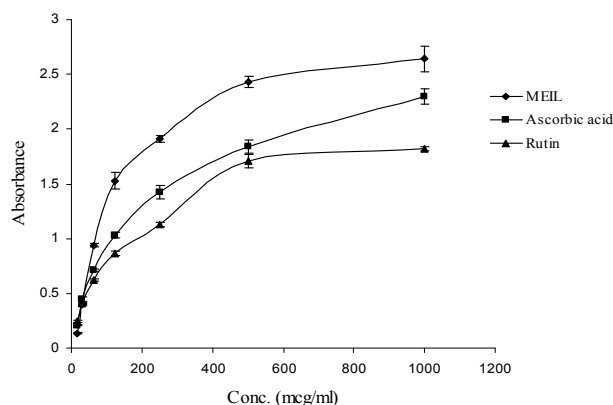


Fig.4. Effect of MEIL on Iron Reducing Power Assay

**DPPH Radical Scavenging Activity**

The DPPH radical scavenging assay showed that MEIL eliminated DPPH radical in a dose dependent manner (Fig.2). IC<sub>50</sub> values for MEIL, ascorbic acid and rutin were 15.39 ± 0.85, 9.03 ± 0.025 and 9.65 ± 0.19 µg/ml, respectively.

### Nitric oxide Scavenging Assay

The extract exhibits a strong nitric oxide scavenging activity and which was comparable to the standards ascorbic acid and rutin (Fig.3). The IC<sub>50</sub> values of MEIL, ascorbic acid and rutin were found to be 62.24 ± 1.78, 129.06 ± 5.54 and 54.27 ± 1.00 µg/ml, respectively.

### Superoxide and Hydrogen peroxide radical Scavenging Activity

Superoxide radical scavenging activity of MEIL was assessed by alkaline DMSO method. The plant extract moderately inhibit the superoxide radical generation. In hydrogen peroxide radical scavenging assay, the extract was found to be equipotent with rutin but less potent when compared to ascorbic acid. The values were tabulated in Table 1.

**Table 1. Effect of MEIL on Superoxide and Hydrogen peroxide Radical Scavenging Activity**

Drug/Standard	IC <sub>50</sub> (µg/ml)*	
	Superoxide Radical Scavenging by Alkaline DMSO Method	Hydrogen peroxide Radical Scavenging Method
MEIL	121.7 ± 1.38	123.08 ± 5.85
Ascorbic acid	>1000	31.41 ± 1.9
Rutin	>1000	127.63 ± 3.20

\* Mean of three determinations

Data were expressed as mean ± SEM

### Hydroxyl radical Scavenging Assay

Hydroxyl radical scavenging activity of MEIL was measured by p-NDA method and deoxyribose method. In p-NDA method, the extract showed very potent activity when compared to standards used. In deoxyribose method, the inhibitory concentration was comparable to that of rutin. The IC<sub>50</sub> values were presented in Table 2.

**Table 2. Effect of MEIL on Hydroxyl Radical Scavenging Activity**

Drug/Standard	IC <sub>50</sub> (µg/ml)*	
	Hydroxyl Radical Scavenging	
	p-NDA Method	Deoxyribose Method
MEIL	74.63 ± 7.74	58.8 ± 2.92
Ascorbic acid	>1000	-
Rutin	>1000	32.82 ± 0.60

\* Mean of three determinations

Data were expressed as mean ± SEM

### Iron Reducing Power Assay

Fig.4 shows the reductive ability of MEIL compared to ascorbic acid and rutin. Absorbance of the solution was increased when the concentration increased. A higher absorbance indicates a higher reducing power.

### Discussion

In the last two decades there has been an explosive interest in the role of oxygen free radicals, more generally known as “reactive oxygen species” (ROS) and of “reactive nitrogen species” (RNS) in experimental and clinical medicine [1]. ROS/RNS are known to play a dual role in biological systems, since they can be either harmful or beneficial to living systems [26]. Beneficial effects of ROS involve physiological roles in cellular responses to noxia, as for example in defense against infectious agents and in the function of a number of cellular signaling systems. One further beneficial example of ROS at low concentrations is the induction of a mitogenic response. In contrast, at high concentrations, ROS can be important mediators of damage to cell structures, including lipids and membranes, proteins and nucleic acids termed as oxidative stress [27]. Oxidative stress has been implicated in the pathology of many diseases such as inflammation, cancer, diabetes, neurodegenerative disorders and aging. Reactive oxygen species and reactive nitrogen species such as superoxide anions, hydroxyl radical and nitric oxide inactivate enzymes and damage intracellular components causing injury through covalent binding and lipid peroxidation. Antioxidants are compounds that hinder the oxidative processes and thereby delay or prevent oxidative stress [28].

The harmful effects of ROS are balanced by the antioxidant enzymes. Despite the presence of the cell's antioxidant defense system to counteract oxidative damage from ROS, oxidative damage accumulates during the life cycle, and radical-related damage to DNA, to proteins and to lipids has been proposed to play a key role in the development of age-dependent diseases such as cancer, arteriosclerosis, arthritis, neurodegenerative disorders and other conditions [1]. The antioxidant activity of the methanol extract of *Indigofera linnaei* was investigated against various *in vitro* models. Since, free radicals are of different chemical entities, it is essential to test the extract against many free radicals to prove their antioxidant activity. Hence, a large number of *in vitro* methods were used for the screening. IC<sub>50</sub> values obtained were compared with the standards used, that is, ascorbic acid and rutin.

ABTS radical scavenging activity is relatively recent one, which involves a more drastic radical, chemically produced and is often used for screening complex antioxidant mixtures such as plant extracts, beverages and biological fluids. The ability in both the organic and aqueous media and the stability in a wide pH range raised the interest in the use of ABTS<sup>+</sup> for the estimation of antioxidant activity [29]. The extract showed potent antioxidant activity in ABTS method which is comparable to the standard used. Here, the extract's radical scavenging activity showed a direct role of its phenolic compounds in free radical scavenging.

The DPPH is a stable free radical, which has been widely accepted as a tool for estimating free radical scavenging activities of antioxidants [30]. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [31]. The reduction capability of DPPH radical is determined by the decrease in absorbance at 517 nm induced by antioxidants. The experimental data of the extract revealed that the extract is likely to have the effects of scavenging free radicals. From the result we observe that a dose dependent relationship in the DPPH radical scavenging activity. The involvement of free radicals, especially their increased production, appears to be a feature of most of the human diseases including cardiovascular diseases and cancer [32]. It has been found that cysteine, glutathione, ascorbic acid, tocopherols, flavonoids, tannins and aromatic amines reduce and decolorize the DPPH by their hydrogen donating ability. Flavonoids and phenolic compounds of MEIL are possibly involved in its antiradical activity.

Superoxide radical is known to be a very harmful species to cellular components as a precursor of more reactive species [1]. The superoxide radical is known to be produced in vivo and can result in the formation of hydrogen peroxide via dismutation reaction. Moreover, the conversion of superoxide and hydrogen peroxide into more reactive species. The extract is found to be an efficient scavenger of superoxide radical generated in alkaline DMSO system. The result clearly indicates that the plant extract have a noticeable effect as scavenging superoxide radical.

Hydrogen peroxide itself is not very reactive, but sometimes is toxic to cell because it may give rise to hydroxyl radical in the cells [33]. Therefore, removing of hydrogen peroxide is very important for antioxidant defense in cell system. Polyphenols have also been shown to protect mammalian cells from damage induced by hydrogen peroxide, especially compounds with the orthohydroxy phenolic compounds like quercetin, gallic acid, caffeic acid and catechin [34]. Therefore, the phenolic compounds of the *Indigofera linnaei* extract may probably be involved in scavenging hydrogen peroxide.

Nitric oxide formed during their reduction with oxygen or with superoxide, such as  $\text{NO}_2$ ,  $\text{N}_2\text{O}_4$ ,  $\text{N}_3\text{O}_4$  are very reactive. These radicals are responsible for altering the structure and functional behavior of many cellular components. The MEIL showed better activity in competing with oxygen to react with nitric oxide and thus the inhibition of generation of anions and the activity is with the standards used. The plant secondary metabolites may have the property to counteract the effect of nitric oxide formation and in turn may be considerable interest in preventing the ill effects of excessive nitric oxide generation in the human body. Further the scavenging activity may also help to arrest the chain or reactions initiated by excess generation of nitric oxide that are dangerous to the human health. Nitric oxide is also implicated for inflammation, cancer and other pathological conditions [35].

Among the oxygen radicals, hydroxyl radical is the most reactive and induces severe damage to adjacent biomolecules [36]. In the present study, the hydroxyl radical scavenging activity of MEIL was assessed by the inhibition of p-NDA bleaching method and deoxyribose degradation method. In p-NDA method, the hydroxyl radical is generated through Fenton reaction. In this reaction, iron-EDTA complex reacts with hydrogen peroxide in presence of ascorbic acid to generate hydroxyl radical which can bleach p-NDA specifically. The extract shows potent scavenging activity by inhibition of bleaching of p-NDA. In deoxyribose method, the sugar is degraded on exposure to hydroxyl radical generated Fenton reaction. The resulting complex mixture of products is heated under acid condition; malondialdehyde (MDA) is formed and detected by its ability to react with thiobarbituric acid to form a pink chromogen. In the deoxyribose method, the plant extract shows potent hydroxyl radical scavenging activity which can be comparable with the standards used. The scavenging activity may be due to the presence of various phytochemicals including polyphenols and flavonoids in MEIL.

In the measurement of the reducing ability, it has been investigated from the  $\text{Fe}^{3+}$  -  $\text{Fe}^{2+}$  transformation.  $\text{Fe}^{3+}$  reduction is often used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action and can be strongly correlated with other antioxidant properties [37]. The reducing properties of the plant extracts are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom [38]. Reductones are also reported to react with certain precursors of peroxide, thus preventing



peroxide formation. The data obtained in the present study suggest that it is likely to contribute significantly towards the observed antioxidant effects. However, the antioxidant activity has been attributed by various mechanisms, like prevention of chain initiation, binding of transition metal ion catalysts, prevention of continued hydrogen abstraction, reductive capacity, radical scavenging activity and decomposition of peroxides. Like the antioxidant activity, the reducing power of the extract increases with increasing concentration.

The systemic literature collection, pertaining to this investigation indicates that the plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical scavengers. Therefore, it is necessary to determine the total amount of phenols and flavonoids in the plant extract chosen for the study. Flavonoids are the most diverse and widespread group of natural compounds and are likely to be the most important natural phenolics. These compounds possess a broad spectrum of chemical and biological activities including radical scavenging activity. The content of total phenols and flavonoids were estimated by the standards curves and expressed as gallic acid equivalents for total phenols and rutin equivalents for flavonoids. The extract contains more than 20% of total flavonoids and rich in phenols. Previous literatures showed that high phenol and flavonoid content increases the antioxidant activity [39] and there is a linear relation between the phenol and flavonoid contents and antioxidant activity [40].

Phenolic compounds are commonly found in both edible and medicinal plants and they have been reported to have various biological effects including antioxidant activity. The antioxidant activities of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides [41]. The methanolic extract of *Indigofera linnaei* showed strong antioxidant activity in various *in vitro* systems tested. The antioxidant effect of *I. linnaei* is may be due to the phenolic compounds present in it. To our knowledge this is the first report on the antioxidant potential of *Indigofera linnaei*.

### Conclusion

The results from various free radicals scavenging systems reveal that methanol extract of *Indigofera linnaei* have significant antioxidant activity. The extract is found to have different levels of antioxidant activity in all the methods tested. IC<sub>50</sub> values obtained were comparable with that of the standards used, that is, ascorbic acid and rutin. Since, free radicals are of different chemical entities, it is essential to test the extracts against many free radicals to prove their antioxidant activity. Hence, a large number of *in vitro* methods were used for the screening. However, the difference in the activity in extract may be due to the different chemical entities of the free radicals and the diverse chemical nature of the extract. According to this study, a significant and linear relationship was found between the antioxidant activity and total phenol and flavonoid contents, indicating that these compounds could be major contributors to antioxidant activity. Further studies in our laboratory are in progress for the isolation and identification of phytochemical compounds and to ensure the medicinal properties of the plant *in vivo* correlate with its antioxidant activity.

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