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PHYTOCHEMICAL INVESTIGATION AND ANTI-OXIDANT ACTIVITY OF INDIGOFERA ASTRAGALINA

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ABSTRACT

The plants aerial parts of *Indigofera astragalin* was selected for the present studies**.** Fresh and mature plant plants of *I. astragalina* were collected from tiruchengode, Tamilnadu. 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.: PCIAS 007). The *in vitro* methods include the determination of Total phenolic content and Total flavonoid content. Besides these, the antioxidant activity of the extracts and fractions were determined in different *in-vitro* experimental methods like Diphenyl-picryl-hydrazyl (DPPH) radical, Superoxide free radical (O_2•−), Peroxide radical (H_2O_2), ABTS radical cation scavenging activity and Nitric oxide (NO) free radical scavenging activity with reference to standard antioxidant ascorbic acid. The capabilities of ethanolic and aqueous extracts of aerial parts of *Indigofera astragalin* to scavenge DPPH were measured *in-vitro* the related IC_{50} values and the % scavenging. The chloroform fraction of *Indigofera astragalin* was found to be an effective superoxide anion scavenger to scavenge the superoxide anions as compared to ascorbic acid which is measured in terms of IC_{50} (87.76 µg/ml) were obtained.

Key words: Anti-oxidant, *I. astragalina,* DPPH, Superoxide free radical, ABTS

1. INTRODUCTION:

Species of Indigofera are mostly shrubs, though some are small trees or herbaceous perennials or annuals. Most have pinnate leaves. Racemes of flowers grow in the leaf axils, in hues of red, but there are a few white- and yellow-flowered species. The fruit is a legume pod of varying size and shape. Many of the species will also succeed in drier conditions and in poor soils. This species has a symbiotic relationship with certain soil bacteria; these bacteria form nodules on the roots and fix atmospheric nitrogen. Some of this nitrogen is utilized by the growing plant but some can also be used by other plants growing nearby. Sandy grounds muddy sand, sand on sandstone often ruderal rocaille waste or cultivated ground heavely grazed grassland; at elevations up to 1,800 metres**.**

2. MATERIAL AND METHODS:

Plant material:

The selection of plant species for our study was based on their traditional use for Antioxidant treatment, the information being gathered from published sources and traditional healers. The plants aerial parts of *Indigofera astragalin* was selected for the present studies.

Collection, identification and authentification of plant parts

Fresh and mature plant plants of *I. astragalina* were collected from Tiruchengode, Tamilnadu. 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.: PCIAS 007). After due authentication, fresh matured aerial parts *Indigofera astragalin* L was collected in bulk, initially rinsed thoroughly with distilled water, shade dried for 15 days. The shade dried materials were coarsely powder by a mechanical grinder and preserved in a nylon bag in a deep freezer, till further use.

Preparation of extracts

The extraction yield of the extracts from plant species is vastly depends on the solvent polarity, which find out both qualitatively and quantitatively the extracted compounds. Ethanol and water are the commonly used solvent for the extraction because of their low toxicity and high extraction yield with the advantage of modulating the polarity of the solvent by using mixtures at different ratios (Jackson et al, 1996). The plant materials (1 kg) were initially defatted with petroleum ether and

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then extracted with alcohol and water using a Soxhlet apparatus. The yield of the plant extracts ethanol (95%) and aqueous measured about 20 g each after evaporating the solvent using water bath. The standard extracts obtained from *Indigofera astragalin* were then stored in a refrigerator at 4°C for further use for phytochemical investigation and pharmacological screening (Akueshi et al, 2002).

Anti-oxidant activity *in-vitro*

The *in vitro* methods include the determination of Total phenolic content and Total flavonoid content. Besides these, the antioxidant activity of the extracts and fractions were determined in different *in-vitro* experimental methods like Diphenyl-picryl-hydrazyl (DPPH) radical, Superoxide free radical (O_2•−), Peroxide radical (H_2O_2), ABTS radical cation scavenging activity and Nitric oxide (NO) free radical scavenging activity with reference to standard antioxidant ascorbic acid.

Estimation of total phenolics content of the extracts

Principle: Phenols react with phosphomolybdic acid in Folin-Ciocalteau‟s reagent in alkaline medium to produce a blue-colored complex (molybdenum blue) which can be estimated spectrophotometrically at 760 nm.

The extracts were oxidized with Folin-Ciocalteau's reagent and were neutralized with sodium carbonate. The absorbance of the resulting blue colour solution was measured at 765 nm after 60 min using gallic acid (GA) as standard. Total Phenolic content was expressed as mg gallic acid equivalents (mg.GAE)/g of the samples.

Procedure: Total phenolics in ethanolic and aqueous extracts of both plant extracts were determined with Folin-Ciocalteu‟s reagent. One ml of each extract (1mg/ml in distilled water) was taken in the separate volumetric flask and one ml of Folin-Ciocalteu reagent was added in both flasks. After three minutes, three ml of 2% Na2CO3 was added. Subsequently, the mixture was shaken for two hours at room temperature and absorbance was read at 760nm and the experiment was performed in triplicate (singleton et al, 1999).

Estimation of total flavonoids content (Nguyen, 2011**)**

Principle: Total flavonoids content in test extracts of both plants was measured by the aluminum chloride colorimeter assay. Flavonoids react with aluminum chloride reagent to produce a colored product which can be measured spectrophotometrically at 510nm.

Procedure:

Extract solution (0.25ml, 1mg/ml) of each plant extract was added to 1.25 ml of distilled water. Sodium nitrite solution (0.075ml, 5%) was then added to the mixture followed by incubation for 5 minutes, after which 0.15ml of 10% aluminium chloride was added. The mixture was allowed to stand for 6min at room temperature before 0.5ml of 1 M sodium hydroxide was finally added and the mixture diluted with 0.275 ml distilled water. The absorbance of the reaction mixture was measured at 510 nm with a UV/VIS spectrophotometer immediately. Quercetin was used as the standard for the calibration curve. Flavonoid contents were expressed as mg quercetin equivalent (QE)/g dry weight.

***In vitro* antioxidant activity of Indigofera astragalin by using DPPH (α, α- diphenyl α-picryl hydrazyl) Free Radical Scavenging Activity**

Principle: Free radical scavenging potentials of the extracts were tested (*in vitro*) against a methanolic solution of α,α-diphenyl-β-picryl hydrazyl (DPPH). Antioxidants react with DPPH and convert it to α,α-diphenyl-β-picryl hydrazine (Fig. 4.1). The DPPH is stable free radical which is purple in colour and upon reaction with an antioxidant; it becomes colourless and the degree of discoloration indicates the scavenging potentials of the antioxidant extract. The change in the absorbance produced at 517nm has been used as a measure of antioxidant activity.

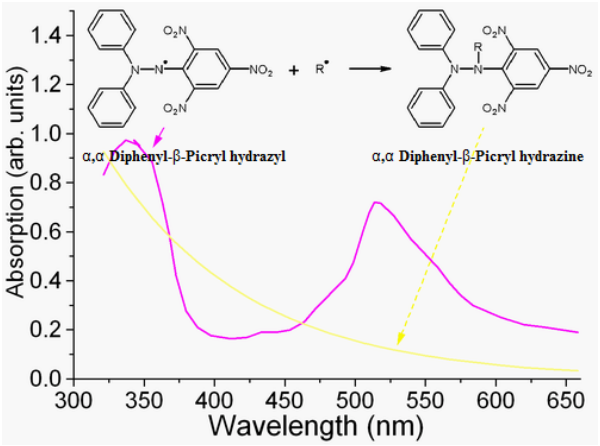

Fig.1: Reduction of DPPH free radical

The IC_{50} value, defined as the amount of the sample sufficient to elicit 50% reduction of the initial DPPH concentration, was calculated from the linear regression of plots of concentration of test

compounds against the mean percentage of antioxidant activity obtained from the three replicate tests. The free radical scavenging activity of ascorbic acid (Vitamin C) was also measured under the same condition to serve as +ve control.

Procedure:

DPPH stock solution (0.3 mM): 11.8 mg of DPPH was dissolved in 100 ml of analytical grade ethanol.

Preparation of stock solutions of test fraction:

The stock solutions of extracts/fraction and standard were prepared with analytical grade ethanol and further test solutions of different concentration of test fractions of *Indigofera astragalin* (5-160 µg/ml) were prepared. All solutions were prepared with ethanol.

Method: The DPPH scavenging activity was done using the method of Chang et al, 2002 and Umamaheswari et al, 2008**.** A total 1ml of 0.3mM DPPH solution was added to 2.5 ml of sample/standard solution of different test concentrations and allowed to react at room temperature. After 30 min, the absorbance values were measured at 517nm and converted into the percentage antioxidant activity using the following equation.

$$\% \text{ Anti radical activity (I)} = \frac{\text{Control Abs} - \text{Sample Abs}}{\text{Control Abs}} \times 100$$

Ethanol (1ml) plus plant test fraction solution (2.5 ml) was used as a blank, while DPPH solution plus methanol was used as a negative control. The positive controls were DPPH solution plus each ml of standard (Ascorbic acid). The IC_{50}values were calculated by linear regression of plot, where the abscissa represents the concentration of tested plant extracts/standard and the ordinate the average % of scavenging capacity from three triplicates. Each experiment was carried out in triplicate and IC_{50} (µg/mL) of the chloroform fraction of *Indigofera astragalin* were reported.

Nitric oxide scavenging activity

Principle: Nitric oxide (NO) is an extremely unstable free radical generated from sodium nitroprusside at physiological pH, which can be determined by Griess reagent. It reacts with O_2 to produce the stable product nitrates and nitrite through intermediates like NO_2, N_2O_4 and N_3O_4. In the presence of test compound, which is a scavenger, the amount of nitrous acid will decrease. The degree of decrease will reflect the amount of scavenging, which is measured at 546 nm (Shirwaikar et al, 2006).

Method: The reaction solution comprising of 2.5 ml of different concentrations of chloroform fraction of *Indigofera astragalin* (5-160 µg/ml) and 0.75ml of 5mM of sodium nitroprusside. The test tubes were incubated at 25^0C for 5hours. After 5 hrs, 0.5ml of Griess reagent was added. For control, Griess reagent and sodium nitroprusside solution was quantified spectrophotometrically at 546nm. The experiment was performed in triplicate (Shirwaikar et al, 2006).

ABTS scavenging activity

Principle: In this method scavenging of ABTS [2, 2" azino bis (3- ethylbenzthiazoline-6-sulfonic acid) diammonium salt] radical cation take place. The principle involves the reaction between ABTS and sodium persufate, ABTS is converted to its radical cation by addition of sodium persulfate. In the presence of antioxidant reductant, the coloured radical is converted back to colourless ABTS; the absorbance is read at 745 nm (Shirwaikar et al, 2006).

Procedure for preparation of ABTS radical cation: The ABTS radical cation was prepared by reacting ABTS solution (7mM) with 2.45mM of ammonium persulphate and the mixture was allowed to put in dark at room temperature for 12-16 hrs prior to use.

Method: 1.5ml of different concentration of chloroform fraction of *Indigofera astragalin* (5-160 µg/ml) was added with 0.9ml of ABTS radical cation. The absorbance was read at 745nm and all experiments were performed in triplicate (Shirwaikar et al, 2006).

Super oxide radical scavenging activity

Principle: Superoxide radical is known to be very harmful to the cellular components. The assay was based on the capacity of sample to inhibit farmazan formation by scavenging the superoxide radicals generated in riboflavin-light- nitro blue tetrazolium (NBT) system (Kumaran and Karunakaran, 2007).

Method: Each 3ml reaction mixture contained 50 mM sodium phosphate buffer (pH 7.6), 20 µg riboflavin, and 12 mM EDTA and 0.1 mg NBT and 1ml of sample solution. Reaction was started by illuminating the reaction mixture with different concentrations of test fractions and standard ascorbic acid solution (5-160 µg/ml) for 5min. Immediately after illumination, the absorbance was

measured at 590 nm. Identical tubes with reaction mixture and 1ml of methanol were kept in the dark along and served as control. The % scavenging activity at different concentrations was determined and the IC_{50} value of the chloroform fraction of *Indigofera astragalin* was compared with that of ascorbic acid, which was used as the standard.

The percentage inhibition of superoxide anion generation was calculated from

$$\% \text{ Scavenging} = \frac{\text{Absorbance of Control - Absorbance of Test}}{\text{Absorbance of Control}} \times 100$$

Procedure: Acetic acid 1.5ml (20%, pH 3.5), 1.5 ml of thiobarbituric acid (0.8%) and 0.2 ml sodium dodecyl sulphate (8.1%) was added to 0.1ml of supernatant and heated at 100°C for 60min. Mixture was cooled and five ml of n-butanol pyridine (15:1) mixture was added with one ml of distilled water and vortex vigorously. After centrifugation at 1200 x g for 10 minutes, the organic layer was separated and absorbance was measured at 532 nm. It was calculated using a molar extinction coefficient of 1.56 x 105 M-1 cm-1 and expressed as nanomoles of TBARS, mg of protein (Mukherjee et al, 2007).

Calculations were made as per the formula

Inhibitory rate = [1 - (A1 – A2) / A0] X 100

Where A0 was the absorbance of control (without extract) and A1 was the absorbance in the presence of the extract, A2 was the absorbance without tissue homogenates.

3. RESULT AND DISCUSSION:

Preliminary phytochemical study of the *I. astragalin* extracts/fractions

The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents. The preliminary phytochemical screening was carried out to assess the qualitative chemical composition of crude extracts and fractions from *I. astragalin* by using precipitation and coloration reaction to identify the major natural chemical groups (Harborne, 1998; Kokate et al., 2003). General reactions in this analysis revealed the presence or absence of these compounds in the crude extracts and fractions tested. Summary of preliminary phytochemical screening of different extracts and fractions is depicted in Table-1.

Table 1: Phytochemical screening of extracts and fractions of *I. astragalin*

Chemical constituents	Chemical Test	Extracts/Fractions			
		Ethanol extract	Aqueous extracts	Chloroform fraction	Aqueous fraction
Alkaloids	Mayer's	+	+	+	+
	Dragendorff's	+	+	+	+
	Wagner's	-	-	-	-
	Hager's	+	+	+	+
saponin	Foam	-	+	-	-
	Haemolytic	-	-	-	-
Phenolic compounds and Tannins	Ferric Chloride	+	+	+	+
	Gelatin	-	-	-	
	Lead acetate test	+	+	+	+
Proteins	Million's	+	-	+	-
	Biuret	+	+	+	-
	Xanthoprotein	-	-	-	-
Flavonoids	Ferric Chloride	+	+	-	+
	Shinoda	-	-	-	-
	Lead Acetate	+	+	+	+
Glycoside	Baljet's	-	-	-	-
	Legal's	-	-	-	-
	Borntrager's	-	-	-	-
	Killer killani	-	-	-	-
Fixed oil	Spot	-	-	-	-
Carbohydrate	Molisch's	-	-	-	-
	Fehling's	+	+	+	+
	Benedict's	-	-	-	-
	Barfoed's	+	+	+	+

	Cobalt-chloride	-	-	-	
Gums and mucilage	*Swelling Index*	-	-	-	-
Amino Acids	*Ninhydrin*	-	-	-	-
	Tyrosin	-	-	-	-
	Tryptophan	-	-	-	-
Sterols and triterpenes	Liebermann-Burchard's	+	+	+	-
	Salkowski's	+	-	+	-

Key (+) = Presence, (-) = Absent

Determination of total phenolic and flavonoid content of ethanolic and aqueous extracts of *I. astragalin*

The perusal of table-2 showed that the total phenolic contents of ethanolic and aqueous extracts of aerial parts of *A. mexicana* found to 56.75mg and 113.12mg of gallic acid equivalent (GAE)/g, respectively and total flavonoids content of both test extracts of *Indigofera astragalin* found to be 6.86mg and 8.34mg equivalent of quercetin /gm of the dry weight basis which is quantitatively a greater value.

Table 2: Determination of Total Phenolic and Flavonoid content of ethanolic and aqueous extracts of *I. astragalin*

Groups	Total Phenolic content (mg.GAE/g)	Total Flavonoid content (mg QE /g)
***I. astragalin* Ethanolic Extract**	56.75 ± 1.12	6.86 ± 0.32
***I. astragalin in* Aqueous Extract**	113.12 ± 1.74	8.34 ± 1.21

Values are expressed in MEAN ± S.E.M (n =3).

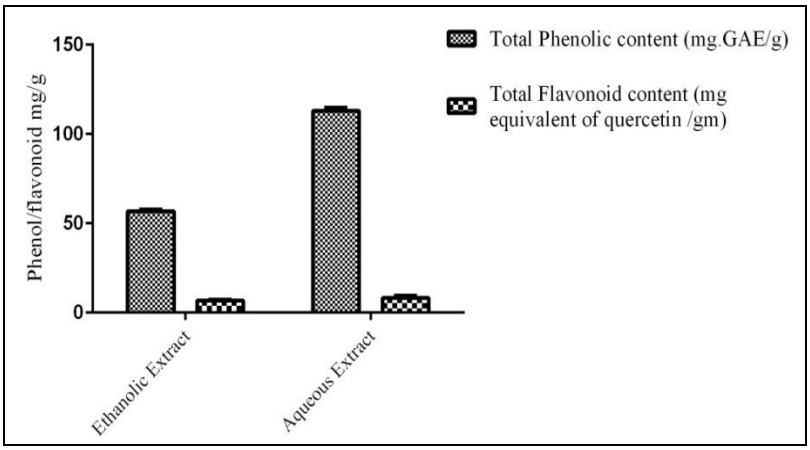

Fig.3: Total phenolic and flavonoid content of ethanolic and aqueous extracts of *I. astragalin*

Effect of extracts of *Indigofera astragalin* in 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method

The DPPH is stable free radical which reacts with appropriate reducing agent (hydrogen), to become paired off (diamagnetic molecules) and solution be converted into colourless stoichometrically depending on the number of electron taken up (Shirwaikar et al., 2006).

The capabilities of ethanolic and aqueous extracts of aerial parts of *Indigofera astragalin* to scavenge DPPH were measured *in-vitro* the related IC_{50} values and the % scavenging results are mentioned in table-3.

Both the extracts, *Indigofera astragalin* scavenges DPPH radical in a concentration dependent way. The antioxidants react through DPPH, a purple colored stable free radical and convert it into a colorless α-α-diphenyl-β-picryl hydrazine. The quantity of DPPH reduced could be quantified by measuring a decrease in absorbance at 517 nm. The IC_{50} value was found to be 45.21and 40.11µg/ml for ethanolic extracts and aqueous extracts while the IC_{50} value of ascorbic acid was 18.53µg/ml significantly reduced DPPH radical by bleaching it. From the results, it may be postulated that ethanolic and aqueous extracts of aerial parts of *I. astragalin* have hydrogen donors thus, scavenging the free radical DPPH.

Table 3: **Antioxidant activity of various extracts of *Indigofera astragalin* in DPPH radical scavenging method**

Sample	Concentration µg/ml	% inhibition	IC $_{50}$ value µg/ml
Ethanolic Extract	20	29.21 ± 2.14	45.21
	40	47.53 ± 1.85	
	60	59.21 ± 2.46	
	80	74.72 ± 2.68	
	100	87.17 ± 1.45	
Aqueous extract	20	25.21 ± 2.04	40.11
	40	50.53 ± 1.65	
	60	61.21 ± 2.46	
	80	78.72 ± 2.49	
	100	90.17 ± 1.35	
Ascorbic acid			18.53

Values are expressed in MEAN ± S.E.M (n =3).

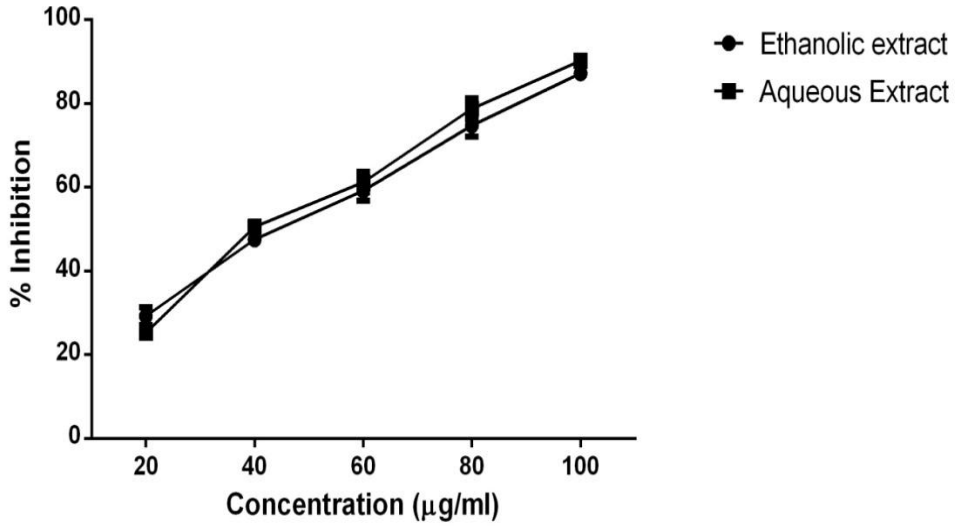

Fig.4: Effect of various extracts of *Indigofera astragalin* in DPPH radical scavenging method

Effect of various extracts of *Indigofera astragalin* in hydrogen peroxide (H_2O_2) radical scavenging method

Hydrogen peroxide, although not a radical species play a role to contribute oxidative stress. The generation of even low levels of H_2O_2 in biological systems may be important. Naturally-occurring iron complexes inside the cell believed to react with H_2O_2 in vivo to generate highly reactive hydroxyl radicals and this may be the origin of many of its toxic effects.

Scavenging of hydrogen peroxide of the both extracts of *Indigofera astragalin* is presented in table 4. The IC_{50} value for *I. astragalin* was found to be 56.02 and 56.24µg/ml for ethanolic extracts and aqueous extracts while the IC_{50} value of ascorbic acid was 21.41µg/ml significantly and concentration dependently scavenges H_2O_2 radical. Though, ethanolic and aqueous extracts of aerial parts of *I. astragalin* are having significant potential in scavenging the free radicals in the above experiments.

Table 4: Effect of various extracts of *Indigofera astragalin* in hydrogen peroxide (H_2O_2) radical scavenging method

Sample	Concentration µg/ml	% inhibition	IC $_{50}$ value µg/ml
	20	11.16 ± 2.12	
	40	24.12 ± 1.62	
Ethanolic Extract	60	56.25 ± 1.12	56.02
	80	71.15 ± 1.24	
	100	84.25 ± 1.62	
	20	16.12 ± 2.14	
	40	29.15 ± 1.31	
Aqueous extract	60	55.21 ± 2.42	56.24
	80	69.15 ± 2.21	
	100	75.27 ± 1.91	
Ascorbic acid			21.41

Values are expressed in MEAN ± S.E.M (n =3).

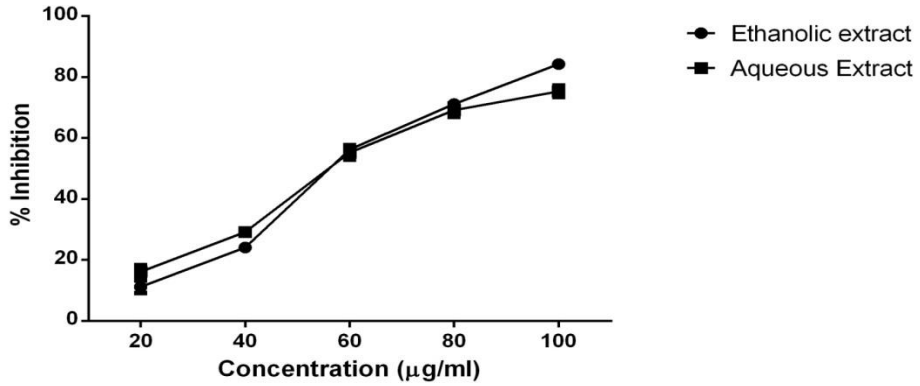

Fig.5: Effect of various extracts of *Indigofera astragalin* in hydrogen peroxide (H_2O_2) radical scavenging method

Effect of chloroform fraction of *Indigofera astragalin* in 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical, Super oxide radical, ABTS radical cation and Nitric oxide radical scavenging method

The capacity of chloroform fraction of *I. astragalin* to scavenge DPPH, super oxide radical, ABTS radical cation and nitric oxide radical were measured *in-vitro*; the related IC_{50} values and the % scavenging results are mentioned in table-6 respectively.

DPPH is a stable free radical, which has been widely used in phytomedicine for the assessment of scavenging activities of bioactive fractions. The DPPH radical is considered to be a model of a stable lipophilic radical. This reaction has been widely used to test the ability of compounds to act as free-radical scavengers or hydrogen donors and to evaluate the antioxidative activity of plant extracts/fractions. The scavenging activity of chloroform fraction of *I. astragalin* was determined using free radicals of 1, 1-diphenyl 1-2-picryl-hydrazyl (DPPH). Results showed that chloroform fraction of *Indigofera astragalin* (IC_{50} 61.89 µg/ml) possessed the good antioxidant activity. While standard drug ascorbic acid have 10.05 µg/ml IC_{50} value.

Oxidation is life, but except of so many necessary processes of life, during normal metabolism of oxygen, various free radicals as well as superoxide are produced continuously. The high level of this superoxide radical is known to be harmful to cellular ingredients as, contributing to tissue damage and various diseases. The superoxide radicals generated from dissolved oxygen by PMS-NADH coupling can be measured by their ability to reduce NBT. The decrease in absorbance at 560 nm with the plant fraction and the reference compound indicates their abilities to quench superoxide radicals in the reaction mixture. The chloroform fraction of *Indigofera astragalin* was found to be an effective superoxide anion scavenger to scavenge the superoxide anions as compared to ascorbic acid which is measured in terms of IC_{50} (87.76 µg/ml).

The 2, 2 azobis-(3-ethylbenzothiozoline-6-sulphonic acid (ABTS) assay is based on the inhibition of the absorbance of the radical cation $ABTS^{\bullet+}$ which is quantified at 734 nm. It is a decolorization assay, thus the

radical cation is prepared prior to addition of the antioxidant test system, quite than the generation of the radical taking place repetitively in presence of antioxidants (Youdim and Joseph, 2001). These results, obtained that chloroform fraction of *I. astragalin* with IC_{50} of 115.29µg/ml, where ascorbic acid had IC_{50} of 7.71µg/ml, significantly showed activity which is either by inhibiting or scavenging the ABTS radicals in a concentration dependant manner.

Nitric oxide (NO) is a main chemical moderator generated by endothelial cells, macrophages, neurons etc. and regulates the various physiological processes. Reactive Oxygen Species like oxygen reacts with excess NO to generate RNS like NO_2, N_2O_4 and peroxynitrite, together ROS and RNS attack and damage cellular molecules including lipids, protein, nucleic acids and carbohydrates (Pacifici and Davies, 1991).The chloroform fraction of *I. astragalin* with IC_{50} value of 82.12µg/ml where ascorbic acid had IC_{50} value of 7.92µg/ml significantly scavenges RNS. The chloroform fraction of *I. astragalin* owing radical scavenging ability may provide protection against oxidative damage induced to the biomolecules. It may also scavenge free radicals due to presence of antioxidant principles which compete with oxygen to react with nitric oxide and thus inhibiting the generation of RNS.

Table 6: Effect of chloroform fraction of *Indigofera astragalin* in 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical, super oxide radical, ABTS radical cation and Nitric oxide radical scavenging method

Sl. No	Concentration (µg/ml)	DPPH (% inhibition)	Superoxide radical (% inhibition)	ABTS (% inhibition)	Nitric oxide radical (% inhibition)
1	5	15.58 ± 0.33	11.75 ± 0.19	8.01 ± 0.30	12.11 ± 0.64
2	10	24.58 ± 0.12	19.52 ± 0.15	14.81 ± 0.28	22.08 ± 0.47
3	20	37.98 ± 0.19	28.11 ± 0.02	21.22 ± 0.11	29.16 ± 0.29
4	40	47.55 ± 0.25	34.56 ± 0.03	32.10 ± 0.22	38.14 ± 0.26
5	80	67.63 ± 0.28	58.43 ± 0.16	47.47 ± 0.12	58.48 ± 0.64
6	160	83.52 ± 0.16	71.03 ± 0.16	58.57 ± 0.14	73.56 ± 1.75
IC_{50} (µg/ml)		61.89	87.76	115.29	82.12
Ascorbic Acid IC_{50} (µg/ml)		10.05	15.54	7.71	7.92

Values are expressed in MEAN ± S.E.M (n =3).

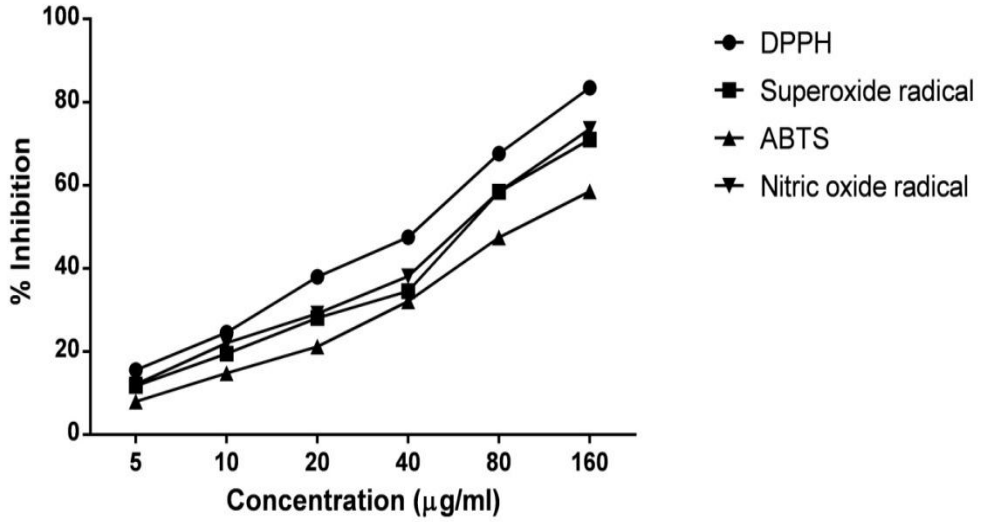

Fig.5: Effect of chloroform fraction of *Indigofera astragalin* in 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical, super oxide radical, ABTS radical cation and Nitric oxide radical scavenging method

4. CONCLUSION:

Conversely the extracts and fractions of both plants exert very good potentials to scavenge toxic free radicals along with the inhibition of the liver lipid peroxidation products and activation of the enzymatic antioxidant defense mechanism in diabetic rats that might be due to the presence of high levels of sterols. The chloroform fraction of *I. astragalin* with IC_{50} value of 82.12µg/ml where ascorbic acid had IC_{50} value of 7.92µg/ml significantly scavenges RNS. The chloroform fraction of *I. astragalin* owing radical scavenging ability may provide protection against oxidative damage induced to the biomolecules. It may also scavenge free radicals due to presence of antioxidant principles which compete with oxygen to react with nitric oxide and thus inhibiting the generation of RNS.

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