

PRELIMINARY PHYTOCHEMICAL SCREENING AND IN VITRO ANTIOXIDANT ACTIVITIES OF INDIGOFERA MYSORENSIS

¹Gullepelli Poojasri, ²Syeda Nishat Fathima

Department of Pharmacology
Jayamukhi college of pharmacy
Arsampet, Chennaraopet road,
Warangal, Telangana, India.

Correspondence to Author: Gullepelli Poojasri

Abstract- Multiple free radical scavenging assays show that *Indigofera mysorensis* leaf extracts in different solvents are very effective antioxidants. Across all assays, the extracts exhibited varying degrees of antioxidant activity. Results showing IC₅₀ values similar to those of ascorbic acid and rutin, the standards utilized, were obtained. To demonstrate the extracts' antioxidant activity, it is necessary to test them against a wide variety of free radicals, since these molecules might have varying structures. As a result, the screening process made extensive use of in vitro methodologies. Nonetheless, the fact that the free radicals and the extracts themselves are chemically distinct could account for the observed variation in their activities. The results showed a strong correlation between total phenol and flavonoid concentration and antioxidant activity, suggesting that these chemicals may play a key role in this process. Our team is currently doing more investigations to identify and isolate phytochemical components, as well as to determine which therapeutic qualities of the plant correlate with its antioxidant activity in vitro.

Key words: Antioxidant activity, Free Radical Scavenging Activities, Various solvents extract, *Indigofera mysorensis*.

INTRODUCTION

Oxidative stress is the major driving factor responsible for the initiation and progression of cancer, diabetes mellitus, cardiovascular diseases, neurodegenerative diseases, and inflammatory diseases among other syndromes [1]. The condition is brought by excessive generation of free oxygen and nitrogen species or their inefficient quenching in the cell. Free oxygen and nitrogen species are unstable molecules that are present in the environment (exogenous) and are also generated in the body (endogenous) during the normal aerobic metabolic processes in the body [2]. Exogenous sources of free radicals include cigarette smoke, exposure to ozone, ionizing radiation such as X-rays, and drugs among others. On the other hand, endogenous sources of free radicals include the electron transfer chain reactions in the mitochondria, xanthine oxidase pathway, during disease states such as inflammation, ischemia, and reperfusion injury [3].

The body possesses a complex antioxidant defense system, comprising of enzymatic and nonenzymatic pathways, which in the normal physiologic state, maintain a steady equilibrium between prooxidants and antioxidants, thereby ensuring well-being [1]. The enzymatic antioxidants comprise the catalase, glutathione peroxidase, and superoxide dismutase. Conversely, nonenzymatic antioxidants employed by the body include the bilirubin, uric acid, and lactoferrin among others. However, during disease states, the endogenous antioxidant systems are overwhelmed, leading to accumulation of excessive free radicals, which in turn cause oxidative stress-associated damage to cellular machinery, as implicated in various diseases [4].

Conventionally, oxidative stress is managed using various synthetic antioxidant compounds such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propyl gallate (PG). Despite their usage, these synthetic antioxidant compounds have been associated with undesirable effects [5]. For instance, BHT and BHA cause hepatotoxicity and have been demonstrated to be carcinogenic. Additionally, synthetic antioxidants are inaccessible, unaffordable, and labile, thus limiting their utilization [3]. Therefore, due to the profound consequences of oxidative stress and the drawbacks of synthetic antioxidants, the need for alternative antioxidants, which are safer, easily accessible, and potent, are warranted [6], hence the current study.

Considering the available alternative and complementary strategies, medicinal plants stand a better chance of providing potent, safer, affordable, and easily accessible therapies for oxidative stress-related maladies [7]. Medicinal

plants contain various secondary metabolites, which have demonstrated a wide spectrum of pharmacologic activities. Antioxidant properties of plants have been demonstrated to play a protective role in the body against diseases, since their consumption lowers the risk of cancer, heart disease, hypertension, dementia, and stroke [8].

The major groups of phytochemicals that contribute to antioxidant capacity of plants include polyphenols and vitamins (A, C, and E). Phenolic compounds of plants are hydroxylated derivatives of benzoic acid and cinnamic acids, which possess antioxidant and anticarcinogenic effects [3]. They include phenols, flavonoids, coumarins, tannins, and anthocyanidins. These phytoactive complexes are important in plant defense mechanisms against biotic and abiotic stresses [9]. When plants or plant products rich in these phytoactive principles are consumed, they are deemed to confer the same beneficial effects to humans [8]. For instance, flavonoids have for long been recognized to possess anti-inflammatory, antiallergic, antiviral, immunomodulatory, antiaging, and antiproliferative properties [10]. *Indigofera mysorensis* Wight & Arn. is a plant of the genus *Marsdenia* belongs to *Asclepiadaceae* family. *Indigofera mysorensis* is one such rare medicinal twining shrub found in Tamilnadu and Karnataka states of India. It has long been used by tribes and native medical practitioners to treat various chronic disorders

The aim of present study is In Vitro Antioxidant and free radical scavenging activities of various solvent extracts of *Indigofera mysorensis*

MATERIALS AND METHODS

Collection of Plant and its Authentication

The plant *Indigofera mysorensis* has been collected from Chittoor district. The collected plant and its various parts were authenticated.

Extraction with Solvents

The solvents were chosen and used based on its polarity increasing order in nature. The extracted extract is filtered to remove solid particles and by using rota-vapor equipment and the extracts concentrated to dryness at low temperature & pressure. The temperature used here is 40-50°C. The obtained residues are conditioned in vacuum entrusted desiccators. The percentage (%) of total yield in different solvent extracts is being calculated as per the equation given below:

$$\% \text{ yield} = \left[\frac{\text{Weight of crude extract}}{\text{Weight of raw material}} \right] \times 100$$

The extractive yield, colour, odour and nature of the various solvent extracts of *Indigofera mysorensis* were tabulated.

Qualitative Phytochemical Analysis

The various active constituents of *Indigofera mysorensis* are identified by subjecting to the extracts to chemical tests.

Analysis of Activity for *in-Vitro* Antioxidant Property

The *in-vitro* assessment is based on the concept of inhibition technique. The prepared sample is added in to the free radical system, the depth of inhibition of free radical process is noted and the found inhibition level is depends on antioxidant property of the tested sample.

The assessment method is differing based on the following conditions:

- Based on generated free radical,
- Capacity of reproducibility in the generation stage,
- Type of endpoint used for assessment

The obtained data through *in-vitro* assessment is tricky to use in system for biological activity at the same time it does not produce same activity. Due to presence of constituents of phytochemical in the different extracts solution of *Indigofera mysorensis*, the possibility of antioxidant property of the different extracts are assessed using various methods available in research technique. The various concentrations of the solution of extract and standard (1000, 500, 250, 125, 62.5,

31.25 and 15.6256 microgram per milliliter) utilized in this study is being measured and analysed.

The observed absorbance through spectrophotometric technique is interpreted with the corresponding standard and blank solution.

The total percentage (%) of inhibition is calculated based on the following equation.

$$\text{Radical scavenging activity (\%)} = \frac{\text{OD control} - \text{OD sample}}{\text{OD control}} \times 100$$

Scavenging of 2-2'-azino bis (3-ethylbenzothiazoline sulphonic acid) ABTS

This 2-2'-azino-bis (3-ethylbenzothiazoline sulphonic acid) assay is a novel method and it involves sudden radicals and used for assessment of complexed anti-oxidant solutions like extracts of plant. Which can be used in almost

many ranges of pH, henceforth its application in research of pharmacy discipline in order to study the activity of antioxidant property is dominant area.

Evaluation of 2,2-diphenyl picrylhydrazyl (DPPH) compound

The 2,2-diphenyl picrylhydrazyl (DPPH) compound reacts with H donors and it undergoes reduction stage resulting which it produces respected hydrazine molecule. The changes to hydrazine causes for colour changes from purple to yellowish colour since originally radical of DPPH is purple shade and upon reduction it turns in to yellow colour. This assay also called as discoloration evaluation. It is estimated through adding of anti-oxidant compound into 2, 2-diphenyl-1-picrylhydrazyl solution in either C₂H₅OH or CH₃OH and the resultant reduction in light absorbent are measured in the wavelength of 490 nano meter.

Hydroxyl radical scavenging of by p-NDA technique

The inhibition of p-Nitroso Diamine bleaching technique is used to measure the hydroxyl radical. The fenton treatment is to evolve the generation of hydroxyl radicals through reaction between H₂O₂ and Fe-EDTA compounds in the presence of acid like ascorbic acid and resulting which, it can bleach the compound generated through the reaction called as fenton reaction. In this reaction, ferrous iron-Ethylene di-amine tetra adipic acid complex reacts with the hydrogen peroxide in presence of ascorbic acid in order to generate hydroxyl compound, the formed hydroxyl compound shall decolourise the p-Nitroso Diamine purposely. In this reaction, the OH radical scavenger determines the scavenging functions through inhibition of bleaching and nonetheless, the percentage of scavenging is determined by absorbing the wavelength at 440 nano meter. The sequence of procedure is given below,

Superoxide radical Scavenging through alkaline dimethyl sulphoxide technic (DMSO)

In this technique, superoxide radical is produced by the adding up of NaOH and dimethyl sulphoxide. The produced superoxide will not reduced in the aqueous solution and at RT reduction of nitro-blue tetra-zolium is takes place resulting which formazan is produced and the absorbance is measured at 550 to 560 nanometer. H₂O₂ Scavenging H₂O₂ is produced *in-vivo* through several enzymes which are oxidase based. and it is found to be that H₂O₂ generation of hydroxyl ions is takes place either in alkaline medium or acidic medium and the formed hydroxyl ions causes for damage in biological systems. In this process, the scavenging is done with H₂O₂ and the loss of H₂O₂ is evaluated spectrophotometrically in the range of 230 nanometre⁷⁰. The sequence of process is given below,

Evaluation of activity for Reductive capability

The evaluation of activity for Reductive capability is carried out by the transforming of metal ions (Ferrous) from ferrous to ferric when the extract is there in the mixture of solution.

RESULTS AND ANALYSIS

Extraction

Different types of extracts of *Indigofera mysorensis* were prepared and the percentage yield was calculated. Among the prepared extracts, maximum yield was obtained from ethanol and water. Percentage yields, colour and nature of the prepared plant extracts are presented in **Table 2**.

Table 1. Color, nature and percentage yields of *Indigofera mysorensis*

Extract	Color	Nature	Yield
Hexane	Dark greenish	Sticky semisolid	2.6%
Chloroform	Dark greenish Yellow	Solid	1.5%
Ethyl acetate	Greenish yellow	Solid	6.3%
Ethanol	Yellowish brown	Solid	8.6%
Water	Brown	Solid	10.4%

Qualitative Phytochemical Analysis

The screening of preliminary phytochemical of the extracts of plant - *Indigofera mysorensis* demonstrate the existence of diverse phytochemical components. phyto-sterols, fixed oils and fats were found in hexane extract, whereas chloroform extract restrain phytosterols and gives a very low extractive yield when compared to other extracts of plant. The extract of ethyl acetate consists of glycosides, flavonoids and phenolic compounds. Glycosides,

phytosterols, tannins, flavonoids, polyphenolics, proteins & amino acids and carbohydrates were found in ethanol extract. Aqueous extract contains many polar constituents such as phenolic compounds, glycosides, proteins & amino acids, flavonoids, tannins and carbohydrates. Alkaloids, mucilage gums and terpenoids are not present in all the prepared extracts. The results are more clearly proved that the existence of each phytoconstituents depends upon the phytochemicals solubility in the particular solvents. Many of the constituents were extracted by ethanol. The results are displayed in **Table 2**.

Table 2. Preliminary Phytochemical Studies of *Indigofera mysorensis*

Constituents	Extract				
	Hexane	Chloroform	Ethyl acetate	Ethanol	Water
Carbohydrates	A	A	A	P	P
Phytosterols	P	P	A	P	A
Alkaloids	A	A	A	A	A
Glycosides	A	A	P	P	P
Terpenoids	A	A	A	A	A
Proteins & amino acids	A	A	A	P	P
Saponins	A	A	A	A	A
Tannins	A	A	A	P	P
Phenolic compounds	A	A	P	P	P
Flavonoids	A	A	P	P	P
Fixed oils & Fats	P	A	A	A	A
Gums & Mucilages	A	A	A	A	A

(P) Presence

(A) Absence

***In vitro* Antioxidant Activity**

The activity of antioxidant property of various solvent extracts of *Indigofera mysorensis* was investigated through various *in vitro* models. Since, free radicals are of diverse chemical entities, it is necessary to test the extracts against many free radicals to check their antioxidant activity. Hence, a large number of *in vitro* methods were employed for the screening. IC₅₀ values acquired were compared with the standards ascorbic acid and rutin. In the current work, all the prepared extracts apart from hexane extract, exhibiting radical scavenging activity against ABTS radical. The IC₅₀ values of ethyl acetate and ethanol extracts were found to be $52.35 \pm 2.2 \mu\text{g/ml}$ and $60.33 \pm 5.88 \mu\text{g/ml}$ which showed strong activity against scavenging of ABTS radical. The order of activity was as follows: 1) Ethyl acetate 2) Ethanol 3) Water, 4) Chloroform

5) Hexane. In DPPH radical scavenging assay, ethyl acetate and ethanol extracts are found to be active. The order of activity was as follows: 1) Ethyl acetate 2) Ethanol,

3) Water 4) Chloroform, 5) Hexane. All the results were compared with the standards. The scavenging activities of the MB extracts were displayed in **Table 3**.

In the assay of superoxide scavenging, the various MB extracts displayed moderate activity. Hexane extract does not exhibit much activity against superoxide radicals. Based on the IC₅₀ values, extracts of ethanol and water have an appreciable effect on scavenging superoxide radical. Also in hydrogen peroxide radical scavenging assay, ethanol and ethyl acetate extracts exhibit the IC₅₀ values of $97.46 \pm 3.91 \mu\text{g/ml}$ and $117.83 \pm 11.58 \mu\text{g/ml}$ and showed appropriate activity when compared to other extracts. The results obtained are displayed in **Table 4**.

In the present work, the hydroxyl radical scavenging activity of different extracts of *Indigofera mysorensis* was analysed by the inhibition of p-NDA bleaching method. Among the solvent extracts, ethanol and water extracts show more appropriate activity with the IC₅₀ values of $108.6 \pm 3.7 \mu\text{g/ml}$ and $115.57 \pm 2.31 \mu\text{g/ml}$ when compared to other solvent extracts of MB. The hydroxyl radical scavenging activity of diverse extracts of *Indigofera mysorensis* is presented in **Table 5**.

In the total reducing power assay, the absorbance is increased with increase in concentration. This increase in the absorbance indicates higher reducing activity. Among the tested extracts, ethanol, ethyl acetate and water extracts

shows potent reducing activity whereas hexane and chloroform extracts shown least activity. The result is illustrated in **Figure. 1**.

Table 3. Different Extracts of *Indigofera mysorensis* on ABTS and DPPH Techniques

Extracts	IC ₅₀ (µg/ml)* by method	
	ABTS	DPPH
Hexane	Greater than 1000	Greater than 1000
Chloroform	101.67 ± 10.01	102.59 ± 9.96
Ethyl acetate	52.35 ± 2.20	28.78 ± 5.08
Ethanol	60.33 ± 5.88	30.61 ± 4.15
Water	79.03 ± 3.94	60.99 ± 4.37
Ascorbic Acid	13.63 ± 1.17	7.05 ± 0.76
Rutin	6.61 ± 0.64	8.11 ± 0.42

*Avg. of 3 determinations; Data are mentioned as mean ± SEM

Table 4. Various Extracts of *Indigofera mysorensis* on Scavenging of Superoxide Radical and Hydrogen peroxide techniques

Extracts	IC ₅₀ (µg/ml)* by method	
	Superoxide radical scavenging	H ₂ O ₂ radical scavenging
Hexane	Greater than 1000	Greater than 1000
Chloroform	216.7 ± 17.53	227.4 ± 13.27
Ethyl acetate	134.17 ± 5.43	117.83 ± 11.58
Ethanol	91.88 ± 4.42	97.46 ± 3.91
Water	107.74 ± 10.12	123.8 ± 5.95
Ascorbic Acid	187.53 ± 11.93	75.16 ± 3.78
Rutin	55.97 ± 2.21	32.56 ± 1.05

*Avg. of 3 determinations; Data are mentioned as mean ± SEM

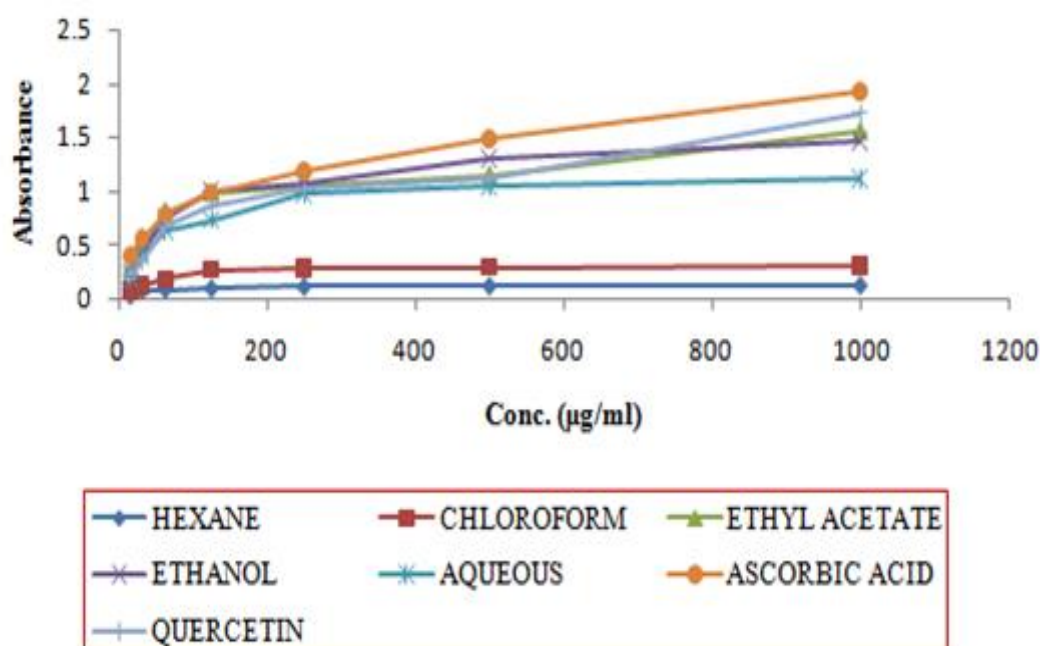
Table 5. Different Extracts of *Indigofera mysorensis* on Hydroxyl-Radical Scavenging Assay

Extracts	IC ₅₀ (µg/ml)*
	Hydroxyl Radical Scavenging (p-NDA method)
Hexane	>1000

Chloroform	211.07±6.67
Ethyl acetate	128.8 ±4.16
Ethanol	108.6 ± 3.7
Water	115.57 ± 2.31
Ascorbic Acid	182.5 ± 4.88
Rutin	44.1 ±4.4

*Average of 3 determinations; Data are expressed as mean ± SEM

Fig. 1. The Total Reducing Power Assay of Various Solvent Extracts



DISCUSSION

It is well-known that reactive nitrogen species can serve as both harmful and helpful agents, and the biochemistry of reactive oxygen species (ROS) including superoxide anion, hydrogen peroxide, hydroxyl radicals, and singlet oxygen is significant in aerobic cellular metabolism. According to Eboh Abraham (2014), oxidative stress can lead to damage to DNA, lipids, proteins, carbohydrates, and cell structure and function when there is an overproduction of reactive oxygen species (ROS) due to mitochondrial electron transport chain leakage or an overstimulation of xanthine oxidase and other oxidative enzymes. At low concentrations, ROS and RNS have favorable impacts on cellular responses to infectious agents, gene expression, cellular growth, the function of several cellular signaling pathways, hypoxia, and respiratory burst, among other physiological processes. The importance of reactive oxygen species in various diseases has been better understood and acknowledged in recent years. Enzymes including catalase, glutathione peroxidases, glutathione reductase, and superoxide dismutases help the body deal with reactive oxygen species and prevent damage (Eboh Abraham., 2014).

There are several physiological roles for antioxidants since they suppress oxidation even at low concentrations. The plant-based antioxidant phytoconstituents assist reduce the reactivity of free radicals by scavenging them and transforming them into less reactive forms. Plants in their whole contain antioxidants. Vitamins, carotenoids, phenols,

flavonoids, intravenous glutathione, and endogenous metabolites are all examples of antioxidants. Studies have demonstrated that antioxidants produced from plants can quench both singlet and triplet oxygen levels, as well as free radicals, peroxide, enzymes, and work in tandem with other antioxidants. Recent studies on antioxidant effect have concentrated on phenolic chemicals, including flavonoids. The antioxidant properties of many plant-based nutrients, including vitamin C, vitamin E, and carotenoids, have only been proven in the last several years. This includes tannins, flavonoids, and other phenolic components.

The oxidative products at both the early and ultimate phases of oxidation were evaluated using antioxidant assays in this work. The *in vitro* models used to study *Indigofera mysorensis* leaf extracts' antioxidant and free radical scavenging activities were varied. To demonstrate the extracts' antioxidant activity, it is necessary to test them against a wide variety of free radicals, since these molecules might have varying structures. As a result, the screening process made extensive use of *in vitro* methodologies. The ascorbic acid and rutin standards were utilized to compare the IC50 values that were obtained.

Screening complicated antioxidant combinations, including plant extracts, drinks, and biological fluids, is facilitated by the more current ABTS radical scavenging activity, which employs a chemically manufactured, more powerful radical. Interest in using ABTS • + for antioxidant activity assessment was piqued due to its stability across a wide pH range and its ability to dissolve in both organic and water-based medium (Nenadis et al., 2004). Using the ABTS method, which is similar to the gold standard, the extracts demonstrated strong antioxidant activity. In this case, the radical scavenging activity of the extract demonstrated that its phenolic components had a direct role in free radical scavenging.

Bleaching a 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) solution purple is one way to evaluate a natural product's electron donating capabilities. The technique relies on DPPH scavenging by discoloring the DPPH solution with the addition of an antioxidant or radical species. The level of color change is directly related to the antioxidants' concentration and efficacy. According to Pratap Chandran et al. (2013), a substantial drop in the absorbance of the reaction mixture suggests that the tested chemical has strong free radical scavenging capabilities.

The results of the experiments showed that the extracts might potentially have free radical scavenging effects. The results show that the DPPH radical scavenging activity is dosage dependant. Most human disorders, including cancer and cardiovascular diseases, seem to include free radicals, particularly their enhanced generation (Deighton et al., 2000). Because of their capacity to donate hydrogen, cysteine, glutathione, ascorbic acid, tocopherols, flavonoids, tannins, and aromatic amines are able to decrease and decolorize the DPPH. The radical scavenging activity of *Indigofera mysorensis* leaf extracts may be due to flavonoids and phenolic substances. As a byproduct of highly reactive species, superoxide radical is well-known to be extremely damaging to biological components (Halliwell and Gutteridge, 2007). One recognized *in vivo* production of superoxide radical is the dismutation reaction, which can lead to the generation of hydrogen peroxide. The evolution of peroxides and superoxide into even more reactive forms is another important step. The results show that the extracts effectively neutralize the superoxide radicals produced in the acidic DMSO solution. The results show that the plant extracts effectively scavenge superoxide radicals.

Despite hydrogen peroxide's lack of reactivity, it can be harmful to cells when it produces hydroxyl radicals within them (Halliwell, 1991). So, for antioxidant defense in the cell system, hydrogen peroxide removal is crucial. Quercetin, gallic acid, caffeic acid, and catechin are just a few examples of the polyphenols that have been demonstrated to shield mammalian cells from the cellular damage that hydrogen peroxide can cause (Nakayama, 1994). Thus, it is likely that the phenolic chemicals found in *Indigofera mysorensis* leaf extracts have a role in hydrogen peroxide scavenging. According to Sakanaka et al. (2005), hydroxyl radicals are the most reactive oxygen radicals and can cause significant damage to nearby biomolecules. This study found that *Indigofera mysorensis* leaf and bark extracts inhibited the p-NDA bleaching and deoxyribose degradation methods, indicating that they scavenged hydroxyl radicals. The Fenton reaction is used to create the hydroxyl radical in the p-NDA technique. The hydroxyl radical, which can bleach p-NDA in particular, is produced when an iron-EDTA complex combines with hydrogen peroxide in the presence of ascorbic acid. By blocking the bleaching of p-NDA, the extracts demonstrate strong scavenging activity. The inclusion of several phytochemicals, such as flavonoids and polyphenols, in the extracts might explain the scavenging activity.

The Fe³⁺ - Fe²⁺ transition has been studied for the purpose of measuring the reducing ability. One of the main ways

in which phenolic antioxidants work is by donating electrons; this activity is closely related to other antioxidant characteristics, and Fe³⁺ reduction is a common sign of it (Dorman et al., 2003). Plant extracts with reducing activities are likely to contain reductones, which have antioxidant effects by donating a hydrogen atom to free radicals, disrupting their chain reaction (Gordon, 1990). It has been found that reductones can inhibit the generation of peroxide by reacting with specific peroxide precursors. Based on what we know from this study, it probably plays a major role in the antioxidant effects we've seen. Different processes, such as the suppression of chain reactions, have been proposed to explain the antioxidant activity. The reducing power of the extracts, similar to their antioxidant activity, grows with increasing concentration.

According to the accumulated research on this topic, plant phenolics are among the most important classes of chemicals that neutralize free radicals and serve as antioxidants. So, it's important to count how much phenols and flavonoids were in the plants used for the research. Flavonoids are the most diverse and widespread group of natural compounds and are proposed to be the most important natural phenolics. These compounds possess a broad spectrum of chemical and biological activities including radical scavenging activity. There are a lot of phenols and total flavonoids in the extracts. Previous literatures showed that high phenol and flavonoid content increases the antioxidant activity (Holasoava et al., 2002) and there is a linear relation between the phenol and flavonoid contents and antioxidant activity (Gheldof and Engeseth, 2002). 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 (Osawa, 1994). The leaf extracts of *Indigofera mysorensis* showed strong antioxidant activity in various in vitro systems tested. The antioxidant effect of *Indigofera mysorensis* is may be due to the phenolic compounds present in it. To our knowledge this is the first report on the antioxidant and radical scavenging potential of *Indigofera mysorensis*.

CONCLUSION

Multiple free radical scavenging assays show that *Indigofera mysorensis* leaf extracts in different solvents are very effective antioxidants. Across all assays, the extracts exhibited varying degrees of antioxidant activity. Results showing IC₅₀ values similar to those of ascorbic acid and rutin, the standards utilized, were obtained. To demonstrate the extracts' antioxidant activity, it is necessary to test them against a wide variety of free radicals, since these molecules might have varying structures. As a result, the screening process made extensive use of in vitro methodologies. Nonetheless, the fact that the free radicals and the extracts themselves are chemically distinct could account for the observed variation in their activities. The results showed a strong correlation between total phenol and flavonoid concentration and antioxidant activity, suggesting that these chemicals may play a key role in this process. Our team is currently doing more investigations to identify and isolate phytochemical components, as well as to determine which therapeutic qualities of the plant correlate with its antioxidant activity in vivo.

REFERENCES:

1. W. Arika, C. M. Kibiti, J. M. Njagi, and M. P. Ngugi, "In vitro antioxidant properties of dichloromethanolic leaf extract of *Gnidia glauca* (Fresen) as a promising antiobesity drug," *Journal of Evidence-Based Integrative Medicine*, vol. 24, 2019. View at: [Publisher Site](#) | [Google Scholar](#)
2. A. H. Bhat, K. B. Dar, S. Anees et al., "Oxidative stress, mitochondrial dysfunction and neurodegenerative diseases; a mechanistic insight," *Biomedicine & Pharmacotherapy*, vol. 74, pp. 101–110, 2015. View at: [Publisher Site](#) | [Google Scholar](#)
3. G. A. Moriasi, A. M. Ireri, and M. P. Ngugi, "In vivo cognitive-enhancing, ex vivo malondialdehyde-lowering activities and phytochemical profiles of aqueous and methanolic stem bark extracts of *Piliostigma thonningii* (schum.)," *International Journal of Alzheimer's Disease*, vol. 2020, Article ID 1367075, 15 pages, 2020. View at: [Publisher Site](#) | [Google Scholar](#)
4. S. Vertuani, A. Angusti, and S. Manfredini, "The antioxidants and pro-antioxidants network: an overview," *Current Pharmaceutical Design*, vol. 10, no. 14, pp. 1677–1694, 2004. View at: [Publisher Site](#) | [Google Scholar](#)
5. A. Ndhlala, M. Moyo, and J. Van Staden, "Natural antioxidants: fascinating or mythical biomolecules?" *Molecules*, vol. 15, no. 10, pp. 6905–6930, 2010. View at: [Publisher Site](#) | [Google Scholar](#)
6. S. K. Mwiha, "In vitro antibacterial and antioxidant activities of methanolic and dichloromethanolic seed extracts of Kenyan *Annona squamosa* Linn," Kenyatta University, Nairobi, Kenya, 2017, Doctoral dissertation. View at: [Google Scholar](#)
7. M. R. Goyal and H. A. R. Suleria, Eds. in *Human Health Benefits of Plant Bioactive Compounds: Potentials and Prospects*, CRC Press, Boca Raton, FL, USA, 2019.

8. C. Ojiewo, A. Tenkouano, J. D. A. Hughes, and J. D. H. Keatinge, "Diversifying diets: using indigenous vegetables to improve profitability, nutrition and health in Africa," *Diversifying Food and Diets: Using Agricultural Biodiversity to Improve Nutrition and Health*, Routledge, Abingdon, UK, 2013. View at: [Google Scholar](#)
9. C. B. Rajashekar, E. E. Carey, X. Zhao, and M. M. Oh, "Health-promoting phytochemicals in fruits and vegetables: impact of abiotic stresses and crop production practices," *Functional Plant Science and Biotechnology*, vol. 3, no. 1, pp. 30–38, 2009. View at: [Google Scholar](#)
10. A. N. Panche, A. D. Diwan, and S. R. Chandra, "Flavonoids: an overview," *Journal of Nutritional Science*, vol. 5, 2016. View at: [Publisher Site](#) | [Google Scholar](#)