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# GC-MS ANALYSIS OF BIO-ACTIVE COMPOUNDS AND *IN-VITRO* ANTIOXIDANT ACTIVITY IN METHANOLIC EXTRACTION OF *INDIGOFERA PROSTRATE*

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**Keywords:**

*Indigofera prostrate*, phytocomponents, GC-MS, Campesterol, Stigmasterol

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**ABSTRACT:** The present study focuses on identification of bioactive compounds from Methanolic extraction of *Indigofera prostrate*, by GC–MS analysis and also evaluated the efficacy of *Indigofera prostrate* for antioxidant activity. Phytochemical screening of the Methanolic extraction of *Indigofera prostrate* revealed the presence of flavonoids, phenolic compounds, triterpenoids, tannins, saponins, amino acids, proteins, Steroids, Gums and carbohydrates. Gas chromatography-mass spectrometry (GC-MS) analysis of the methanol extract of *Indigofera prostrate* was performed on a GC-MS equipment. The GC-MS analysis has shown the presence of different bio active compounds in the Methanolic extract of *Indigofera prostrate*. A total of 14 compounds were identified in methanolic extraction in that the main active compounds are Campesterol, Stigmasterol, γ-Sitosterol, Lupeol. From the results, it is evident that *Indigofera prostrate* contains various phytocomponents and is recommended as a plant of phytopharmaceutical importance.

**INTRODUCTION:** Medicinal plants represent a rich source of novel lead compounds that contribute to various therapeutic and pharmacological activities [1]. Around 25% of the pharmaceutical products used in the modern era were developed from plants [2]. According to WHO, nearly 80% of the world population consume the products of medicinal plants to cure different diseases [3]. In many studies, it is reported that antioxidant, anti-inflammatory, anticancer, antiviral, antibacterial, antifungal, insecticidal, antimalarial, anti-aging, and various other therapeutic activities depend on a significant variety of secondary metabolites (glucosinolates, lycopenes, anthocyanidins, flavonoids, isoflavonoids, polyphenols, limonoids, carotenoids, phytoestrogens, and omega-3 fatty acids, etc.) that are isolated from potential medicinal plants with the help of advanced, sensitive, and sophisticated equipment. Under these characteristics, about 20,000 plant species have been explored for their medicinal purposes [4].

Reactive oxygen species (ROS) are formed by cellular metabolism or some exogenous factors, such as drugs, chemicals, smoke, and environmental stress conditions. The ROS structure contains at least one unpaired electron [5]. The risk is related to the accumulation of these agents in the body, resulting in a radical reactions chain, which degrades many biological vital molecules, namely DNA, proteins, lipids, and carbohydrates [6]. It has been revealed that ROS are associated with some diseases, such as diabetes mellitus, insulin resistance, cardiovascular diseases, Alzheimer's disease, Parkinson's disease, and some types of

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cancer [7]. Indeed, antioxidants of natural origin have received significant interest regarding exploration to identify secondary metabolites for the health and food industry. Antioxidants can maintain health by scavenging radicals and reactive oxygen species [8]. It is reported that two-thirds of all plant species have medicinal value and antioxidant potential [9].

The extraction and characterization of these bioactive compounds have resulted in the delivery of specific medications with a high-activity profile [10]. Fourier-transformed infrared (FTIR) and Gas chromatography-mass spectrometry (GC–MS) have been widely used for observation of functional groups and identification of various bioactive compounds present in plants [11, 12]. GC–MS is a reliable technique for the identification of various compounds such as alkaloids, flavonoids, organic acids, amino acids etc. from plant extracts [13]. Also, computer-based tools have evolved as sophisticated drug discovery approaches that may be used to screen medicines from bioactive compounds present in medicinal plants [14]. Computational prediction models are utilized in the *in-silico* prediction of pharmacological, pharmacokinetic and toxicological production and play a crucial role in the selection of procedure leading to pharmaceutical and technological advancement [15]. Molecular docking is an efficient and low-cost approach for creating and testing pharmaceuticals. This technique gives the knowledge on drug-receptor interactions that may be used to anticipate how the drug model will bind to the target proteins [16] leading to reliable binding at the binding sites of ligands [17].

*Indigofera prostrate* belongs to Fabaceae family, Prostrate spreading branched herbs; stems appressed pubescent. Leaves 3-foliolate; leaflets 0.8-1.8 x 0.4-0.8 cm, obovate or elliptic-obovate, base cuneate, apex obtuse, apiculate, appressed-pubescent, gland-dotted beneath; petiole 0.8-1.3 cm long; stipules subulate. Racemes axillary, c. 5 mm long, 3-6-flowered. Flowers pink or brick-red; pedicels c. 2 mm long. Calyx-tube c. 0.5 mm long; lobes c. 1 mm long, setaceous. Corolla exserted; standard c. 5 mm long, obovate; wings oblong; keels to 4 mm long. Staminal sheath c. 3mm long. Pods 1-1.5 cm long, terete, slender, deflexed, slightly winged, appressed-pubescent. Seeds 4-8, oblong.

The present study was aimed for GC- MS Analysis of Bio- Active Compounds and *in-vitro* Antioxidant Activity in Methanolic Extraction of *Indigofera prostrate.*

## MATERIALS AND METHODS:

**Plant Material:** Seed of *Indigofera prostrta* were obtained from the local places of Tirupati, AP. The plant was authenticated by Dr. K. Madhava Chetty M.Sc., M.Ed., M.Phil., Ph.D., PG DPD.,

**Extraction by Maceration:** Fresh seeds of *Indigofera prostrta* washed with water to get rid of contaminants like dirt and other impurities and were shade-dried. These dried seeds were ground and sieved to get a uniform, coarse powder. Powdered plant material was weighed (1Kg) and is immersed in Methanol and kept for maceration for a period of 7 days with occasional stirring. On the 8$^{th}$ day, the solvent was filtered by pressing with a muslin cloth and was evaporated in a rotary evaporator at 40$^{o}$C. The resultant extract was put in a desiccator to remove any methanol left in it. The dried Methanolic extract of *Indigofera prostrta*. (MEIP) was packed in an air-tight bottle and put in a dry place for further studies.

**Preliminary Phytochemical Analysis:** All the extract/fractions of *Indigofera prostrta* were analyzed for their primary and secondary metabolites to confirm the presence of various primary metabolites, such as carbohydrates, amino acids, proteins, and lipids, and secondary metabolites, such as alkaloids, tannins, phenols, flavonoids, saponins, steroids, glycosides, and resins, according to standard methods.

**Gas Chromatography-mass Spectrometry (GC-MS) Analysis:** GC-MS analysis was carried out in a combined 7890A gas chromatograph system (GCMSQP2010, SHIMADZU) and mass spectrophotometer, fitted with a HP-5 MS fused silica column (5% phenyl methyl siloxane 30.0 m ×250µm, film thickness 0.25µm), interfaced with 5675C Inert MSD with Triple-Axis detector. Helium gas was used as carrier gas and was adjusted to column velocity flow of 1.0 ml/min. Other GC-MS conditions are ion-source temperature, 250 °C; interface temperature, 300 °C; pressure, 16.2 psi; out time, 1.8 mm; and 1µl injector in split mode with split ratio 1:50 with

injection temperature of 300 °C. The column temperature started at 36 °C for 5 min and changed to 150 V at the rate of 4 °C/min. The temperature was raised to 250 °C at the rate of 20 °C/min and held for 5 min. The total elution was 37 min. The relative percent amount of each component was calculated by comparing its average peak area to total areas. MS solution software provided by supplier was used to control the system and to acquire the data.

**Identification of Compounds:** Identification of components was achieved based on their retention indices and interpretation of mass spectrum was conducted using the database of National Institute of Standards and Technology (NSIT). The database consists of more than 62,000 patterns of known compounds. The spectra of the unknown components of *Indigofera prostrta* fraction obtained were compared with the standard mass spectra of known components stored in NIST library (NISTII).

***In-vitro* Antioxidant Screening Assays:**

**DPPH Radical Scavenging Assay:** DPPH radical scavenging activity was assessed according to the method of Blois, 1958. Various concentrations of the plant extract or standard (2 ml) were added to 6 ml of methanolic solution of DPPH (33 mg/l) in a test tube. The reaction mixture was kept at 25°C for an hour in an incubator. The absorbance of the residual DPPH solution was determined at 517 nm in a UV-Visible Spectrophotometer. The experiment was performed in triplicate. Ascorbic acid was used as standard. The inhibition was calculated in terms of percentage inhibition (I %) using following formula and lower $IC_{50}$ value indicates high antioxidant capacity [18].

$$I\ \% = (Abs_{control} - Abs_{sample}) / Abs_{control} \times 100$$

**ABTS Radical Scavenging Activity:** ABTS radical scavenging ability was assessed according to the method of Roberta *et al*., 1999. Initially, ABTS 2 mM (0.0548 gm in 50ml) and potassium per sulphate 70 mM (0.0189 gm in 1ml) were prepared in distilled water. Next, 200 ml of potassium per sulphate and 50 ml of ABTS were mixed and kept aside for 2 hrs. This solution was used for assessing ABTS radical scavenging activity. To the 1 ml of various concentrations of plant extract or standard, 0.6 ml of ABTS radical cation and 3.4 ml of phosphate buffer pH 7.4 were added and the absorbance was measured at 734 nm. The experiment was performed in triplicate. Ascorbic acid was used as standard. The percentage of inhibition (I %) was calculated using following formula and lower $IC_{50}$ value indicates high antioxidant capacity.

$$I\ \% = (Abs_{control} - Abs_{sample}) / Abs_{control} \times 100$$

**Metal Chelating Assay:** Metal chelating ability was carried out according to the Dinis *et al*., 1994. In this assay, 10 ml of plant extract or standard, 0.2 ml of 2 mM ferric chloride and 0.4 ml of ferrozine solution were mixed and kept aside for 10 min at room temperature with continuous shaking. The absorbance was measured at 562 nm. The experiment was performed in triplicate. EDTA was used as standard. The percentage inhibition was calculated using following formula and lower $IC_{50}$ value indicates high antioxidant capacity.

$$I\ \% = (Abs_{control} - Abs_{sample}) / Abs_{control} \times 100$$

**Total Antioxidant Activity:** The total antioxidant activity was eluted by using the method described by Prieto *et al*., 1999. In this process, 0.2 ml of various concentrations of plant extract or standard was added to the 6 ml of reagent solution (0.6 M Sulphuric Acid, 28 mM Sodium Phosphate 4mM Ammonium molybdate) and solution was incubated at $95^0$C for 1 h 30 min. After incubation, solution was cooled to room temperature and then the absorbance of the solution was measured using UV-Visible spectrophotometer at 695 nm. The experiment was performed in triplicate. Ascorbic acid was used as standard. The total antioxidant ability of the plant extract was expressed as ascorbic acid equivalents in microgram per milligram of extract.

**Reducing Power Assay:** Reducing power assay was carried out according to the method of Manisha *et al*., 2009. In this method, 2.5 ml of various concentrations of plant extract were mixed with 2.5 ml of phosphate buffer (0.2 M $P^H$ 6.6) and 2.5 ml of 1 % potassium ferricyanide. This solution was incubated at $50^0$ C for 20 min. After incubation, 2.5 ml of 10 % of trichloroacetic acid was added to reaction mixture and centrifuged at 3500 rpm for 10 min. Next, 2.5ml of supernatant was added to 2.5 ml of distilled water and 0.5 ml of

freshly prepared 0.1% of ferric chloride. The absorbance of the solution was measured using UV-Visible spectrophotometer at 700 nm. The experiment was performed in triplicate. The total reducing power ability was calculated using standard ascorbic acid graph. The total reducing ability of the plant extract was expressed as ascorbic acid equivalents in micrograms per milligrams of the extract [19].

**Total Phenol Content:** Total phenolic content was determined according to the Folin ciocalteu method. 0.4 ml of plant extract was added to 2 ml of folin ciocalteu reagent and 1.6 ml of 7.5 % sodium carbonate. Then the solution was mixed and kept aside for 30 min at room temperature. The absorbance of the solution was measured at 765 nm using UV-Visible spectrophotometer. The experiment was performed in triplicate. The total flavonoid content was calculated using standard gallic acid graph. The total phenol content of the plant extract was expressed as gallic acid equivalents in micrograms per milligrams of the extract.

**Total Flavonoid Content:** Total flavonoid content was quantified according to the modified method of Zhishen *et al.*, 1999. 1ml of plant extract, 1 ml of distilled water and 0.075 ml of 5% sodium nitrite were added in the test tube. After 5 min 0.075 ml of 10% aluminium chloride was added to it. After 5 min 0.5 ml of 1M NaOH was added. The solution was mixed well and allowed to stand for 15 min. The absorbance was measured at 510 nm. The experiment was performed in triplicate. The total flavonoid content was calculated using standard quercetin graph. The total flavonoid content of the plant extract was expressed as quercetin equivalents in micrograms per milligrams of the extract.

## RESULTS:

**TABLE 1: RESULTS OF PHYTOCHEMICAL SCREENING**

| S. no. | Name of the Phytochemical | MEIP |
|---|---|---|
| 1 | Carbohydrates | + |
| 2 | Amino acids | + |
| 3 | Proteins | + |
| 4 | Alkaloids | + |
| 5 | Cardiac glycosides | + |
| 6 | Triterpenoids | + |
| 7 | Saponins | + |
| 8 | Flavonoids | + |
| 9 | Phenolic compounds | + |
| 10 | Tannins | + |
| 11 | Steroids | + |
| 12 | Gums | + |

Where, + means positive and - means negative.

In the present study, the investigation of Methanolic extraction *Indigofera prostrta* revealed the presence of various presences of various phytoconstituents like flavonoids, phenolic compounds, triterpenoids, tannins, saponins, amino acids, proteins, Steroids, Gums and carbohydrates results were showed in **Table 1.**

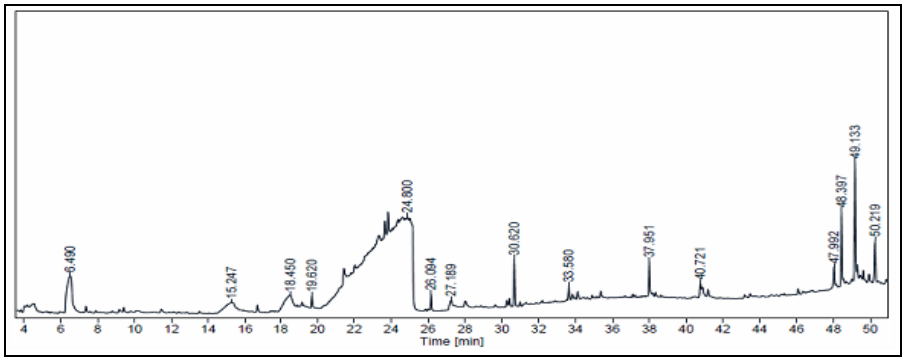
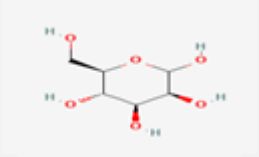
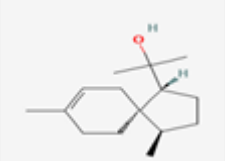
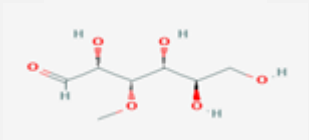
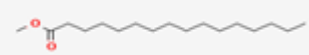
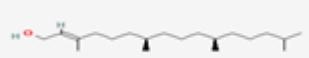
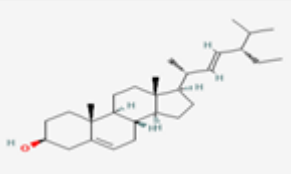
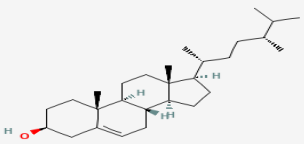
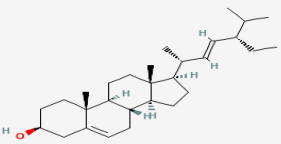
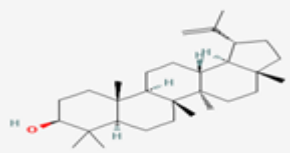



**FIG. 1: GC-MS CHROMATOGRAM OF METHANOLIC EXTRACT OF *INDIGOFERA PROSTRTA* (MEIP)**

**TABLE 2: BIOACTIVE COMPOUNDS FOUND IN METHANOLIC EXTRACT OF *INDIGOFERA PROSTRTA* (MEIP]**

| S. no. | R. Time | Area% | Compound name | Molecular Formula | M.W g/mol | Structure of Compound |
|---|---|---|---|---|---|---|
| 1 | 6.494 min | 19.34 | 1-Butanol, 3-methyl-, formate | C6H12O2 | 116.16 |  |
| 2 | 15.246 min | 8.06 | d-Mannose | C6H12O6 | 180.156 |  |
| 3 | 19.621 min | 0.59 | β-Acorenol | C15H26O | 222.37 |  |
| 4 | 24.797 min | 7.55 | 3-O-Methyl-d-glucose | C7H14O6 | 194.18 |  |
| 5 | 26.091 min | 1.56 | Hexadecanoic acid, methyl ester | C17H34O2 | 270.5 |  |
| 6 | 27.191 min | 6.49 | n-Hexadecanoic acid | C16H32O2 | 256.42 |  |
| 7 | rt: 30.623 min | 4.70 | Phytol | C20H40O | 296.5 |  |
| 8 | rt: 33.580 min | 0.79 | E-8-Methyl-9-tetradecen-1-ol acetate | C17H32O2 | 268.4 |  |
| 9 | rt: 37.950 min | 4.11 | Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester | C19H38O4 | 330.5 |  |
| 10 | 40.719 min | 5.43 | 9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester | C21H38O4 | 354.5 |  |
| 11 | 47.989 min | 1.21 | Campesterol | C28H48O | 400.7 |  |
| 12 | 48.395 min | 6.42 | Stigmasterol | C29H48O | 412.7 |  |
| 13 | 49.133 min | 12.59 | γ-Sitosterol | C29H50O | 414.7 |  |

| | | | | | | |
|---|---|---|---|---|---|---|
| 14 | 50.214 min | 8.35 | Lupeol | C30H50O | 426.7 |  |

The chromatogram of GC-MS displayed in **Fig. 1** whereas the chemical constituents with their Retention Time (RT), atomic equation, Molecular weight (MW) and Area (%) within the MEMM is displayed in **Table 2.** The following bioactive compounds were present in the GC-MS analysis carried on methanolic fraction of *Indigofera prostrta* was found the following bio active compounds 1-Butanol, 3-methyl-, formate, d-Mannose, β-Acorenol, 3-O-Methyl-d-glucose, Hexadecanoic acid, methyl ester, n-Hexadecanoic acid, Phytol, E-8-Methyl-9-tetradecen-1-ol acetate, Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, 9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester, Campesterol, Stigmasterol, γ-Sitosterol and Lupeol

***In-vitro* Antioxidant Assays:**

**DPPH Radical Scavenging Assay:** It is an extensively used, relatively rapid and accurate method for the assessment of free radical scavenging activity. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. Antioxidant donates the electron or hydrogen atom after interaction with DPPH radical and thus neutralizing free radical character of the DPPH and convert it to 1-1,diphenyl-2- picryl hydrazine and the degree of discoloration indicates the scavenging activity of the drug. The reduction capacity of DPPH radical is determined by the decrease in its absorbance at 517 nm induced by antioxidants. The decrease in absorbance of DPPH radical caused by antioxidants because of the reaction between antioxidant and radical progress which results in the scavenging of the radical by hydrogen donation. It is visually evident as change in color from purple to yellow. Hence DPPH is usually used as a substance to evaluate the antioxidant activity. The $IC_{50}$ values of the MEIP (Methanolic extraction of *Indigofera prostrta*) was found to be 380.09 µg/ml. $IC_{50}$ value for the Vitamin C (Standard) was found to be 6.8µg/ml and also presented in **Table 3**.

**TABLE 3: DPPH RADICAL SCAVENGING ASSAY OF MEIP**

| Extract/Standard | Concentration(µg/ml) | % Inhibition | $IC_{50}$ Value |
|---|---|---|---|
| MEIP (Methanolic extraction of *Indigofera prostrta* ) | 100 | 30.16±3.79 | |
| | 200 | 37.72±1.22 | |
| | 300 | 45.85±3.29 | 380.09 µg/ml |
| | 400 | 53.59±1.94 | |
| | 500 | 55.30±2.91 | |
| | 200 | 43.37±2.26 | |
| | 300 | 79.44±3.46 | |
| | 400 | 84.75±1.50 | |
| | 500 | 90.87±2.14 | |
| Ascorbic acid | 1 | 4.16 ± 0.27 | |
| | 2 | 16.22± 2.09 | |
| | 4 | 28.88± 3.95 | 6.8 µg/ml |
| | 6 | 44.95± 2.96 | |
| | 8 | 57.02± 3.98 | |
| | 10 | 66.12± 2.76 | |

**ABTS Radical Scavenging Assay:** It is one of the most commonly used assays in food industry for the measurement of antioxidant ability of foods. In this, ABTS is converted to its radical cation by addition of potassium per sulfate. This radical cation is blue in color and absorbs light at 734 nm. The ABTS radical cation is reactive towards most antioxidants including polyphenols, thiols and ascorbic acid. During this reaction, the blue ABTS radical cation is converted rear to its colorless neutral form. The $IC_{50}$ values of the MEIP (Methanolic extraction of *Indigofera prostrta*) was found to be 191.23µg/ml. $IC_{50}$ value for the Vitamin C (Standard) was found to be 14.1µg/ml and also presented in **Table 4.**

**TABLE 4: ABTS RADICAL SCAVENGING ASSAY OF MEIP**

| Extract/Standard | Concentration (µg/ml) | % Inhibition | $IC_{50}$ value |
|---|---|---|---|
| MEIP (Methanolic extraction of *Indigofera prostrta* ) | 100 | 37.25±2.75 | |
| | 200 | 52.81±3.66 | |
| | 300 | 61.75±4.54 | 191.23 µg/ml |
| | 400 | 81.21±0.99 | |
| | 500 | 91.77±1.55 | |
| | 200 | 17.73±1.87 | |
| | 300 | 28.64±4.11 | |
| | 400 | 35.97±1.02 | |
| | 500 | 47.26±4.04 | |
| | 750 | 60.33±3.73 | |
| | 1000 | 76.52±3.46 | |
| ASCORBIC ACID | 10 | 36.70 ±2.19 | |
| | 20 | 72.63± 3.91 | |
| | 30 | 88.69± 2.85 | 14.1 µg/ml |
| | 40 | 92.18± 1.02 | |
| | 50 | 98.11± 0.97 | |

**Metal Chelating Assay:** Ferrous iron can initiate lipid peroxidation by the Fenton reaction as well as accelerating peroxidation by decomposing lipid hydro peroxides into peroxyl and alkoxyl radicals. Ferrozine can make complexes with ferrous ions. From the result it was evident that MEIP (Methanolic extraction of *Indigofera prostrta) possessed* $Fe^{2+}$ chelating activity and might play a protective role against oxidative damage induced by metal catalyzed decomposition reactions. The $IC_{50}$ values of the MEIP (Methanolic extraction of *Indigofera prostrta*) was found to be 482.09µg/ml. $IC_{50}$ value for the EDTA (Standard) was found to be 76.19µg/ml and also presented in **Table 5.**

**TABLE 5: METAL CHELATION ASSAY OF MEIP**

| Extract/Standard | Concentration (µg/ml) | % Inhibition | $IC_{50}$value |
|---|---|---|---|
| MEIP (Methanolic extraction of *Indigofera prostrta* ) | 100 | 12.64 ± 0.04 | |
| | 200 | 22.19± 1.27 | |
| | 300 | 33.48±1.46 | 482.09 µg/ml |
| | 400 | 43.09±2.11 | |
| | 500 | 51.20±0.64 | |
| | 200 | 39.14±0.29 | |
| | 300 | 48.73±1.43 | |
| | 400 | 61.74±1.85 | |
| | 500 | 74.28±1.65 | |
| EDTA | 10 | 16.20± 0.83 | |
| | 20 | 27.11±1.05 | |
| | 40 | 35.39±0.05 | 76.19 µg/ml |
| | 60 | 42.73±1.64 | |
| | 80 | 54.18±1.21 | |
| | 100 | 61.32 ± 0.26 | |

**Total Antioxidant Activity:** The assay was based on the reduction of Mo (VI)-Mo (V) by the extracts and subsequent formation of a green phosphate/Mo (V) complex at acidic pH.

Total antioxidant activity of the hydroalcoholic extract of MEIP (Methanolic extraction of *Indigofera prostrta*) was found to be 194.10 ± 0.03 µg vitamin C equivalents per mg of plant extract. Total antioxidant activity of the standard vitamin C was specified in the **Fig. 2.**

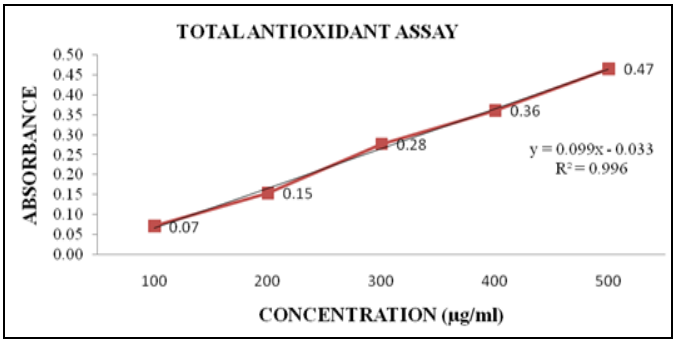



**FIG. 2: TOTAL ANTIOXIDANT ABILITY OF STANDARD ASCORBIC ACID**

**Reducing Power Assay:** In the present assay, the reducing ability of the plant extract was confirmed by transformation of $Fe^{3+}$ to $Fe^{2+}$. The reducing ability of a substance may serve as a significant indicator of its potential antioxidant activity.

However, the activity of antioxidants has been assigned to various mechanisms such as prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging. The Reducing power ability of the *Indigofera prostrta* was found to be 29.00 ± 0.093 µg Vitamin C equivalents per mg of plant extract. The reducing power ability of standard Ascorbic acid was specified in the **Fig. 3.**

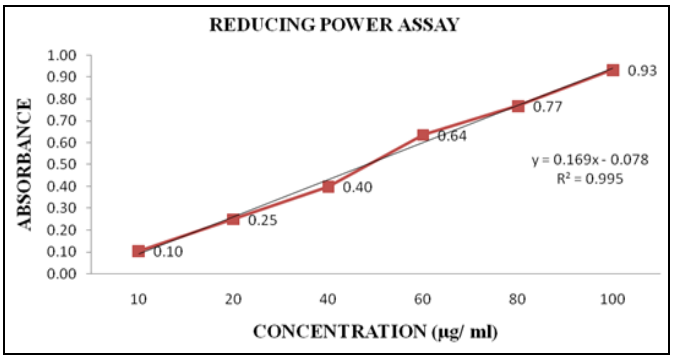



**FIG. 3: REDUCING POWER ASSAY OF STANDARD ASCORBIC ACID**

**Total Phenol Content:** The antioxidant activity of phenolics are 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. Total phenol content of the *Indigofera prostrta* was found to be 170.02± 0.052µg Gallic acid equivalents per mg of plant extract. The total phenol content of Standard Gallic acid was shown in the **Fig. 4.**

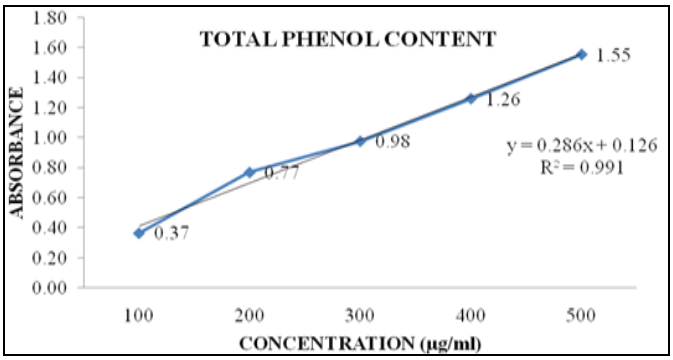



**FIG. 4: TOTAL PHENOL CONTENT OF STANDARD GALLIC ACID**

**Total Flavonoid Content:** Total flavonoid content of standard Quercetin was specified in the Figure. The principle of this method is that aluminum chloride forms acid stable complexes with C-4 keto group and either the C-3 or C-5 hydroxyl groups of flavones and flavonols. In addition, aluminum chloride forms acid stable complex with ortho- di hydroxyl groups in the A or B rings of the flavonoids. Total flavonoid content of the *Indigofera prostrta* was found to be 29.5±0.007µg Quercetin equivalents per mg of plant extract.

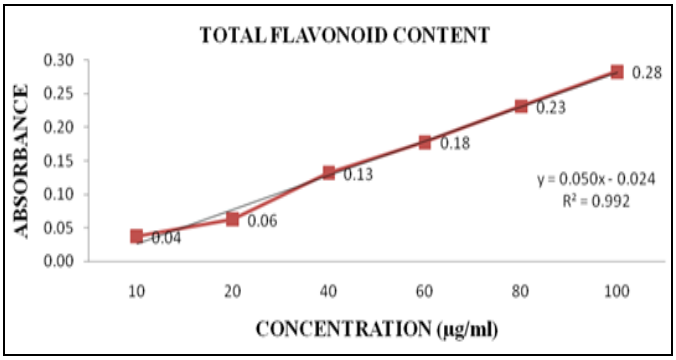



**FIG. 5: TOTAL FLAVONOID CONTENT OF STANDARD QUERCETIN**

**DISCUSSION:** Phenolics compounds are well known as antioxidant and scavenging agents against free radicals associated with oxidative damage [20]. The presence of these compounds such as tannins, flavonoids, proanthocyanidins and phenols in *Indigofera prostrta* extract may give credence to its local usage for the management of oxidative stress induced ailments. Tannins have been used traditionally for the treatment of diarrhoea, hemorrhage and detoxification [21, 22]. The composition of tannins as observed in this study may justify its traditional usage for the management of diarrhoea. Flavonoids are important secondary metabolite of plant modulating lipid peroxidation involved in atherogenesis, thrombosis and carcinogenesis. It has been confirmed that pharmacological effect of flavonoids is correlating with their antioxidant activities [23]. Furthermore, the ethnomedicinal usage of *Indigofera prostrta* extract might be attributed to the high concentration of flavonoids and therefore it could support its usage for the management of hypertension, obesity and diabetes. The antioxidant activity of proanthocyanidins has been demonstrated to be 50 times greater than vitamin C and 20 times greater than vitamin E. It has also been shown that proanthocyanidins help to protect body from tissue

damage, cancer, and to improve blood circulation by strengthening the capillaries, arteries and veins [23]. Therefore, the concentration of this compound as shown in this study could contribute synergistically to the significant antioxidant potency of this plant and thus may support the local usage for the treatment of radical related diseases. Alkaloids and saponins have a history of pharmacological effects for their analgesic and antispasmodic effects thus it explains why traditional healers of South Africa used *Indigofera prostrta* extract for the management of chest pain and arthritis among other diseases [24, 25].

The reducing power of the extract was evaluated by the transformation of Fe3+ to Fe2+ through electron transfer ability which serves as a significant indicator of its antioxidant activity. The reductive activity of the extract and the standard drugs was increased with increasing concentration which is confirmed with increasing absorbance at 700 nm. The antioxidant activity of plant extract was significantly higher than that of the standard drugs used in this study. Findings from this study showed that the antioxidant activity is well correlated with the amount of phenolics constituent found in the extract. Therefore, phenolics compounds as depicted in *S. latifolia* are good electron donors and could terminate the radical chain reaction by converting free radicals to more stable products. The reaction of plant extract with purple coloured DPPH radical converted the radical to α, α diphenyl-β-picryl hydrazine due to the extract antioxidant property. The degree of discolouration indicates the potential of the plant extract to scavenge free radical due to its ability to donate hydrogen proton. The concentration-dependent curve of DPPH radical scavenging activity of *S. latifolia* compared well with ascorbic acid, gallic acid and BHT used as standard drugs. The result obtained from this study concurred with the findings of Igbinosa *et al* [26, 27].

The strong antioxidant activity of *Indigofera prostrta* as shown in the present study might be related to the high contents of phenolics compounds. ABTS radical is a blue chromophore produced by the reaction of ABTS and potassium persulphate after incubation in the dark environment. The reactions of extract with this pre-formed radical cation discolorized the blue chromophore with increasing concentrations. The scavenging activity of ABTS and DPPH radicals by the extract was found to be similar at the highest concentration. This is contrary to the several opinions that plant with DPPH scavenging ability may not inhibit ABTS radical which is due to their different system of preparation and solubility [28, 29].

**CONCLUSION:** The present investigation was focused on identification of various bioactive compounds from the Methanolic extraction of *Indigofera prostrta* for the first time by GC–MS analysis. These compounds are responsible for the different therapeutic and pharmacological properties. We have also provided the evidence of Methanolic extraction of *Indigofera prostrta* for its antioxidant activity.

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**CONFLICT OF INTEREST:** Nil

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