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Screening Of Phytochemical Content and Antioxidant Activity Of Some Selected Indian Plants

Anjali Singh[1*], Ashish Jain[2]

[1*,2]School of Pharmacy, LNCT University, J.K. Town, Kolar Road, Bhopal, M.P. (India)

***Corresponding Author:** Anjali Singh
*Email: anjalisinghabhi002@gmail.com

ABSTRACT
This study investigates the antioxidant potential of five medicinal plants—*Cordia obliqua*, *Lantana camara*, *Grewia obliqua*, *Sphaeranthus indicus*, and *Tinospora cordifolia*—which are traditionally valued for their health-promoting properties. Phytochemical screening was conducted to identify the presence of bioactive compounds, particularly phenolics and flavonoids, using qualitative and quantitative methods. Extracts were prepared using Soxhlet extraction with a 70% methanol solvent, yielding higher concentrations of antioxidants compared to petroleum ether extracts. Total phenolic content (TPC) and total flavonoid content (TFC) were assessed using the Folin-Ciocalteu method and aluminum chloride colorimetry, respectively. *Sphaeranthus indicus* exhibited the highest TPC (127.80 mg/g) and TFC (160.85 mg/g), indicating its significant antioxidant potential. Additionally, the DPPH, super oxideradical scavenging and reducing power assays demonstrated the extracts' capacity to neutralize free radicals, supporting their use in natural antioxidant formulations for food, cosmetic, and nutraceutical applications.

Keywords: Phytochemical screening, Total phenolic content, Folin-Ciocalteu method,antioxidants, free radical scavenging.

1. INTRODUCTION

There is an increasing trend to replace synthetic antioxidants, which are of safety concern **(Tripathi et al., 2007),** with the natural antioxidants available from plant extracts or isolated products of plant origin. Many plants, particularly medicinal plants, have been extensively studied for their antioxidant activity in recent years. It is believed that an increased intake of food rich in natural antioxidants is associated with lower risks of degenerative diseases, particularly cardiovascular diseases and cancer **(Pérez-Jiménez et al., 2008).** Antioxidants from aromatic, spicy, medicinal, and other plants were studied to develop natural antioxidant formulations for food, cosmetic, and other applications **(Miliauskas et al., 2004).** There are three major classes of plant chemicals: terpenoids, phenolic metabolites, and alkaloids **(Harborne, 1999)**. Among these three groups, phenolic compounds are the most important for dietary applications and the most extensively researched **(King et al., 1999).** Phenolic compounds include phenolic acids (hydroxybenzoic and hydroxycinnamic acids), polyphenols (hydrolyzable and condensed tannins), and flavonoids. These compounds protect plants, fruits, and vegetables from oxidative damage and have been used as antioxidants by humans.

There are many techniques to recover antioxidants from plants, such as Soxhlet extraction, maceration, supercritical fluid extraction, subcritical water extraction, and ultrasound-assisted extraction. However, extraction yield and antioxidant activity not only depend on the extraction method but also on the solvent used for extraction. The presence of various antioxidant compounds with different chemical characteristics and polarities may or may not be soluble in a particular solvent **(Turkmen et al., 2006).**

The medicinal plants selected for the present investigation, which included leaves of *Cordia obliqua*, *Lantana camara*, *Grewia obliqua*, Fruits of *Sphaeranthus indicus and* seeds of *Tinospora cordifolia* have long been used in the folk medicine due to their potential health promoting and pharmacological attributes, which are mainly ascribed to the presence of antioxidant constituents such as phenolic acids and flavonoids. It is important to establish appropriate means to evaluate and quantify effective antioxidant principles of medicinally or economically viable plant materials. The present study therefore was conducted with the main objective of investigating the potent antioxidant compounds, especially phenolics from different parts of selected medicinal plants.

2. MATERIAL AND MEHODS

2.1 Collection and authentication plant material

The plants were collected from local area of Bhopal, Madhya Pradesh, India and authenticated by at the Department of Botany, Govt. College Khimlasa, Sagar. The reference number given for each plant is listed:

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Plant samples	Plant Part	Reference No.
Lantana camara	Leaves	2023056
Cordia obliqua	Leaves	2023055
Grewia obliqua	Leaves	2023057
Sphaeranthus indicus	Fruits	2023058
Tinospora cordifolia	Seed	2023059

2.2 Sample preparation
Fresh plant parts were washed and dried. The dried samples were ground into powder using a kitchen milling machine and then passed through a 60-mesh sieve.

2.3Physicochemical evaluation (WHO, 2002; Harborne, 1998)
2.3.1 Determination of extractive values
a) Determination of water soluble extractive value:
4 gm of dried powder was soaked in 100 ml of water for 1 hr. and mixed properly. The mixture was boiled (100 0C) on water bath and then filtered. Filtrate was evaporated in pre weighed porcelain dish and dried at 105 ^{0}C. Water soluble extractive value was calculated.

b) Determination of alcohol soluble extractive value:
4 gm of powdered material was macerated with 100 ml of alcohol in shaking condition and allowed to stand for 16 hr. and filtered. Filtrate was than evaporated in pre weighed porcelain dish and dried at 105 0C. Alcohol soluble extractive value was calculated.

2.3.2 Determination of Ash values
Ash values like total ash, acid insoluble ash and water soluble ash of plant samples were determined by following methods:

a) Determination of Total ash
2 gm of powder was taken in pre weighed silica crucible and incinerated in a muffle furnace at 500 ^{0}C- 600 ^{0}C till carbon free ash was obtained. Percentage of ash was calculated with reference to initial weight of dried powder.
b) Determination of acid insoluble ash
Ash obtained from total ash was boiled for 5 min. with 25 ml of 1 N HCl and filtered using ashless filter paper to collect insoluble matter. The filter paper was transferred into a pre weighed silica crucible and incinerated at 650 0C in muffle furnace until free from carbon. Percentage of acid insoluble ash was calculated with reference to dried powder.

c) Determination of water soluble ash value
Ash obtained from total ash was boiled for 5 min. with 25 ml of water. Soluble matter was collected on an ashless filter paper. The filter paper was transferred into pre weighed silica crucible and incinerated at 450 0C in muffle furnace. Percentage of water soluble ash was measured with reference to dried powder.

2.4 Soxhlet extraction:
Dried and powered leaves of *Cordia obliqua, Lantana camara, Grewia obliqua,* Fruits of *Sphaeranthus indicus and* seeds of *Tinospora cordifolia* successively defatted with petroleum ether and then placed in a thimble of Soxhlet apparatus. The extraction was carried out using 70% methanol (hydroalcoholic) solvent system at 40-60^{o}C temperature of the heating mantle for 8-10 hours. After the extraction process, the extract of sample was filtered and concentrated to dryness. Extracts were collected in air tight container **(Alara et al., 2019).** Extraction yield of all extracts were calculated using the following equation below:

$$\text{Formula of Percentage yield} = \frac{\text{Actual yield}}{\text{Theoretical yield}} \times 100$$

2.5 Qualitative Phytochemical Estimation of Extracts
The qualitative tests for phytochemicals were performed according to several previously published standard protocols **(Khandelwal, 2008; Kokate et al., 2008).**

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2.6 Quantitative Phytochemical estimation-

2.6.1 Total phenol content

Total phenol content (TPC) in each extract was determined using the folin ciocalteu (FC) method described by Do et al. **(Do et al., 2014)**, with minor modifications. The dried extract was dissolved in distilled water to a concentration of 1 mg/mL. The calibration curve was established using gallic acid (20–100 µg/mL). The diluted extract or gallic acid (0.5 mL) was added to 2.5 mL FC reagent (5-fold diluted with distilled water) and mixed thoroughly for 3 minutes. Sodium carbonate (2 mL, 10% w/v) was added to the mixture and the mixture was allowed to stand for 45 minutes at room temperature. The absorbance of the mixture was measured at 760 nm using a UV–VIS spectrophotometer (Shimadzu-1700). TPC was expressed as milligram gallic acid equivalent per gram extract (mg GAE/g).

2.6.2 Total flavonoid content

The total flavonoid content (TFC) of each extract was investigated using the aluminum chloride colorimetry method described by Chang et al **(Chang et al., 2002)**. with slight modifications. In brief, the extract sample was diluted with methanol until 1 mg/mL. The calibration curve was prepared by diluting rutin in methanol (20–100 µg/mL). The diluted extract or rutin (0.5 mL) was mixed with 0.15 mL of 10% (w/v) aluminum chloride solution and 0.15 mL of 0.1 mM potassium acetate solution. The mixture was kept at room temperature for 30 minutes. Then the maximum absorbance of the mixture was measured at 510 nm using a UV–VIS spectrophotometer. TFC was expressed as milligram rutin equivalent per gram extract (mg RU/g).

2.7 *In vitro* anti oxidant activity

2.7.1 DPPH radical scavenging activity

The antioxidant activity of the extract was measured with the DPPH method **(Do et al., 2014)** with slight modifications. A solution of DPPH was freshly prepared by dissolving 6 mg DPPH in 50 mL methanol (about 0.3 mM). The extract with varying concentrations (20–100 µg/mL) and DPPH solution (2.5 mL) was mixed together in a test tube. The test tube was then incubated in the dark for 20 minutes at room temperature. The decrease in absorbance was measured at 517 nm using a UV–VIS spectrophotometer. The percentage inhibition of radicals was calculated using the following formula:

$$\%\ \boldsymbol{Inhibiton} = \frac{\boldsymbol{A\ control - A\ sample}}{\boldsymbol{A\ control}} \times \mathbf{100}$$

where *A*control is the absorbance of DPPH solution without extract and *A*sample is the absorbance of sample with DPPH solution. The half-maximal inhibitory concentration (IC_{50}) was reported as the amount of antioxidant required to decrease the initial DPPH concentration by 50%. All tests were performed at least in triplicate.

2.7.2 Reducing power

The method described by Chu et al **(Chu et al., 2000)** was applied in this work to determine the reducing power of dried extract. This reducing power was investigated by observing the transformation of Fe^{3+} to Fe^{2+}. The extract was diluted with distilled water (20–100 µg/mL). The diluted extract was mixed with phosphate buffer (2.5 mL, pH 6.6) and potassium ferricyanide (2.5 mL, 1% w/w) in a test tube, followed by incubating in a water bath at 50°C for 30 minutes. After the tube was removed from the water bath, trichloroacetic acid (2.5 mL, 10% w/v) was added into the tube and centrifuged (13,000*g*, 10 minutes). The supernatant (2.5 mL) was diluted with distilled water (2.5 mL), and freshly prepared ferric chloride (0.5 mL, 0.1% w/w) was added. The mixture was mixed thoroughly and its absorbance was measured at 700 nm using a UV–VIS spectrophotometer**(Do et al., 2014)**.

2.7.3 Superoxide anion radical scavenging activity

Superoxide anion-scavenging activity determined according to the method of Chavan et al., with some modifications **(Chavan et al., 2018).** The reaction mixture consisted of 1 ml of NBT (156 µM in 0.1 M potassium phosphate buffer pH 7.4), 1.0 ml of NADH (468 µM in 0.1 M potassium phosphate buffer pH 7.4) and 0.5 ml of an appropriately diluted sample. The reaction was initiated by addition of 100 µl of PMS (60 µM in 0.1 M potassium phosphate buffer pH 7.4) to the mixture. The tubes were incubated at ambient temperature for 5 min and the absorbance was measured at 560 nm. Decreased absorbance of the reaction mixture indicated increased superoxide anion-scavenging activity. The percentage inhibition of superoxide anion generation was calculated using the following formula:

% Inhibition = [(A0 – AS) / A0] × 100,

Where, A0 is absorbance of the control and AS is absorbance of the sample.

3. RESULTS AND DISCUSSION

3.1 Pharmacognostical evaluation

Ash values and extractive values of medicinal plants are essential parameters for assessing purity, quality, and the presence of organic and inorganic compounds. These values offer insights into the plant's composition and potential

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therapeutic efficacy. In this study, five medicinal plants were analyzed for their total ash value, water-soluble ash, acid-insoluble ash, water extractive value, and alcoholic extractive value, as presented in the accompanying data.

Table 1 Pharmacognostical evaluation of *Cordia Obliqua*plant sample

Parameters	**Value in percentage (%)**				
	Cordia oblique	***Lantana camara***	***Grewia obliqua***	***Sphaeranthus indicus***	***Tinosporacordifolia***
Total ash value	3.89	4.56	3.45	3.97	4.56
Water soluble ash	3.67	3.34	2.78	3.67	3.87
Acid insoluble ash	1.13	1.52	1.84	1.45	1.34
Water extractive value	3.56	3.19	3.12	2.89	3.34
Alcoholic extractive value	1.78	1.45	1.56	1.68	1.23

3.2 Percentage yield

Table 2 Percentage yield of extracts

S. No.	**Plant name**	Solvent	**Color of extract**	**% Yield**
1.	*Cordia obliqua* leaves	Petroleum ether (COPE)	Light yellow	0.225
2.	*Cordia obliqua* leaves	Hydroalcoholic 70% Methanol (COHE)	Brownish	3.75
3.	*Lantana camara* leaves	Petroleum ether (LCPE)	Yellow brownish	0.275
4.	*Lantana camara* leaves	Hydroalcoholic 70% Methanol (LCHE)	Dark greenish	5.325
5.	*Grewiaobliqua* Leaves	Petroleum ether (GOPE)	Light yellow Greenish	0.262
6.	*Grewiaobliqua* Leaves	Hydroalcoholic 70% Methanol (GOHE)	Brownish	2.275
7.	*Sphaeranthus indicus* fruits	Petroleum ether (SIPE)	Dark yellow	0.416
8.	*Sphaeranthus indicus* fruits	Hydroalcoholic 70% Methanol (SIHE)	Yellow brownish	4.701
9.	*Tinospora cordifolia* seeds	Petroleum ether (TCPE)	Light green Brownish	0.262
10.	*Tinospora cordifolia* seeds	Hydroalcoholic 70% Methanol (TCPE)	Brownish	4.328

Lantana camara (LCHE)had the highest overall yield, positioning it as a particularly promising candidate for further phytochemical and pharmacological research. The extraction efficiency was notably influenced by the choice of solvent, with 70% hydroalcoholic methanol consistently yielding higher results across all the studied plants compared to petroleum ether. This indicates that most of the bioactive compounds in these plants are likely polar, making hydroalcoholic extraction more effective for isolating therapeutic compounds.

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3.3 Qualitative Phytochemical Analysis of extracts

Experiment	**COPE**	**COHE**	**LCPE**	**LCHE**	**GOPE**	**GOPE**	**SIPE**	**SIHE**	**TCPE**	**TCPE**
Test for Carbohydrates										
Molisch's Test	-	+	-	+	-	-	-	+	-	+
Fehling's Test	-	+	-	-	-	-	-	-	-	-
Benedict's Test	-	+	-	-	-	-	-	-	-	+
Bareford's Test	-	+	-	+	-	-	-	+	-	+
Test for Alkaloids										
Mayer's Test	-	+	-	-	-	-	-	-	-	-
Hager's Test	-	+	-	+	-	-	-	-	-	-
Wagner's Test	-	+	-	+	-	-	-	+	-	+
Test for Steroid &Terpenoids										
Salkowski Test	-	-	-	+	-	-	-	+	-	+
Libermann-Burchard's Test	-	-	+	+	+	+	+	+	+	+
Test for Flavonoids										
Lead Acetate Test	-	+	-	+	-	-	-	+	-	+
Alkaline Reagent Test	-	+	-	+	-	-	-	+	-	+
Shinoda Test	-	+	-	+	-	-	-	+	-	+
Test for Tannins and Phenolic Compounds										
$FeCl_3$ Test	+	+	-	+	-	-	-	-	-	-
Lead Acetate Test	+	+	-	+	-	-	-	+	-	+
Gelatine Test	+	+	-	+	-	-	-	-	-	-
Test for Saponins										
Froth Test	+	+	-	-	-	-	-	-	-	-
Test for Protein and Amino acids										
Ninhydrin Test	-	-	-	+	-	-	-	+	-	+
Biuret's Test	-	-	-	+	-	-	-	-	-	-
Test for Glycosides										
Legal's Test	-	-	-	+	-	-	-	+	-	+
Keller Killani Test	-	-	-	+	-	-	-	+	-	+
Borntrager's Test	-	-	-	+	-	-	-	-	-	-

Phytochemical screening revealed that the hydroalcoholic 70% methanol extracts of the tested plants generally contained a richer diversity of bioactive compounds, including carbohydrates, alkaloids, steroids, terpenoids, flavonoids, tannins, saponins, proteins, amino acids, and glycosides. In contrast, the petroleum ether extracts exhibited minimal presence of these compounds, underscoring the importance of solvent selection in phytochemical extractions. These phytochemicals are known for their therapeutic activity, which justifies their use in traditional medicine. Sharmila et al. documented that these phytoconstituents may be responsible for various pharmacological activities, such as wound healing, cholesterol-lowering, and antidiabetic effects. It is also well-established that plant steroids, flavonoids, and phenols possess antioxidant properties**(Sharmila et al., 2007).**

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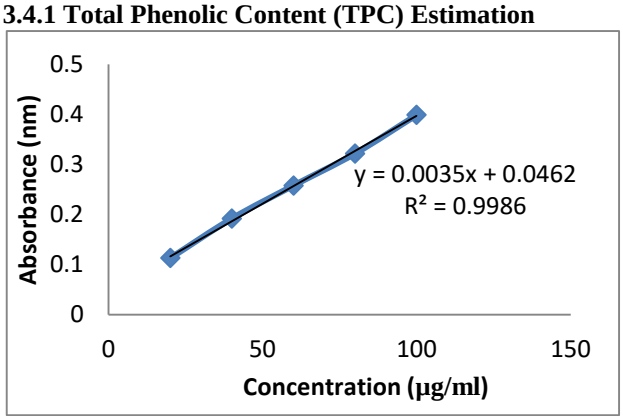
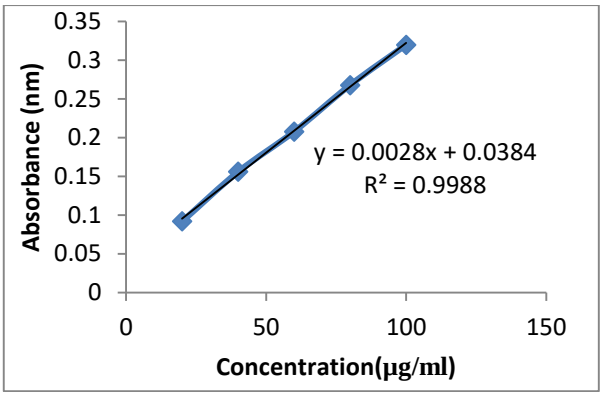
3.4 Quantitative Phytochemical analysis Hydro alcoholic extract:-
3.4.1 Total Phenolic Content (TPC) Estimation

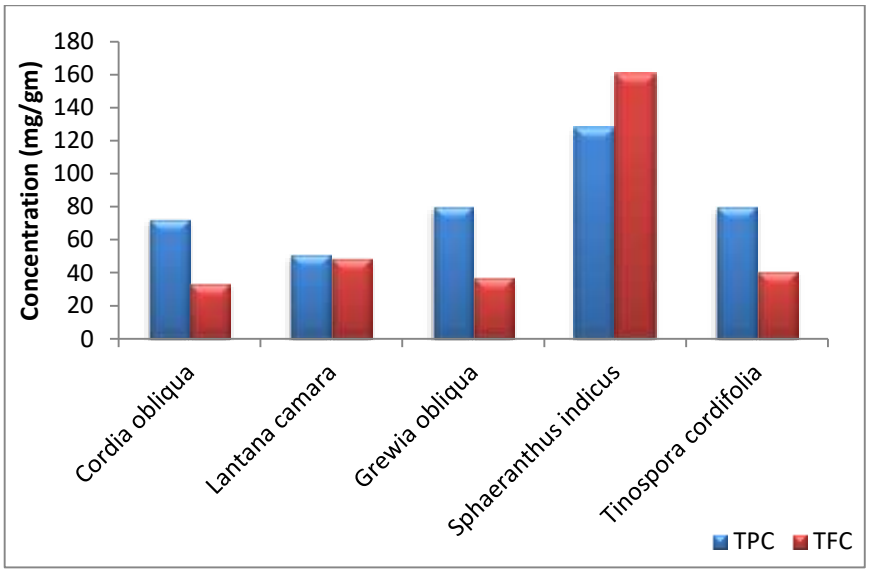




Graph 1 Graph represent standard curve of Gallic acid and Rutin

Table 3 Total Phenolic and Total Flavonoid Content in extracts

Total phenolic content (mg/gm equivalent to Gallic acid)					
Hydro alcoholic extract	***Cordia obliqua***	***Lantana camara***	***Grewia obliqua***	***Sphaeranthus indicus***	***Tinospora cordifolia***
Absorbance Mean±SD	0.2639±0.005	0.1986±0.004	0.2864±0.006	0.4294±0.004	0.2870±0.003
TPC	72.63	50.86	80.13	127.80	80.33
Total flavonoid content (mg/gm equivalent to Rutin)					
Hydro alcoholic extract	***Cordia obliqua***	***Lantana camara***	***Grewia obliqua***	***Sphaeranthus indicus***	***Tinospora cordifolia***
Absorbance Mean±SD	0.1049±0.004	0.1360±0.003	0.1122±0.004	0.3597±0.005	0.1198±0.004
TFC	33.45	49.00	37.10	160.85	40.90



Graph 2 Graph represent Total Phenolic and Total Flavonoid Content in Hydro alcoholic extracts

The total phenolic content (TPC) and total flavonoid content (TFC) of hydroalcoholic extracts from five different plants were evaluated, revealing significant variations in their phytochemical compositions. The highest phenolic content was observed in *Sphaeranthus indicus* (127.80 mg/g), followed by *Grewia obliqua* (80.13 mg/g) and *Tinospora cordifolia* (80.33 mg/g). *Cordia obliqua* and *Lantana camara* exhibited relatively lower phenolic content, with values of 72.63 mg/g and 50.86 mg/g, respectively. Phenolic compounds are well-known for their potent antioxidant properties, suggesting that *Sphaeranthus indicus* may exhibit strong antioxidant activity due to its high phenolic content.

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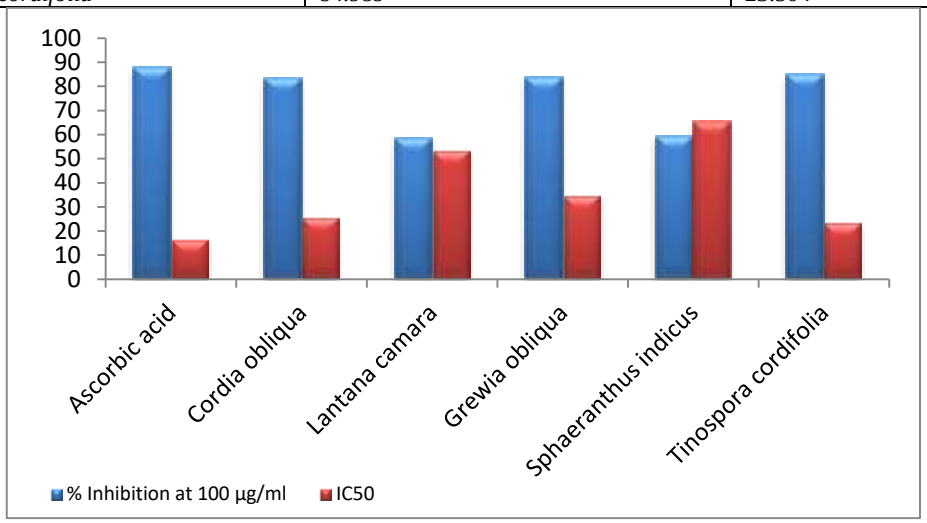
Similarly, *Sphaeranthus indicus* showed the highest flavonoid content (160.85 mg/g), which was considerably greater than that of the other plants. *Lantana camara* followed with 49.00 mg/g, while *Cordia obliqua*, *Grewia obliqua*, and *Tinospora cordifolia* displayed moderate flavonoid content, ranging from 33.45 to 40.90 mg/g. Since flavonoids are powerful antioxidants and anti-inflammatory agents, this further supports the potential of *Sphaeranthus indicus* as a rich source of therapeutic compounds.

3.5*In vitro* anti oxidant activity

3.5.1 DPPH Assay

Table 4 DPPH Activity of extracts

Plant name	% Inhibition at 100 µg/ml	IC50
Ascorbic acid	87.904	16.49
Cordia obliqua	83.261	25.7
Lantana camara	58.639	53.62
Grewia obliqua	83.909	34.735
Sphaeranthus indicus	59.179	65.51
Tinospora cordifolia	84.989	23.504



Graph 3 Graph represent DPPH Activity of extracts

Ascorbic acid, a well-known antioxidant, exhibited the highest percentage of inhibition (87.904%) and the lowest IC50 value (16.49µg/ml), setting a benchmark for comparing the antioxidant activities of the plant extracts. Among the tested plants, *Tinospora cordifolia*, *Cordia obliqua*, and *Grewia obliqua* displayed notable antioxidant activities, reflected by their low IC50 values, which indicate strong free radical scavenging abilities. These plants present significant potential for further investigation as natural antioxidants. In contrast, *Lantana camara* and *Sphaeranthus indicus* showed relatively lower antioxidant activity, which may limit their effectiveness in applications that require potent antioxidant properties.

3.5.2 Reducing Power Assay

Table 5Reducing Power Activity of extracts

Concentration (µg/ml)	**Ascorbic acid**	***Cordia obliqua***	***Lantana camara***	***Grewia obliqua***	***Sphaeranthus indicus***	***Tinospora cordifolia***
20	0.139	0.102	0.084	0.093	0.073	0.116
40	0.186	0.124	0.112	0.124	0.092	0.149
60	0.254	0.15	0.129	0.147	0.116	0.171
80	0.307	0.173	0.148	0.166	0.139	0.204
100	0.361	0.203	0.181	0.194	0.148	0.241

The results from the reducing power assay indicate that *Tinospora cordifolia*, *Cordia obliqua*, and *Grewia obliqua* possess significant electron-donating capacities, reflecting their strong antioxidant potential. In contrast, *Lantana camara* and *Sphaeranthus indicus* exhibited weaker reducing powers, suggesting lower antioxidant activities. These findings are consistent with the results of other assays, further identifying *Tinospora cordifolia* and *Cordia obliqua* as promising sources of antioxidants.

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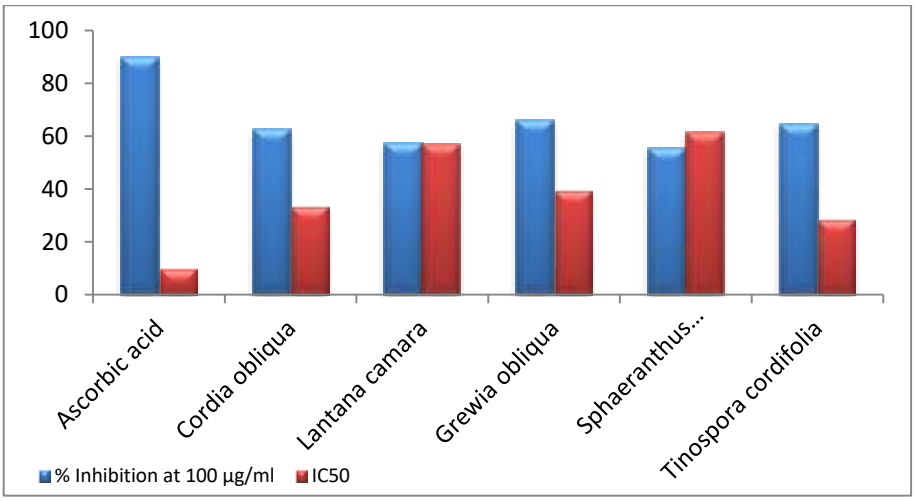
3.5.3 Superoxide free radical scavenging activity

Table 6Superoxide free radical scavenging activity of extracts

Plant name	% Inhibition at 100 µg/ml	IC50
Ascorbic acid	89.905	9.93
Cordia obliqua	62.452	33.457
Lantana camara	57.358	57.023
Grewia obliqua	66.132	39.659
Sphaeranthus indicus	55.660	61.52
Tinospora cordifolia	64.622	28.6



Graph 4Graph represent Superoxide free radical scavenging activity of extracts

The superoxide free radical scavenging activity assay evaluates the ability of plant extracts to neutralize superoxide radicals, reactive oxygen species (ROS) that can cause oxidative stress and damage biological molecules. A higher percentage of inhibition indicates better scavenging activity, with ascorbic acid serving as the standard for comparison. Ascorbic acid demonstrated an impressive superoxide scavenging ability, achieving 89.905% inhibition at a concentration of 100 µg/ml, and its low IC50 value of 9.93 µg/ml underscores its potency as a superoxide radical scavenger. The assay results indicate that *Tinospora cordifolia* and *Grewia obliqua* exhibit strong antioxidant potential, positioning them as promising candidates for further exploration in natural antioxidant therapies. Conversely, *Lantana camara* and *Sphaeranthus indicus* showed weaker scavenging abilities, suggesting that their efficacy in antioxidant applications may be limited.

4. CONCLUSION

The findings of this study underscore the potential of *Cordia obliqua*, *Lantana camara*, *Grewia obliqua*, *Sphaeranthus indicus*, and *Tinospora cordifolia* as valuable sources of natural antioxidants. Phytochemical analysis revealed a rich diversity of bioactive compounds, especially phenolics and flavonoids, which are closely linked to antioxidant activity. *Sphaeranthus indicus* emerged as a particularly promising candidate due to its high concentrations of these compounds, highlighting its potential for further pharmacological research and development of natural antioxidant products. The study also emphasizes the critical role of solvent selection in optimizing extraction yields and underscores the therapeutic relevance of these plants in traditional medicine. Future research should focus on isolating specific compounds and investigating their mechanisms of action to validate their efficacy and ensure safety in various applications.

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