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Antioxidant Efficacy And Phytochemical Composition Of Selected Herbal Extracts Used In Indian Medicine

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ABSTRACT

The current study investigates the phytochemical composition and antioxidant activities of five medicinal plants traditionally used in Indian medicine for the treatment of inflammation, cancer, and oxidative stress-related conditions. The selected plant species (Lantana camara, Ipomoea cairica, Callicarpa macrophylla, Cordia obliqua, and Aloe barbadensis miller) were subjected to hydroalcoholic extraction. Phytochemical screening revealed the presence of key bioactive compounds such as flavonoids, terpenoids, phenols, tannins, alkaloids, and glycosides, all of which are associated with notable antioxidant, antimicrobial, and anticancer properties. The total phenolic and flavonoid contents were also determined; with Aloe barbadensis miller exhibiting the highest phenolic content (116.34 mg/g) and flavonoid content (88.64 mg/g). The antioxidant potential was evaluated using DPPH free radical scavenging, hydrogen peroxide scavenging, and reducing power assays. Aloe barbadensis miller displayed the highest antioxidant activity, with strong radical scavenging ability, followed by Callicarpa macrophylla and Lantana camara. These findings suggest that the studied plant species, particularly Aloe barbadensis miller, have strong potential as natural antioxidants and could be valuable in developing therapies targeting oxidative stress-related diseases.

1. INTRODUCTION

Medicinal plants have amazing health benefits as they are natural healers and are used for healthcare in both developed and developing countries. Herbal medicine is traditionally known to be harmless and is generally used for longstanding diseases. A number of drugs of plant origin are included in modern pharmacotherapy **(Welz et al. 2018).** Herbs are easily absorbed by our body as they are natural and get assimilated quickly without any side-effects. Synthetic drug assimilation by our body is not complete and remnants of them lead to harmful interactions causing various side-effects such as allergic reactions. Plants adapt too many physical and physiological defense mechanisms by production of secondary metabolites such as environmental stress, stress against pathogens etc. Until the 18th century, method of treatment using therapeutic properties of many plants and their effect on the human organism was known but the active compound responsible for cure was unknown **(Salmerón-Manzano and Manzano-Agugliaro 2020).**

Medicinal plants gained recognition in research of present times as they protect human body from damage of free radicals obtained by various oxidative stress factors. The overproduction of reactive oxygen species (ROS) has been implicated in the development of various chronic and degenerative diseases such as cancer, respiratory,

neurodegenerative, and digestive diseases **(Liu et al., 2018).** Under physiological conditions, the concentrations of ROS are subtlety regulated by antioxidants, which can be either generated endogenously or externally supplemented. A combination of antioxidant-deficiency and malnutrition may render individuals more vulnerable to oxidative stress, thereby increasing the risk of cancer occurrence **(Liu et al., 2018).** In addition, antioxidant defense can be overwhelmed during sustained inflammation such as in chronic obstructive pulmonary diseases, inflammatory bowel disease, neurodegenerative disorders, cardiovascular diseases, and aging **(Chelombitko, 2018).** Certain antioxidant vitamins, such as vitamin D, are essential in regulating biochemical pathways that lead to the proper functioning of organs. Antioxidant supplementation has been shown to attenuate endogenous antioxidant depletion thus alleviating associated oxidative damage in some clinical research **(Forman and Zhang, 2021).** Increasing trends of microbial resistance to antibiotics and various chronic and degenerative pathologies of humans caused by reactive oxygen species (ROS) have triggered the search for bioactive compounds from plants with alternative mechanisms of action to counteract pathogenic microbes and natural antioxidants capable of protecting the body against oxidative stress and free radical-induced damage **(Oluwajuyitan et al., 2021).** The proper use of medicinal plants requires accurate scientific information and an understanding of their chemical constituents. The therapeutic effects in plants are due to the chemical compounds therein. Medicinal plants play a very important role in the development of alternative drugs without the adverse effects of synthetic drugs **(Bisso et al., 2022).**

The plant species under investigation were chosen based on their history in traditional medicine and they include *Lantana camara, Ipomoea cairica, Callicarpa macrophylla*, *Cordia obliqua* and *Aloe barbadensis miller*. These plant species are widely used in Indian traditional medicine for treatment of inflammation and cancer, which are both linked to oxidative stress. These plant species also possess antioxidant, antimicrobial, anti-inflammatory and anticancer properties **(Kalita et al., 2012; Srivastava and Shukla, 2015; Soni et al., 2014; Gupta and Gupta, 2015; Ojha and Ghule, 2022 and Sharma et al., 2014).** Thus, the present study aimed to investigate the phytochemical contents and antioxidant activities of selected herbal plants.

2. MATERIAL AND METHODS

2.1 Chemicals

DPPH (2, 2-diphenyl-1-picrylhydrazyl), (±)-α-tocopherol, Folin-Ciocalteu's reagent, dimethyl sulfoxide (DMSO), p-iodonitrotetrazolium chloride (INT), rutin, gallic acid, and ascorbic acid, were purchased from Sigma-Aldrich. The solvent and all reagents used in the analysis were of analytical grade.

2.2 Plant collection and Authentication

Five fresh plants (*Lantana camara, Ipomoea cairica, Callicarpa macrophylla*, *Cordia obliqua* and *Aloe barbadensis miller*) **(Table 1)** were collected from various areas of Bhopal, Madhya Pradesh. The plants were authenticated at the Department of Botany, Govt. College Khimlasa, Sagar. The reference number given for each plant is listed in

Table 1.

Table 1 List of Plant and reference number:

Plant samples	Plant Part	Reference No.
Lantana camara	Leaves	2023/061
Cordia obliqua	Leaves	2023/062
Aloe barbadensis miller	Pulp	2023/063
Ipomoea cairica	Leaves	2023/064
Callicarpa macrophylla	Flower	2023/065

2.3 Extracts preparation

The collected plant parts were washed with water and dried in the shade at room temperature. Extraction was done using method described by **Kaur et al., 2019**. The sun dried powdered plant material were extracted in the soxhlet apparatus with 70% methanol, the boiling temperature was maintained at 40 ºC to 60 ºC. The flask containing the extraction solvent was heated to reflux. The extraction was continued for 48 h. After extraction the solvent was removed. The non-soluble portion of the extracted solid remained in thimble and was discarded. Ultimately the extract was collected from the distillation flask and was filtered using filter paper. The filtrate was collected in the beaker was kept in water bath at 67 ºC to remove the solvent so as to obtain a semi solid extract.

2.4 Preliminary Phytochemical Screening

The presence or absence of different constituents, such as alkaloids, steroids, glycosides, flavonoids, tannins, saponins, and terpenoids in each plant extract was determined using the method of **Harbone (1998).** Determination of the total phenolic content (TPC) and total flavonoid content (TFC) were performed using the method.

2.5 Determination of total flavonoid content

The total flavonoid content was determined by the aluminum chloride colorimetric method as described by Chang et al. with some modifications **(Chang et al., 2002).** Aliquot of 0.5 mL of various extracts (1 mg/mL) were mixed with 1.5 mL of methanol, followed by the addition of 0.15 ml of 10% $AlCl_3$, 0.15 ml of 5% sodium nitrite and incubated for 6 min. Then, 2 ml of 4 % sodium hydroxide was added. After 15 min, absorbance of mixture was estimated at 510 nm using UV spectrophotometer. The calibration curve (20–100 µg/mL) was plotted using rutin as a standard. The total flavonoids were expressed as mg of rutin equivalent/gram dry weight.

2.6 Determination of total phenolic content

The amount of total phenolic content was determined according to the Velioglu method using the Folin–Ciocalteu reagent **(Velioglu et al., 1998)**. Aliquot of 0.2 mL of various extracts (1 mg/mL) was mixed with 2.5 mL of Folin–Ciocalteu reagent (10-fold diluted with dH_2O). The mixture was kept at room temperature for 5 min and 2 mL of 7% sodium carbonate was added. After 90 min of reaction, its absorbance was recorded at

760 nm. The standard calibration (20–100 µg/mL) curve was plotted using gallic acid. The total phenolics were expressed as mg gallic acid equivalent/gram dry weight. Negative control was prepared by adding 0.1 mL of solvent instead of extract.

2.7 Anti oxidant activity

2.7.1 DPPH Radical Scavenging Assay

The DPPH assay was performed using the method described by **Dzoyem and Eloff, 2015**. Briefly, 2 ml of DPPH solution (0.1 mM) prepared in methanol was mixed with 1 ml of each plant extract sample at various concentrations (20 to 100 μg/mL). After incubation in the dark at room temperature for 30 min, the absorbance of the mixture was measured at 517 nm using a spectrophotometer. Ascorbic acid was used as a positive control, methanol as a negative control, and extract without DPPH as a blank. The percent of inhibition of DPPH radical scavenging (%I) was calculated using the formula:

$$\%I = ((Absorbance_{Control} - Absorbance_{Sample})/Absorbance_{Control})) \times 100$$

The concentration of each plant extract necessary to scavenge 50% of radicals (IC_{50}) was calculated by plotting inhibition percentages against concentrations of each sample.

2.7.2 Hydrogen peroxide scavenging assay

The ability of the extract to scavenge hydrogen peroxide (H_2O_2) was determined according to the method of **Bhatti et al., 2015.** Aliquot of 0.1 mL of extracts (20–100 µg/mL) was transferred into the eppendorf tubes and their volume was made up to 0.4 mL with 50 mM phosphate buffer (pH 7.4) followed by the addition of 0.6 mL of H_2O_2 solution (2 mM). The reaction mixture was vortexed and after 10 min of reaction time, its absorbance was measured at 230 nm. Ascorbic acid was used as the positive control. The ability of the extracts to scavenge the H_2O_2 was calculated using the following equation:

H_2O_2 scavenging activity percentage = $[(A0-A1)/A0]\times100$

2.7.3 Reducing power assay

The reducing power was determined according to the Oyaizu et al. method with some modifications **(Oyaizu et al., 1986).** Aliquot of 1 mL of various concentrations of the extracts (20–100 µg/mL) were mixed separately with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated in a water bath at 50°C for 20 min. After cooling at room temperature, 2.5 mL of 10% trichloroacetic acid was added to it followed by centrifugation at 3,000 rpm for 10 min. Supernatant (2.5 mL) was collected and mixed with 2.5 mL of distilled water. Ferric chloride (0.2 mL of 0.1%) was added to it and the mixture was left at room temperature for 10 min. The absorbance was measured at 700 nm. Ascorbic acid was used as positive control.

3. RESULT AND DISCUSSION

3.1 Plant Collection

Table 2 Plant collection

S. No.	Plant name	Plant part used	Weight (in gm)
1.	*Lantana camara*	Leaves	200
2.	*Ipomoea cairica*	Leaves	50
3.	*Callicarpa macrophylla*	Flower	250
4.	*Cordia obliqua*	Leaves	80
5.	*Aloe barbadensis miller*	Pulp	80

3.2 Percentage yield

Table 3 Percentage yield of extracts

S. No.	Plant name	Solvent	Color of extract	Theoretical weight (gm)	Yield (gm)	% Yield
1.	*Lantana camara*	Hydroalcoholic	Dark Greenish	150	20.34	13.56
2.	*Cordia obliqua*	Hydroalcoholic	Brownish	50 gm	6.120	12.24
3.	*Aloe barbadensis miller*	Hydroalcoholic	Dark Greenish	50 gm	6.010	12.02
4.	*Ipomoea cairica*	Hydroalcoholic	Brownish	50 gm	7.388	14.77
5.	*Callicarpa macrophylla*	Hydroalcoholic	Brownish	100 gm	4.257	4.25

In this study, five different plant species (*Lantana camara*, *Cordia obliqua*, *Aloe barbadensis miller*, *Ipomoea cairica*, and *Callicarpa macrophylla*) were subjected to hydroalcoholic extraction to evaluate their extract yields. The color of the extracts varied between dark greenish and brownish shades, reflecting the diverse chemical compositions of the plants. Among the plants, *Ipomoea cairica* exhibited the highest yield at 14.77%, followed by *Lantana camara* (13.56%), *Cordia obliqua* (12.24%) and *Aloe barbadensis miller* (12.02%). The

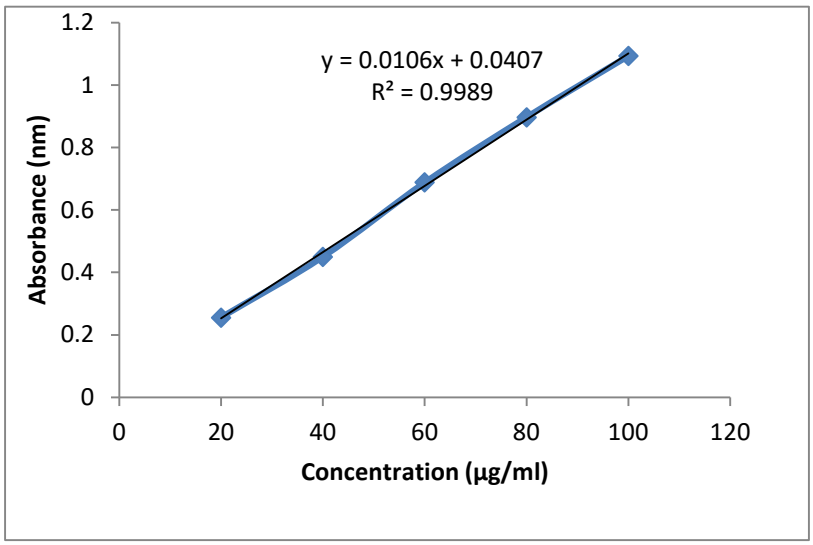
lowest yield was observed in *Callicarpa macrophylla*, with only 4.25%.

3.3 Qualitative Phytochemical Analysis

The phytoconstituents found in plants were flavonoid, terpenoid, phenols, tannin, steroid, alkaloid, and glycosides. The phytochemicals polyphenols, and flavonoids are highly present in all plants. Previous studies have indicated that cytotoxic effect is shown by phytochemicals like tannin, flavonoid, and alkaloid **(Blois, 1958).** The anticancer properties are reported for flavonoids, while antiviral properties and antibacterial effects are reported for the presence of saponin **(Azhdari et al., 2019).** By inhibitory activity toward growth, tannin also indicated the anticancer properties **(Chowdhury et al., 2017).**

3.4 Quantitative Phytochemical analysis

3.4.1 Total Phenolic Content (TPC) Estimation



Graph 1 Graph represent standard curve of Gallic acid

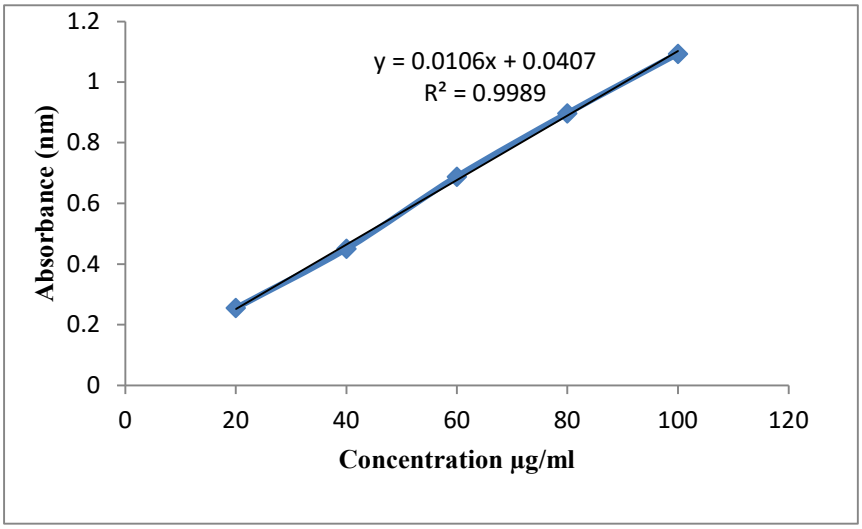
Table 4 Total Phenolic Content in extracts

Total phenolic content (mg/gm equivalent to Gallic acid)					
Hydro alcoholic extract	***Lantana camara***	***Ipomoea cairica***	***Callicarpa macrophylla***	***Cordia oblique***	***Aloe barbadensis miller***
Absorbance Mean±SD	0.813±0.005	0.499±0.002	0.861±0.003	0.493±0.003	1.203±0.004
TPC	67	45.90	82.10	45.32	116.34

The absorbance and corresponding TPC values in mg/g equivalent to gallic acid are presented.

Among the plants, *Aloe barbadensis miller* exhibited the highest total phenolic content with 116.34 mg/g, correlating with the highest absorbance value of 1.203±0.004. This suggests that *Aloe barbadensis miller* may contain a higher concentration of phenolic compounds, known for their antioxidant properties. *Callicarpa macrophylla* also showed a relatively high phenolic content of 82.10 mg/g, with an absorbance value of 0.861±0.003. On the other hand, *Lantana camara, Ipomoea cairica* and *Cordia oblique* exhibited lower phenolic contents, with values of 67 mg/g, 45.90 mg/g and 45.32 mg/g, respectively, despite showing similar absorbance values (0.813±0.005, 0.499±0.002 and 0.493±0.003). This indicates a relatively lower presence of phenolics in these extracts compared to *Aloe barbadensis miller* and *Callicarpa macrophylla*. The variation in TPC values across different plant species could be due to differences in the type and quantity of phenolic compounds present, which play a significant role in their antioxidant activities. Plants with higher TPC values, such as *Aloe barbadensis miller* and *Callicarpa macrophylla*, may offer greater potential as sources of natural antioxidants.

3.4.2 Total Flavonoid Content (TFC) Estimation



Graph 2 Graph represent standard curve of Rutin

Table 5 Total Flavonoid Content in extract

Total phenolic content (mg/gm equivalent to Gallic acid)					
Hydro alcoholic extract	***Lantana camara***	***Ipomoea cairica***	***Callicarpa macrophylla***	***Cordia oblique***	***Aloe barbadensis miller***
Absorbance Mean±SD	0.766±0.004	0.311±0.003	0.610±0.002	0.310±0.002	0.926±0.003
TPC	72	27.10	57.00	27.03	88.64

Flavonoids, being vital plant secondary metabolites, are associated with numerous health benefits, including antioxidant, anti-inflammatory, and cardioprotective activities. *Aloe barbadensis miller* had the highest absorbance (0.926±0.003), indicating the greatest total flavonoid content of 88.64 mg/g. This suggests that *Aloe barbadensis miller* is a rich source of flavonoids, which are known for their potent antioxidant and anti-inflammatory properties.

Lantana camara and *Callicarpa macrophylla* displayed a significant flavonoid content of 72 mg/g and 57.00 mg/g, with an absorbance of 0.766±0.004 and 0.610±0.002. This indicates a moderate flavonoid presence, which might contribute to its biological activity.

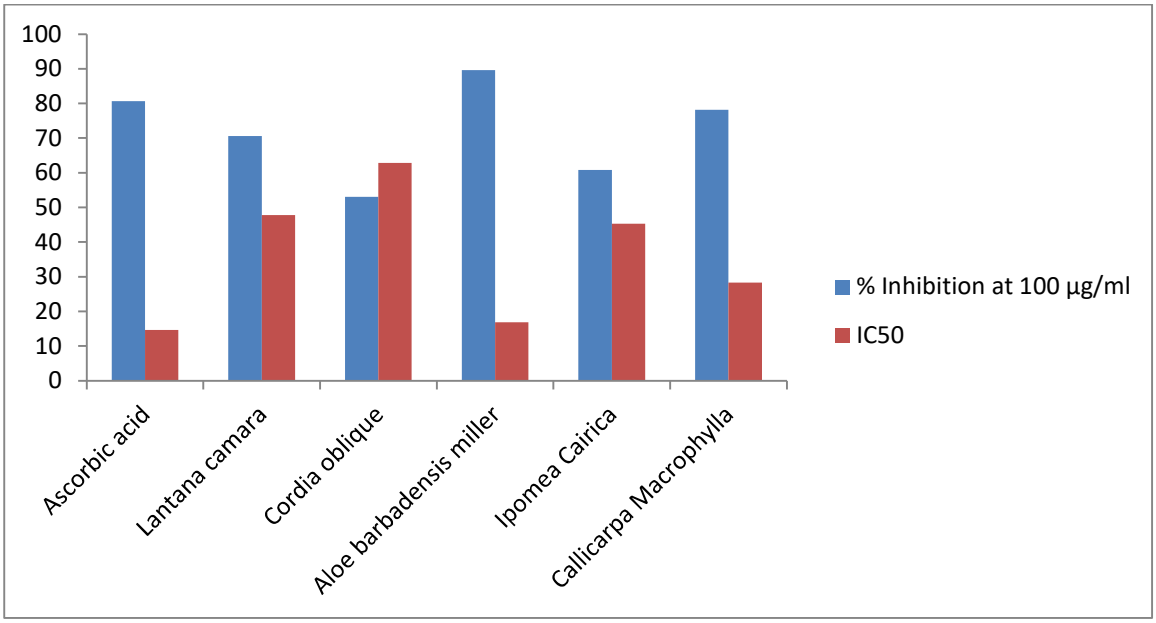
Ipomoea cairica and *Cordia oblique* had relatively lower flavonoid contents, with values of 27.10 mg/g and 27.03 mg/g, respectively. Their absorbance values (0.311±0.003 and 0.310±0.002) were also lower compared to other species in the study.

The variation in TFC among the studied plants highlights the diversity in their phytochemical profiles and potential health benefits.

3.5 Anti oxidant activity

Table 6 DPPH activity of extracts

Sample name	**% Inhibition at 100 µg/ml**	**IC50**
Ascorbic acid	80.642	14.662
Lantana camara	70.562	47.775
Cordia oblique	53.081	62.875
Aloe barbadensis miller	89.572	16.895
Ipomoea cairica	60.811	45.297
Callicarpa macrophylla	78.168	28.322



Graph 3 Graph represent DPPH activity of extracts

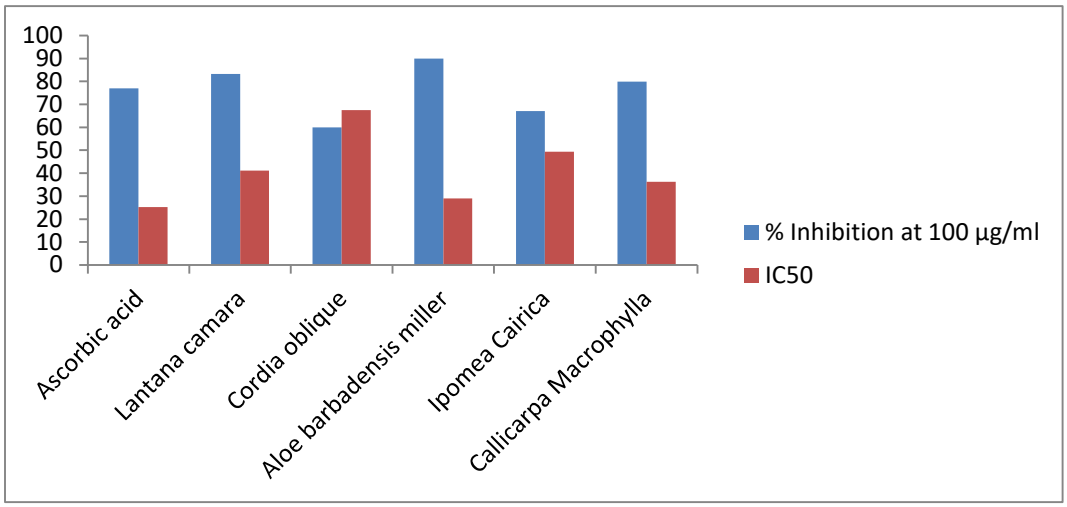
The DPPH activity of various medicinal plant extracts was examined as shown in **Table 6**. The standard antioxidant compound ascorbic acid showed the highest DPPH activity of 14.662µg/mL. The *Aloe barbadensis miller* extract showed the highest antioxidant activity of 16.895 µg/mL followed by *Callicarpa macrophylla* with 28.322 µg/mL, *Ipomoea cairica with* 45.297 µg/mL, *Lantana camara* with 47.775µg/mL, *Cordia oblique* with 62.875 µg/mL.

It was observed that the alcoholic DPPH solution reduction is because of the presence of hydrogen donor antioxidant (AH) which reacts with free radicals and converts it to non-radical DPPHH form. The complete reaction can be expressed by this equation, $DPPH^{\cdot}+AH \rightarrow DPPH\text{-}H^{+}A^{\cdot}$. The DPPH remaining in the reaction mixture measured after some time acts in reverse to the antioxidant radical scavenging activity. The free radical scavenging activity of the plant extracts were evaluated using DPPH antioxidant assay. So, these findings from the present study suggested that relatively higher antioxidant potential of the tested medicinal plant samples were involved in the decolorization of the stable DPPH radical solution **(Jafri et al., 2022)**.

Table 7 Hydrogen peroxide scavenging activity

Sample name	**% Inhibition at 100 µg/ml**	**IC50**
Ascorbic acid	77.003	25.184
Lantana camara	83.306	41.143
Cordia oblique	60.029	67.438
Aloe barbadensis miller	89.93	28.971
Ipomoea Cairica	67.022	49.39

Callicarpa Macrophylla	79.94	36.27



Graph 4 Graph represent Hydrogen peroxide (H_2O_2) activity of extracts

Hydrogen peroxide (H_2O_2) is a reactive oxygen species that can easily penetrate biological membranes and generate harmful hydroxyl radicals, contributing to oxidative stress, cell damage, and various diseases. The hydrogen peroxide scavenging assay measures the ability of compounds to neutralize H_2O_2, indicating their antioxidant capacity. In this analysis, the antioxidant activities of different plant extracts were compared with ascorbic acid, a known standard antioxidant, based on the percentage inhibition at 100 µg/ml and their IC50 values. Among the tested samples, *Aloe barbadensis miller* emerged as the most potent antioxidant, exhibiting the highest percentage inhibition and a low IC50, indicating strong H_2O_2 scavenging activity. *Lantana camara* and *Callicarpa macrophylla* also showed strong antioxidant potential, with high inhibition percentages and moderate IC50 values, making them promising candidates for further exploration in antioxidant therapies. While *Ipomoea cairica* and *Cordia obliqua* demonstrated moderate to lower scavenging activities, they still offer some antioxidant potential, though they may not be as effective as the top-performing extracts in this context.

Table 8 Reducing Power Assay

Concentration (µg/ml)	**Ascorbic acid**	***Lantana camara***	***Cordia oblique***	***Aloe barbadensis miller***	***Ipomoea cairica***	***Callicarpa macrophylla***
20	0.123	0.026	0.214	0.447	0.052	0.507
40	0.187	0.057	0.243	0.559	0.089	0.599
60	0.273	0.093	0.267	0.679	0.108	0.669

80	0.350	0.126	0.288	0.763	0.151	0.753
100	0.436	0.153	0.294	0.883	0.223	0.823

The antioxidant activity of the plant extracts were evaluated using Reducing power assay. Thus, the sequence for the reducing power based on these experimental results can be organized as: *Aloe barbadensis miller* > *Callicarpa macrophylla* > Ascorbic acid > *Cordia oblique* > *Ipomoea cairica* > *Lantana camara*. It was observed that the reductones present in these medicinal plants may be responsible for the reducing properties of the extracts and the reaction may be dependent for inhibiting the free radical chain by providing a hydrogen atom or may be by reaction with certain compounds of peroxides to avoid the peroxide development **(Trpevski et al., 2007).**

4. CONCLUSION

This study highlights the phytochemical diversity and significant antioxidant potential of five medicinal plant species traditionally used in Indian medicine. Among the tested plants, *Aloe barbadensis miller* consistently demonstrated the highest antioxidant activity across various assays, making it a promising candidate for further research into its therapeutic potential. *Callicarpa macrophylla* and *Lantana camara* also exhibited strong antioxidant capacities, suggesting their potential for use in oxidative stress-related treatments. The presence of phenolic compounds and flavonoids, known for their antioxidant and anti-inflammatory properties, further supports the medicinal value of these plants. These results contribute to the growing body of evidence supporting the use of natural antioxidants in combating diseases linked to oxidative stress, including inflammation and cancer. Further studies, including in vivo analysis and clinical trials, are recommended to validate the therapeutic potential of these plant species.

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