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Comparative Study of Antioxidant Properties in Different Extracts of *Ipomoea Cairica*

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ABSTRACT

Ipomoea cairica, a widely distributed medicinal plant, is known for its diverse therapeutic properties, including significant antioxidant activities. This study aims to comparatively evaluate the antioxidant properties of different extracts obtained from Ipomoea cairica using soxhlet extraction method. Ethyl acetate, Methanolic, Petroleum ether extracts were prepared, Ethyl acetate and Methanolic extract were analyzed for their phytochemical content and antioxidant capacities. Standard assays such as H2O2 and SOS radical scavenging activities were employed to quantify the antioxidant activities. The results demonstrated that the methanolic extract exhibited the highest total phenolic content, total flavonoid content and superior antioxidant activity across all assays, followed by the ethyl acetate extract. The study underscores the potential of Ipomoea cairica as a rich source of natural antioxidants and highlights the influence of extraction solvents on the efficacy of antioxidant compounds. These findings contribute to the optimization of extraction processes for enhancing the therapeutic application of Ipomoea cairica in pharmaceutical formulations.

Keywords:*Ipomoea cairica, Antioxidant activity, Extraction method, Phytochemical analysis.*

1. INTRODUCTION

A perfect example of the remarkable phenomenon of symbiosis is usually found in nature. There is a growing interest in natural product therapies with a fundamental respect for nature in the western world as people become more conscious of the effectiveness and adverse effects of synthetic pharmaceuticals. Herbal remedies have long been used to treat a wide range of infectious disorders in human history. The significance and contribution of numerous plants have been emphasized by a number of scientific studies **(Verma 2008).**

Any plant or portion of a plant utilized for flavor, aroma, or medicinal purposes is considered a herb. One class of nutritional supplements is herbal medicine. In addition to fresh or dried plants, they are offered for sale as tablets, capsules, powders, teas, and extracts. To strive to preserve or enhance their health, people take herbal medications **(Thakur and Yadav, 2016).** A lot of individuals think that goods using the label "natural" are invariably healthy and safe. That might not always be the case. Herbal medications are exempt from the same testing requirements as pharmaceuticals. Certain plants, like ephedra, can be extremely harmful **(Kamboj, 2000).**

A multitude of biological activities, including the potential for antioxidant activity, have been discovered in plants and their products, which are also significant sources of phytochemicals **(Harman, 1992).**Consumer preference has led to a significant demand for natural antioxidants as food additives, biopharmaceuticals, and nutraceuticals. Elevated levels of free radicals in the body can lead to a number of illnesses in humans, including cancer, arthritis, Alzheimer's disease, atherosclerosis, and more. The most common pro-oxidants, reactive oxygen species (ROS) and nitrogen species (RNS), are either produced naturally during metabolism or are brought on by UV rays and other contaminants. Consuming antioxidant compounds can help to largely minimize the harmful effects of altered antioxidant-prooxidant balance **(Ghosh *et al.,* 2008; Ognjanovic *et al.,* 2008).** Plant-based materials and supplements have been discovered to contain antioxidants. Compared to synthetic antioxidants, those derived from plants are more beneficial because of their natural source **(Rohman *et al.,* 2010; Zheng and Wang, 2001).** While synthetic antioxidants have been shown to have genotoxic effects, using natural antioxidants derived from plants does not cause any negative effects **(Chen *et al.,* 1992; Kahl and Kappus, 1993).** As a result, there have been several studies looking into the biological activity and chemical makeup of medicinal plants as a possible source of free radicals.

A perennial herb belonging to the Convolvulaceae family, *Ipomoea cairica* grows from a tuberous footstock. It has huge palmate leaves with five to seven lobes and displays spectacular white to lavender blooms. It grows prostrately or twines with other vegetation. Every fruit has hairy seeds and develops to be around 1 cm across. Although many species can also be found in temperate zones, the genus Ipomoea is found worldwide in the tropics **(Cao *et al.,* 2005).**The essential oil of this species presented larvicidal properties against larvae of Culex tritaeniorhynchus, Aedes aegypti, Anopheles stephensi and Culex quinquefasciatus **(Thomas *et al.,* 2004).** The world's tropical and subtropical climates are home to the Convolvulaceae family. *Ipomoea cairica*L. Sweet (Syn.Ipomoeaturberculata (Desr.) Roem. & Schult.) (Convolvulaceae) is a climbing plant widely distributed around almost all tropical regions and is used in folk medicine all over the world **(Ferreira *et al.,* 2006).** Although *Ipomoea cairica* is widely used in folk medicine, few data about its biological activities are found in literature. In this study we have performed the extraction of plant and evaluated the anti-oxidant acivities of plant extract.

2. MATERIAL AND METHOD

2.1 Materials

All the solvents used for extraction and isolations were distilled prior to their used and were obtained from commercial source and were of analytical grade. All the chemicals were purchased from Merck Specialities Pvt. Ltd., Mumbai and Sigma Aldrich, Bangalore.

2.2 Plant collection

The medicinal plant *Ipomoea cairica* whole plant were collected from Bhopal, M.P., and dried under shade for 3 days and then dried in an oven at 45°C. The dried parts were stored in air-tight containers to prevent contamination. A plant taxonomist authenticated the plant to confirm their identity and purity.

2.3 Soxhlet extraction:

Powdered plant sample were placed in a thimble of soxhlet apparatus. The extraction was carried out using different organic solvents; petroleum ether, ethyl acetate and methanol for 8-10 hours and 40-60°C temperature

of the heating mantle were adjusted. After the extraction process, the extract of sample were 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:

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

2.4 Qualitative Phytochemical Estimation of Extracts

Detailed phytochemical testing was performed to identify presence or absence of different phytoconstituents in extracts by using standard procedures **(Kokate*et al.*, 2006).**

2.5 Quantitative Phytochemical estimation

2.5.1 Spectrophotometric Quantification of Total Phenolic Content: -

The total phenolic content of extract was determined using the Folin-Ciocalteu Assay. The extracts (0.2 mL from stock solution) were mixed with 2.5 mL of Folin-Ciocalteu Reagent and 2mL of 7.5% sodium carbonate. This mixture was diluted up to 7 mL with distilled water. Then the resulting solutions were allowed to stand at room temperature for 2 hrs before the absorbance was measured spectrophotometrically at 760 nm. Calibration curves were composed using standard solutions of Gallic Acid Equivalent (GAE) mg/gm. Concentration of 20, 40, 60, 80, and 100 µg/mL of Gallic aid was prepared. The Folin-ciocalteu reagent is sensitive to reducing compounds including polyphenols. They produce a blue colour upon reaction. This blue colour was measuredspectrophotometrically **(Singh*et al.*, 2015).**

2.5.2 Spectrophotometric Quantification of Total Flavonoid Content: -

The flavonoid content was determined using Aluminium chloride method. 0.5 ml of extract solution was mixed with 2 ml of distilled water. Then, 0.15 ml of sodium nitrite (5%) was added and mixed properly. After that, wait for 6 minutes before adding 0.15 ml Aluminium chloride (10 %) and allowed to stand for 6 minutes. Then, 2 ml of 4 % sodium hydroxide was added. The mixture was shaken and mixed thoroughly. Absorbance of mixture was estimated at 510 nm using UV spectrophotometer. Calibration curves were composed using standard solutions of Rutin Equivalent (GAE) mg/gm. Concentration of 20, 40, 60, 80, and 100 µg/mL of Rutin was prepared. Total flavonoid content was determined from the calibration curve and results were indicated as mg Rutin equivalent per gram dry extract weight **(Parthasarathy *et al.*, 2015).**

2.6 Activity (*In-vitro* Anti-oxidant Activity)

2.6.1 Superoxide anion radical scavenging activity

1 ml of nitroblue tetrazolium (NBT) (100 µl of NBT in 100 mM phosphate buffer, pH 7.4), 1 ml of NADH (468 µl in 100 mM phosphate buffer, pH 7.4), solution as well as varying volumes of extracts (*sample*) (20, 40, 60, 80 and 100 µg/ml), were mixed well with methanol. The reaction was started by the addition of 1 ml of phenazine methosulfate (PMS) (60 µl/100 mM phosphate buffer, pH 7.4). The reaction mixture was incubated at 30°C for 15 min. The absorbance was measured at 560 nm in a spectrophotometer. Incubation without the sample (extract) was used as a blank sample. Ascorbic acid was used as the standard in comparing the different sample. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity **(Nishikimi*et al.*, 1972).**The percentage scavenging was calculated by using the formula shown below:

% Inhibition = [(Ab of control- Ab of sample/ Ab of control x 100]

2.6.2 Hydrogen peroxide scavenging activity

The ability of the extract to scavenge hydrogen peroxide (H_2O_2) was determined according to the method of Ruch et al. **(Ruch *et al.*, 1989).** Aliquot of 0.1 mL of extracts of *Ipomoea cairica*plant (25–400 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:

% Inhibition = [(Ab of control- Ab of sample/ Ab of control x 100]

3. RESULTS

3.1 Plant Collection

Table 1 Plant collection

S. No.	Plant name	Plant part used	Weight
1.	*Ipomoea cairica*	Whole plant	235 gm

3.2 Percentage yield

Table 2 Percentage yield of *Ipomoea cairica*

S. No.	Solvent	Yield in gms	% Yield
1.	Pet. Ether	0.55	0.23
2.	Ethyl acetate	6.38	2.71
3.	Ethanol	47.34	20.14

3.3 Qualitative Phytochemical Analysis of *Ipomoea cairica* extracts

Table 3 Phytochemical analysis of *Ipomoea cairica* Extract

S. No.	Experiment	Result	
		Ethyl acetate	Methanol
Test for Carbohydrates			
1.	Molisch's Test	-	-
2.	Fehling's Test	-	-
3.	Benedict's Test	+	+
Test for Alkaloids			
1.	Mayer's Test	-	-
2.	Hager's Test	+	-
3.	Wagner's Test	+	-

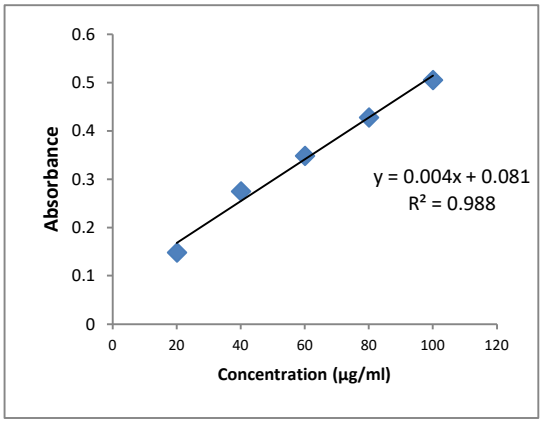
Test for Terpenoids			
1.	Salkowski Test	-	+
Test for Flavonoids			
1.	Lead Acetate Test	-	+
2.	Alkaline Reagent Test	-	+
Test for Tannins and Phenolic Compounds			
1.	$FeCl_3$ Test	+	-
2.	Lead Acetate Test	-	-
3.	Gelatine Test	-	+
Test for Saponins			
1.	Froth Test	-	+
Test for Protein and Amino acids			
1.	Ninhydrin Test	-	+
2.	Biuret's Test	+	+
Test for Glycosides			
1.	Keller Killani Test	+	-
2.	Borntrager's Test	-	+

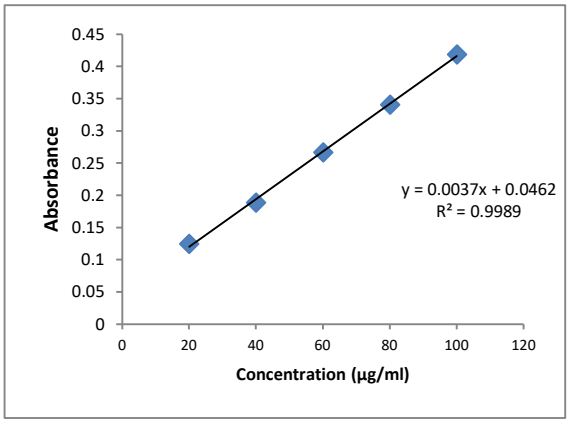
3.4 Quantitative Phytochemical analysis

3.4.1. Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) Estimation

Table 4 Standard table for Gallic acid and Rutin

Concentration (µg/ml)	Absorbance (Gallic acid)	Absorbance (Rutin)
20	0.149	0.125
40	0.275	0.189
60	0.349	0.267
80	0.428	0.341
100	0.506	0.419





Graph 1 Graphs represent standard curve of Gallic acid and Rutin

Table 5TPC and TFC in extracts

Extracts	**Total phenolic content (mg/gm equivalent to Gallic acid)**		**Total flavonoid content (mg/gm equivalent to rutin)**	
	Ethyl acetate extract	**Methanolic extract**	**Ethyl acetate extract**	**Methanolic extract**
Absorbance Mean±SD	0.850	1.507	0.373	0.986
TPC/TFC	192.25	356.5	209	313.33

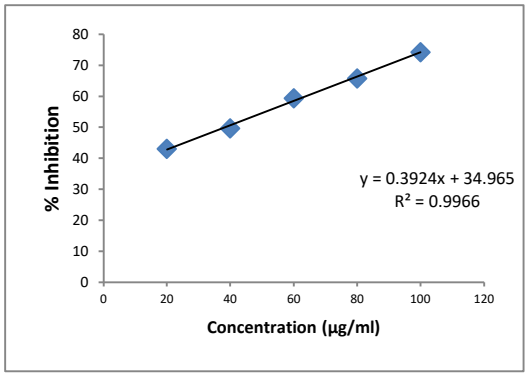
3.5*In-vitro* Anti-oxidant Activity

In the present investigation, the in vitro anti-oxidant activity of extracts of *Ipomoea cairica*whole plant was evaluated by SOS activity and H2O2 activity. The results are summarized in Tables.

3.5.1 H_2O_2 radical scavenging activity

Table 6 H_2O_2 radical scavenging activity of Ascorbic acid

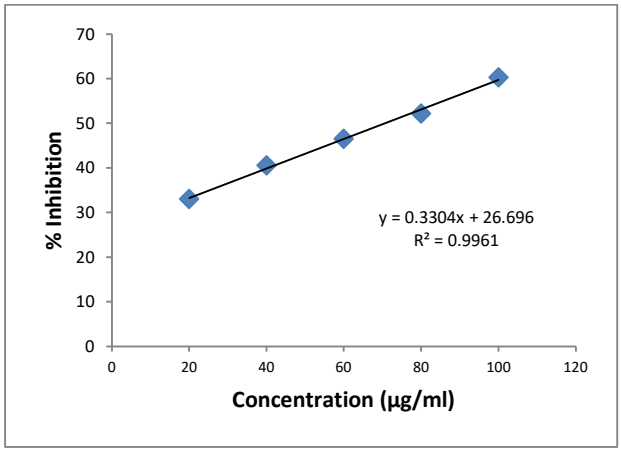
Concentration (µg/ml)	**Absorbance**	**% Inhibition**
20	0.516	43.544
40	0.443	51.531
60	0.375	58.971
80	0.317	65.317
100	0.243	73.413
Control	**0.914**	
IC50		**36.811**



Graph 2 Graph represents the Percentage Inhibition *Vs* Concentration of ascorbic acid

Table 7 H_2O_2 radical scavenging activity of Ethyl acetate extract

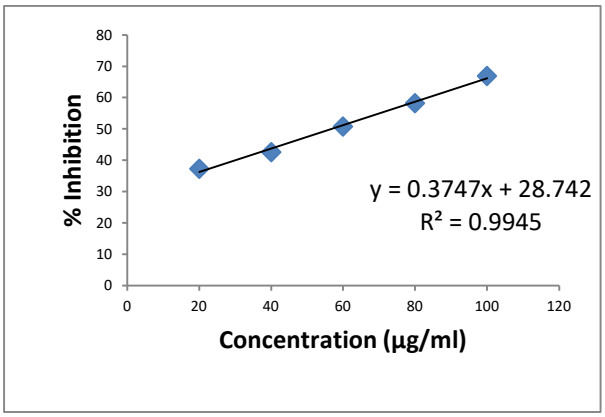
Concentration (µg/ml)	Absorbance	% Inhibition
20	0.612	33.041
40	0.543	40.590
60	0.489	46.498
80	0.437	52.188
100	0.363	60.284
Control	**0.914**	
IC50	**70.636**	



Graph 3 Graph represents the Percentage Inhibition *Vs* Concentration of Ethyl acetate extract

Table 8 H_2O_2 radical scavenging activity of Methanolic extract

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.573	37.308
40	0.524	42.669
60	0.449	50.875
80	0.381	58.315
100	0.302	66.958
Control	**0.914**	
IC50	**56.844**	

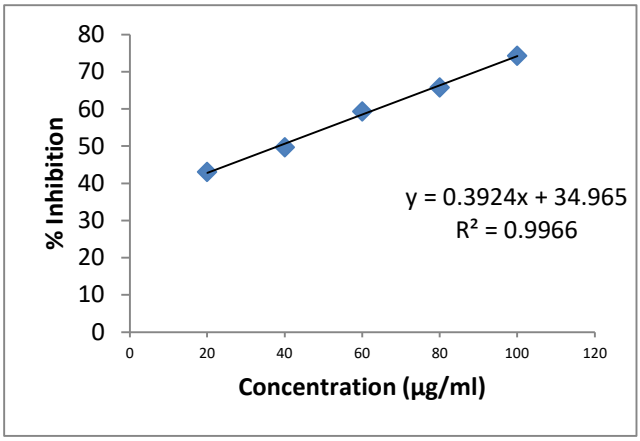


Graph 4 Graph represents the Percentage Inhibition *Vs* Concentration of Methanolic extract

3.5.2 SOS radical scavenging activity

Table 9 SOS radical scavenging activity of Ascorbic acid extract

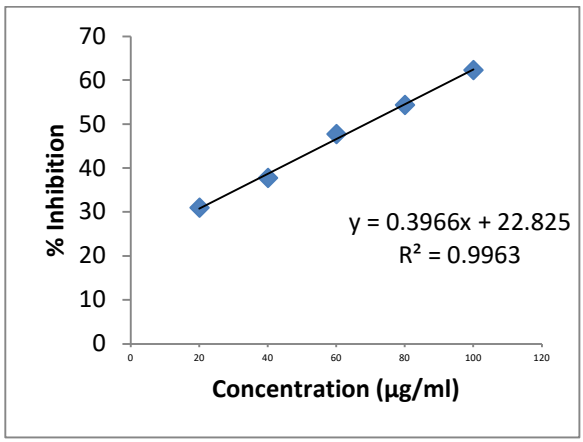
Concentration (µg/ml)	Absorbance	% Inhibition
20	0.481	43.144
40	0.425	49.763
60	0.343	59.456
80	0.289	65.839
100	0.217	74.349
Control	0.846	
IC50	38.367	



Graph 5 Graph represents the Percentage Inhibition *Vs* Concentration of Ascorbic acid extract

Table 10 SOS radical scavenging activity of Ethyl acetate extract

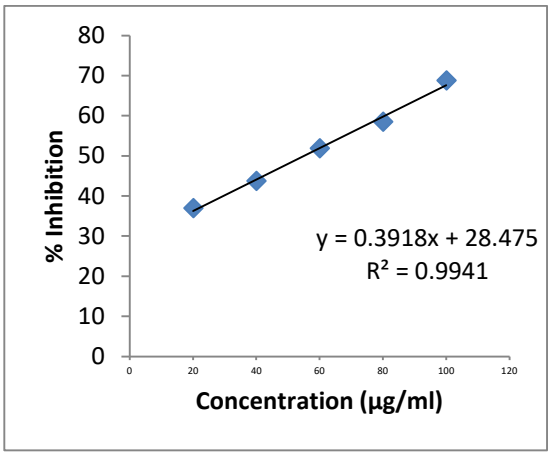
Concentration (µg/ml)	Absorbance	% Inhibition
20	0.584	30.969
40	0.527	37.706
60	0.442	47.754
80	0.386	54.373
100	0.319	62.293
Control	**0.846**	
IC50	**68.636**	



Graph 6 Graph represents the Percentage Inhibition *Vs* Concentration of Ethyl acetate extract

Table 11 SOS radical scavenging activity of Methanolic extract

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.533	36.997
40	0.476	43.735
60	0.407	51.891
80	0.351	58.510
100	0.264	68.794
Control	**0.846**	
IC50	**55.064**	



Graph 7 Graph represents the Percentage Inhibition *Vs* Concentration of Methanolic extract

4. DISCUSSION

The preliminary phytochemical screening of *Ipomoea cairica* extract indicated the existence of bioactive components, including carbohydrates, Alkaloids, Protein, glycosides, phenols, and tannins in the ethyl acetate extract. Additionally, the methanolic extract exhibited the presence of carbohydrates, Terpenoids, flavonoids, Saponins, phenols, tannins, and glycosides **(Table 3).**

The Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) were determined using standard curves derived from absorbance values of Gallic acid and Rutin at different concentrations. The TPC and TFC values in the ethyl acetate and methanolic extracts were quantified. The ethyl acetate extract had an absorbance mean of 0.850 for TPC, equivalent to 192.25 mg/g Gallic acid, and an absorbance mean of 0.373 for TFC, equivalent to 209 mg/g Rutin. The methanolic extract exhibited a higher absorbance mean of 1.507 for TPC, corresponding to 356.5 mg/g Gallic acid, and an absorbance mean of 0.986 for TFC, corresponding to 313.33 mg/g Rutin. These results indicate that the methanolic extract contains higher levels of both phenolic and flavonoid compounds compared to the ethyl acetate extract **(Table no 5, Graph no 1).**

H2O2 radical scavenging activity of Ethyl acetate extract and Methanolic extract of plant demonstrated dose-

dependent scavenging activity against H2O2 radicals. Ethyl acetate extract showed percentage inhibition ranging from 33.041% to 60.284% across concentrations, with an IC50 value of 70.636 µg/ml. Methanolic extract displayed inhibition percentages from 37.308% to 66.958%, with an IC50 value of 56.844 µg/ml.In contrast, the methanolic extract exhibits a slightly higher radical scavenging activity at lower concentrations. Ascorbic acid was used as a reference compound which exhibited percentage inhibition, with values ranging from 43.544% at 20 µg/ml to 73.413% at 100 µg/ml. The IC50 value for ascorbic acid was calculated to be 36.811 µg/ml.

The comparative study of SOS radical scavenging activity for both Ethyl acetate and Methanolic extracts reveals distinct differences in their antioxidant capabilities, as measured by % inhibition across various concentrations. For the Ethyl acetate extract, as the concentration increased from 20 µg/ml to 100 µg/ml, the % inhibition progressively rose from 30.969% to 62.293%, with the absorbance correspondingly decreasing from 0.584 to 0.319. The IC50 value, which is the concentration at which 50% inhibition is achieved, was determined to be 68.636 µg/ml. In contrast, the Methanolic extract demonstrated a more potent antioxidant activity. At the same concentrations, the % inhibition ranged from 36.997% to 68.794%, with a decrease in absorbance from 0.533 to 0.264. The Methanolic extract exhibited a lower IC50 value of 55.064 µg/ml, indicating higher efficacy at lower concentrations compared to the Ethyl acetate extract. Ascorbic acid was used as a reference compound which exhibited an IC50 value of 38.367 µg/ml. The percentage inhibition ranged from 43.144% at 20 µg/ml to 74.349% at 100 µg/ml percentage. Overall, these findings underscore the antioxidant potential of the Ascorbic acid, Ethyl acetate, and Methanolic extracts, with varying degrees of efficacy in scavenging SOS radicals and H2O2 radical scavenging activity.The reducing capacity of a compound indicates its potential antioxidant activity. Comparing it to dietary antioxidants like ascorbic acid, compounds with reducing power act as electron donors, reducing oxidized intermediates in lipid per oxidation processes, acting as primary and secondary antioxidants.

5. CONCLUSION

The study of antioxidant properties in different extracts of *Ipomoea cairica* has provided valuable insights into its potential health benefits. The methanol extract showed the highest antioxidant activity due to its ability to solubilise a broad spectrum of bioactive compounds. Ethyl acetate extract also showed considerable antioxidant properties, though to a lesser extent. The findings emphasize the importance of solvent selection in maximizing the extraction of antioxidant compounds from *Ipomoea cairica*. The results suggest that *Ipomoea cairica* holds promise as a natural source of antioxidants, potentially used in therapeutic agents and functional foods. Further research is needed to isolate and characterize the specific compounds responsible for these antioxidant activities and explore their mechanisms of action in biological systems. This study contributes to the growing body of knowledge on the medicinal potential of *Ipomoea cairica* and can inform future studies and applications aimed at combating oxidative stress-related diseases and promoting overall health.

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