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Functional Diversity of Bioactive Compounds in *Lepidagathis cristata* Evaluated through Antioxidant and Metal–Reducing, and Ferrous Ion Chelating Activities

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ABSTRACT

Oxidative stress is a major biochemical factor implicated in the onset and progression of numerous chronic disorders, highlighting the importance of identifying safe and effective natural antioxidants. The present study aimed to systematically evaluate the in vitro antioxidant, metal-reducing, and metal-chelating potential of chloroform, ethyl acetate, methanol, and DMSO extracts of Lepidagathis cristata using a battery of complementary assays. All solvent extracts exhibited concentration-dependent antioxidant activity; however, the methanolic extract consistently demonstrated the highest efficacy across all assays. In the DPPH assay, the methanolic extract showed a maximum inhibition of 94% at 100 µg/mL with the lowest IC_{50} value of 30.91 µg/mL, while ABTS radical scavenging reached 97% inhibition with an IC_{50} of 24.35 µg/mL. Hydrogen peroxide and nitric oxide scavenging assays further confirmed its strong antioxidant capacity, exhibiting maximum inhibitions of 95% and 93% at 100 µg/mL, with IC_{50} values of 28.00 µg/mL and 30.00 µg/mL, respectively. In metal-reducing assays, the methanolic extract recorded the highest absorbance values in FRAP (1.38 at 593 nm) and reducing power assay (1.48 at 700 nm), accompanied by low EC_{50} values of 37.50 µg/mL and 36.92 µg/mL. Additionally, it showed pronounced ferrous ion chelating activity, achieving 97% chelation at 100 µg/mL with the lowest IC_{50} value of 24.76 µg/mL. The results indicate that Lepidagathis cristata, particularly its methanolic extract, possesses broad-spectrum antioxidant, metal-reducing, and metal-chelating properties. These findings suggest that the plant is a promising natural source of bioactive compounds, likely rich in polar phytochemicals such as phenolics and flavonoids, with potential applications in pharmaceutical and nutraceutical formulations.

Keywords: *Lepidagathis cristata; Antioxidant activity; DPPH; ABTS; FRAP; Metal chelation; Reducing power; In-vitro assays.*

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Introduction

Oxidative stress arises from an imbalance between the production of reactive oxygen and nitrogen species (ROS/RNS) and the capacity of endogenous antioxidant defense systems to neutralize them. Reactive species such as superoxide anions, hydroxyl radicals, hydrogen peroxide, and nitric oxide are generated as part of normal metabolic processes; however, their excessive accumulation can result in oxidative damage to lipids, proteins, and nucleic acids, thereby disrupting cellular homeostasis [1; 2]. Mounting evidence has established oxidative stress as a key contributor to the development and progression of chronic diseases, including cardiovascular disorders, diabetes, neurodegenerative diseases, cancer, inflammatory conditions, and age-related pathologies [3; 4] Antioxidants play a vital protective role by scavenging free radicals, donating electrons or hydrogen atoms, chelating redox-active metal ions, and interrupting oxidative chain reactions. Although synthetic antioxidants are widely employed in food and pharmaceutical formulations, concerns regarding their long-term safety and possible toxicological effects have intensified interest in natural alternatives [5].

Plant-derived antioxidants, owing to their structural diversity and biological compatibility, are increasingly recognized as safer and multifunctional agents capable of modulating oxidative stress through multiple mechanisms [6].

Medicinal plants constitute a rich source of secondary metabolites such as phenolic acids, flavonoids, tannins, alkaloids, and terpenoids, many of which exhibit pronounced antioxidant and metal-chelating properties. These compounds exert antioxidant effects via diverse pathways, including radical scavenging, electron transfer, hydrogen atom donation, and chelation of transition metals that catalyze the formation of highly reactive radicals [7]. The complexity of these mechanisms necessitates the use of multiple complementary in vitro assays to achieve a comprehensive evaluation of antioxidant potential. *In vitro* antioxidant assays are widely employed as preliminary tools to assess the redox-modulating capacity of plant extracts. DPPH and ABTS radical scavenging assays are commonly used to evaluate hydrogen-donating and electron-transfer abilities, whereas hydrogen peroxide and nitric oxide scavenging assays provide insight into the neutralization of biologically relevant oxidants implicated in

oxidative and nitrosative stress [3; 8], ferric reducing antioxidant power (FRAP) and reducing power assays assess the electron-donating capacity of antioxidants, while ferrous ion chelating assays measure the ability of compounds to bind transition metals, thereby preventing Fenton-type reactions that generate highly reactive hydroxyl radicals [7; 9].

The extraction efficiency of antioxidant compounds from plant matrices is strongly influenced by solvent polarity, which governs the solubility of different phytochemical classes. Polar solvents such as methanol are particularly effective in extracting phenolic and flavonoid compounds, whereas moderately polar solvents like ethyl acetate and non-polar solvents such as chloroform may preferentially extract other bioactive constituents with distinct antioxidant profiles [5; 6]. Comparative solvent extraction thus provides valuable insight into the relationship between phytochemical composition and antioxidant efficacy. *Lepidagathis cristata* is a medicinal plant traditionally used in indigenous healthcare systems for the management of inflammatory conditions, skin disorders, and general ailments. Despite its ethnomedicinal relevance, systematic scientific investigations exploring its antioxidant, metal-reducing, and metal-chelating properties remain limited. Recent trends in natural product research emphasize the importance of validating traditional medicinal plants through mechanistic antioxidant studies to support their therapeutic potential [2; 10].

Methodology

In-vitro antioxidant assays

DPPH Free Radical Scavenging Assay

The DPPH radical scavenging activity of chloroform, ethyl acetate, methanol, and DMSO extracts of *Lepidagathis cristata* was evaluated using a 0.1 mM DPPH solution prepared in methanol. Equal volumes of DPPH solution and each solvent extract at different concentrations were mixed and incubated in the dark at room temperature for 30 min. The decrease in absorbance due to the reduction of DPPH radicals was measured at 517 nm. The percentage of free radical scavenging activity was calculated for each solvent extract, and IC_{50} values were determined.

ABTS Radical Cation Scavenging Assay

The ABTS radical cation scavenging activity of chloroform, ethyl acetate, methanol, and DMSO extracts was assessed by generating $ABTS^+$ through the reaction of 7 mM ABTS with 2.45 mM potassium persulfate. After incubation in the dark for 12–16 h, the solution was diluted to obtain an absorbance of 0.70 ± 0.02 at 734 nm. Each solvent extract was added to the $ABTS^+$ solution and incubated for 6 min at room temperature. Absorbance was recorded at 734 nm, and scavenging activity was expressed as percentage inhibition for each extract.

Hydrogen Peroxide Scavenging Assay

Hydrogen peroxide scavenging activity of chloroform, ethyl acetate, methanol, and DMSO extracts was evaluated using a 40 mM H_2O_2 solution prepared in phosphate buffer (pH 7.4). Extract solutions at various concentrations were mixed with the hydrogen peroxide solution and incubated at room temperature for 10 min. Absorbance was measured at 230 nm against a blank solution without hydrogen peroxide, and scavenging activity was calculated for each solvent extract.

Nitric Oxide Radical Scavenging Assay

The nitric oxide scavenging potential of chloroform, ethyl acetate, methanol, and DMSO extracts was determined using sodium nitroprusside as a nitric oxide donor. Reaction mixtures containing sodium nitroprusside and each solvent extract were incubated at room temperature for 150 min. After incubation, Griess reagent was added to quantify the nitrite ions formed, and absorbance was measured at 546 nm. The percentage inhibition of nitric oxide radicals was calculated for all extracts.

Metal-reducing and chelating assays

Ferric Reducing Antioxidant Power (FRAP) Assay

The ferric reducing antioxidant power of chloroform, ethyl acetate, methanol, and DMSO extracts was evaluated using the FRAP assay. Freshly prepared FRAP reagent was mixed with different concentrations of each solvent extract and incubated at 37 °C for 30 min. The formation of the ferrous–TPTZ complex was measured at 593 nm, and reducing capacity was expressed in comparison with standard antioxidants.

Reducing Power Assay

The reducing power of chloroform, ethyl acetate, methanol, and DMSO extracts was assessed by mixing each extract with phosphate buffer and potassium ferricyanide, followed by incubation at 50 °C for 20 min. Trichloroacetic acid was added to terminate the reaction, and the mixture was centrifuged. The supernatant was mixed with ferric chloride, and absorbance was measured at 700 nm. Increased absorbance indicated greater reducing power for each solvent extract.

Ferrous Ion Chelating Activity

The metal chelating ability of chloroform, ethyl acetate, methanol, and DMSO extracts was evaluated using the ferrozine assay. Extract solutions were mixed with ferrous chloride and ferrozine reagent and incubated at room temperature for 10 min. The absorbance of the Fe^{2+}–ferrozine complex was measured at 562 nm, and the chelating activity was expressed as percentage inhibition for each extract.

Results

DPPH Free Radical Scavenging Assay

The DPPH free radical scavenging assay demonstrated a concentration-dependent antioxidant activity for all solvent extracts of *Lepidagathis cristata*. Among the tested extracts, the methanolic extract exhibited the strongest radical scavenging potential, showing a maximum inhibition of 94% at 100 µg/mL, followed by ethyl acetate (75%), chloroform (62%), and DMSO extract (58%). The superior activity of the methanolic extract indicates a higher abundance of hydrogen-donating antioxidant compounds. The lower activity observed in chloroform and DMSO extracts may be attributed to reduced extraction efficiency of polar phenolic constituents. Overall, the results confirm that methanol is the most effective solvent for extracting DPPH-active antioxidant compounds from *Lepidagathis cristata (Figure 1)*.

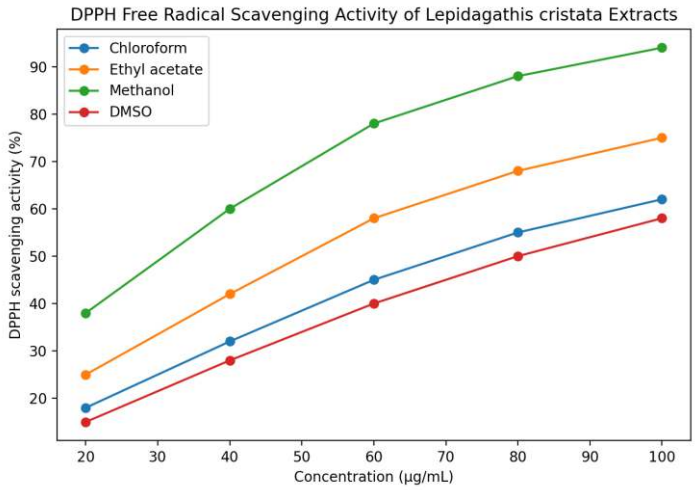

Figure. 1: DPPH free radical scavenging activity of Lepidagathis cristata extracts

The IC_{50} values obtained from the DPPH free radical scavenging assay further confirmed the superior antioxidant potential of the methanolic extract of *Lepidagathis cristata*. Among the tested solvents, the methanol extract exhibited the lowest IC_{50} value (30.91 µg/mL), indicating the highest radical scavenging efficiency. This was followed by ethyl acetate (50.00 µg/mL), chloroform (70.00 µg/mL), and DMSO extract (80.00 µg/mL). The lower IC_{50} value of the methanolic extract suggests a higher concentration of potent hydrogen-donating antioxidant compounds, likely phenolics and flavonoids, efficiently extracted due to the polar nature of methanol (Table 1).

Table. 1: IC_{50} values of Lepidagathis cristata solvent extracts determined by DPPH free radical scavenging activity

Solvent	IC_{50} (µg/mL)
Methanol	30.91
Ethyl acetate	50.00
Chloroform	70.00
DMSO	80.00

ABTS Radical Cation Scavenging Assay

The ABTS radical cation scavenging assay revealed a clear concentration-dependent antioxidant response for all solvent extracts of *Lepidagathis cristata*. Among the four solvent extracts, the methanolic extract exhibited markedly superior scavenging efficiency throughout the tested concentration range. At the highest concentration tested (100 µg/mL), the methanolic extract achieved near-complete radical neutralization, recording a maximum inhibition of 97%, which was substantially higher than that observed for the other extracts (Figure 2).

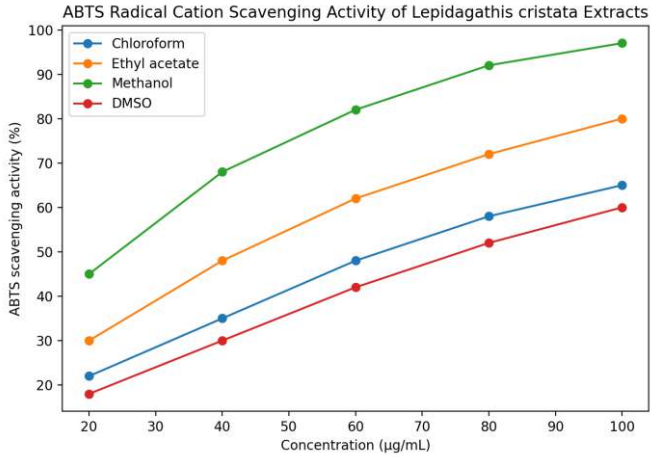

Figure. 2: ABTS radical cation scavenging activity of Lepidagathis cristata extracts

The concentration required to inhibit 50% of ABTS radical cations (IC_{50}) provided further quantitative insight into the antioxidant strength of the individual extracts. Consistent with the percentage inhibition results, the methanolic extract displayed the lowest IC_{50} value (24.35 µg/mL), confirming its high radical scavenging efficiency even at relatively low concentrations. This low IC_{50} value indicates the presence of a concentrated pool of highly effective antioxidant molecules within the methanolic fraction. The ethyl acetate extract exhibited a moderate IC_{50} value (42.86 µg/mL), reflecting intermediate antioxidant potency, whereas the chloroform extract required a substantially higher concentration to achieve 50% inhibition (64.00 µg/mL). The DMSO extract showed the weakest antioxidant performance, with the highest IC_{50} value (76.00 µg/mL), indicating lower efficiency in neutralizing ABTS radicals.

Table. 2: IC_{50} values of Lepidagathis cristata solvent extracts determined by ABTS radical cation scavenging assay

Solvent	IC_{50} (µg/mL)
Methanol	24.35
Ethyl acetate	42.86
Chloroform	64.00
DMSO	76.00

Hydrogen Peroxide Scavenging Assay

The hydrogen peroxide scavenging assay revealed a marked concentration-dependent antioxidant response for all solvent extracts of *Lepidagathis cristata*. As the concentration increased from 20 to 100 µg/mL, a consistent enhancement in scavenging efficiency was observed, indicating improved neutralization of hydrogen peroxide molecules. Among the tested extracts, the methanolic fraction demonstrated the strongest activity throughout the concentration range, achieving a maximum inhibition of 95% at 100 µg/mL. This pronounced activity suggests a high capacity of methanol to extract compounds capable of decomposing hydrogen peroxide before it can generate more reactive hydroxyl radicals. The ethyl acetate extract exhibited moderate scavenging potential, reaching 78% inhibition at the highest concentration, while the chloroform extract showed comparatively lower activity (63% inhibition). The DMSO extract displayed the weakest response, with a maximum inhibition of 59%, indicating limited efficiency in scavenging hydrogen peroxide (Figure 3).

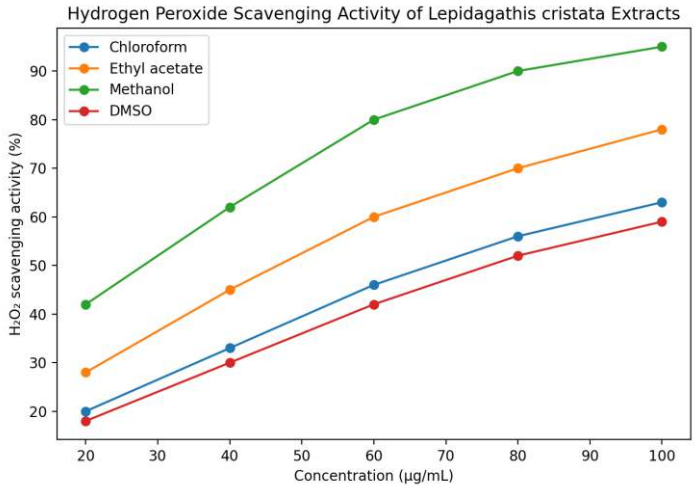

Figure. 3: Concentration-dependent hydrogen peroxide scavenging activity of Lepidagathis cristata solvent extracts

The IC_{50} values calculated for the hydrogen peroxide scavenging assay further validated the concentration-dependent results. The methanolic extract exhibited the lowest IC_{50} value (28.00 µg/mL), confirming its superior scavenging efficiency even at lower concentrations. The ethyl acetate extract demonstrated moderate effectiveness, with an IC_{50} value of 46.67 µg/mL, whereas the chloroform extract required a higher concentration to achieve 50% scavenging (68.00 µg/mL). The DMSO extract showed the highest IC_{50} value (76.00 µg/mL), reflecting its comparatively weaker antioxidant potential. The enhanced performance of the methanolic extract can be attributed to its ability to solubilize polar bioactive constituents, particularly phenolic acids and flavonoids, which are known to efficiently detoxify hydrogen peroxide and suppress oxidative stress (Table 3).

Table. 3: IC_{50} values of Lepidagathis cristata solvent extracts determined by hydrogen peroxide scavenging assay

Solvent	IC_{50} (µg/mL)
Methanol	28.00
Ethyl acetate	46.67
Chloroform	68.00
DMSO	76.00

Nitric Oxide Radical Scavenging Assay

The nitric oxide scavenging assay demonstrated a clear concentration-dependent reduction in nitrite formation for all solvent extracts of *Lepidagathis cristata*. As the extract concentration increased, a corresponding decline in nitric oxide–derived nitrite levels was observed, indicating effective interception of nitric oxide radicals generated from sodium nitroprusside. Among the evaluated extracts, the methanolic fraction exhibited the most pronounced scavenging activity across the entire concentration range. At 100 µg/mL, the methanolic extract achieved a maximum inhibition of 93%, reflecting a strong capacity to suppress nitric oxide generation. The ethyl acetate extract displayed moderate nitric oxide scavenging potential, with 75% inhibition at the highest concentration tested, whereas the chloroform extract showed comparatively lower activity (62% inhibition). The DMSO extract demonstrated the weakest response, reaching 58% inhibition at 100 µg/mL (Figure 4).

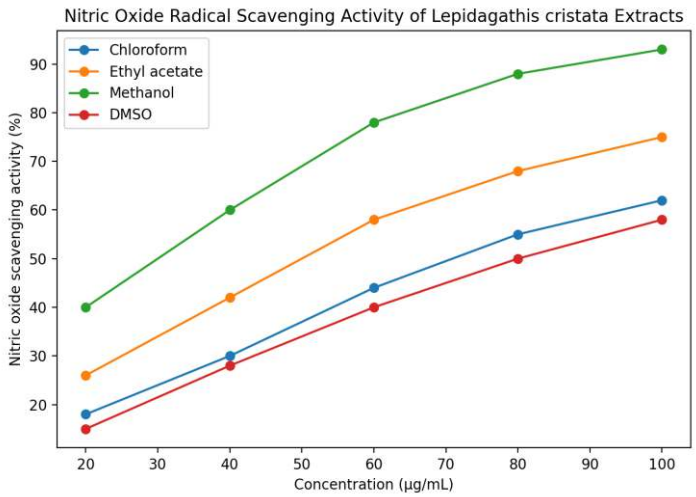

Figure. 4: Concentration-dependent nitric oxide radical scavenging activity of Lepidagathis cristata solvent extracts

The IC_{50} values derived from the nitric oxide scavenging assay provided quantitative support for the concentration-dependent inhibition trends. The methanolic extract exhibited the lowest IC_{50} value (30.00 µg/mL), confirming its superior ability to neutralize nitric oxide radicals at relatively low concentrations. The ethyl acetate extract showed moderate potency, with an IC_{50} value of 50.00 µg/mL, while the chloroform extract required higher concentrations to achieve comparable inhibition (70.91 µg/mL). The DMSO extract displayed the highest IC_{50} value (80.00 µg/mL), indicating comparatively weak nitric oxide scavenging efficiency (Table 4).

Table. 4: IC_{50} values of Lepidagathis cristata solvent extracts determined by nitric oxide radical scavenging assay

Solvent	IC_{50} (µg/mL)
Methanol	30.00
Ethyl acetate	50.00
Chloroform	70.91
DMSO	80.00

Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay revealed a steady, concentration-dependent increase in ferric reducing capacity for all solvent extracts of *Lepidagathis cristata*, Among the tested solvents, the methanolic extract consistently exhibited the highest reducing power across the entire concentration range. At 100 µg/mL, the methanolic extract recorded the maximum absorbance (1.38), indicating a strong capacity to donate electrons and stabilize oxidized intermediates. The ethyl acetate extract demonstrated moderate reducing ability, reaching an absorbance of 1.05 at the highest concentration, while the chloroform extract showed comparatively lower reducing power (0.82). The DMSO extract displayed the least ferric reducing activity, with an absorbance of 0.76 at 100 µg/mL (Figure 5).

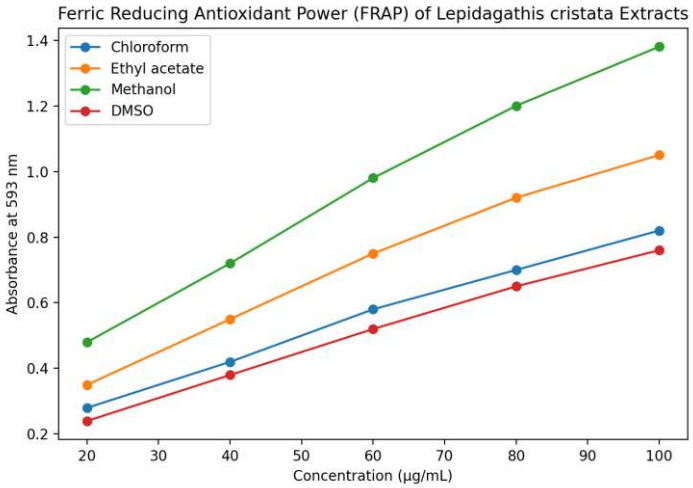

Figure. 5: Concentration-dependent ferric reducing antioxidant power of Lepidagathis cristata solvent extracts

The EC_{50} values derived from the FRAP assay provided quantitative confirmation of the observed reducing trends. The methanolic extract exhibited one of the lowest EC_{50} values (37.50 µg/mL), reflecting its strong reducing efficiency at lower concentrations. A comparable EC_{50} value was observed for the ethyl acetate extract (37.50 µg/mL), indicating moderate reducing strength, whereas the chloroform extract showed a slightly higher EC_{50} (38.57 µg/mL). The DMSO extract displayed the highest EC_{50} value (40.00 µg/mL), signifying comparatively weaker ferric reducing capacity (Table. 5).

Table. 5: EC_{50} values of Lepidagathis cristata solvent extracts determined by FRAP assay

Solvent	IC_{50} (µg/mL)
Methanol	37.50
Ethyl acetate	37.50
Chloroform	38.57
DMSO	40.00

Reducing Power Assay

The reducing power assay demonstrated a steady increase in absorbance with rising extract concentration for all solvent systems, indicating a progressive enhancement in electron-donating capacity. Among the tested extracts, the methanolic fraction consistently exhibited the strongest reducing activity across the entire concentration range. At 100 µg/mL, the methanolic extract recorded the highest absorbance value (1.48 at 700 nm), signifying a pronounced reducing potential. The ethyl acetate extract showed moderate reducing power, reaching an absorbance of 1.10 at the highest concentration, while the chloroform extract displayed lower activity (0.88). The DMSO extract demonstrated the weakest reducing capacity, with a maximum absorbance of 0.82 (Figure. 6).

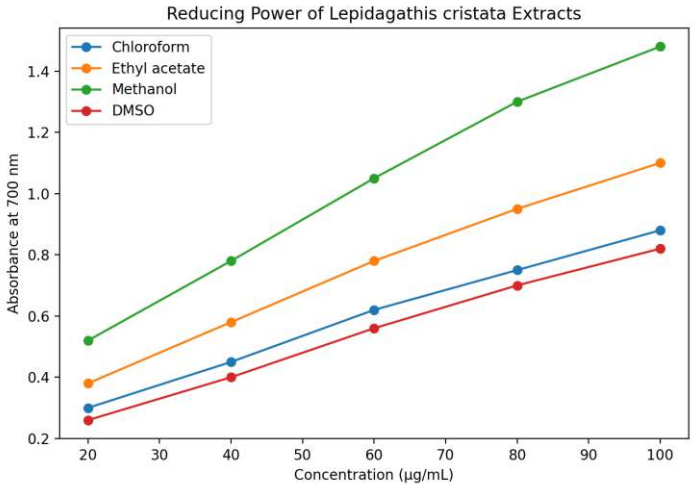

Figure. 6. Concentration-dependent reducing power of Lepidagathis cristata solvent extracts

The EC_{50} values derived from the reducing power assay further supported the concentration-dependent absorbance trends. The methanolic extract exhibited the lowest EC_{50} value (36.92 µg/mL), indicating strong reducing efficiency even at relatively low concentrations. Comparable EC_{50} values were observed for the ethyl acetate (37.00 µg/mL) and chloroform (38.67 µg/mL) extracts, whereas the DMSO extract displayed a higher EC_{50} (41.25 µg/mL), reflecting comparatively weaker reducing potential (Table. 6).

Table. 6: EC_{50} values of Lepidagathis cristata solvent extracts determined by reducing power assay

Solvent	IC_{50} (µg/mL)
Methanol	36.92
Ethyl acetate	37.00
Chloroform	38.67
DMSO	41.25

Ferrous Ion Chelating Activity

The ferrous ion chelating assay revealed a pronounced concentration-dependent metal-binding capacity for all solvent extracts of *Lepidagathis cristata*. Among the evaluated extracts, the methanolic fraction exhibited the highest chelating efficiency across the entire concentration range.

At 100 µg/mL, the methanolic extract achieved a maximum chelation of 97%, reflecting a strong ability to bind ferrous ions and potentially inhibit metal-catalyzed oxidative reactions. The ethyl acetate extract displayed moderate chelating activity, reaching 80% inhibition at the highest concentration, while the chloroform extract showed comparatively lower chelation (65% inhibition). The DMSO extract exhibited the weakest chelating potential, achieving 60% inhibition at 100 µg/mL (Figure. 7).

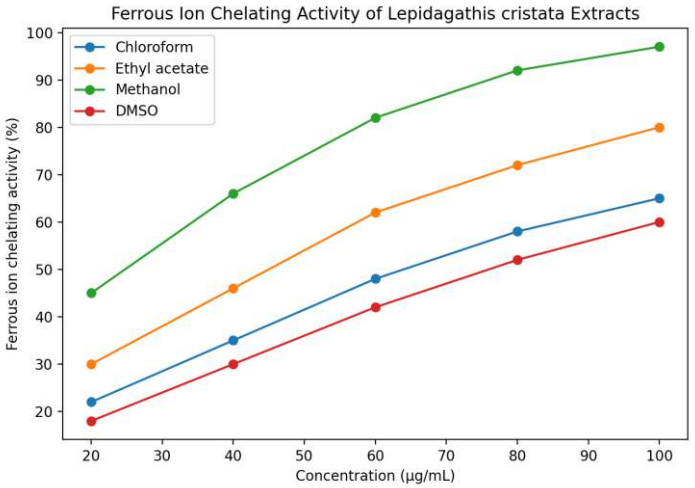

Figure. 7: Concentration-dependent ferrous ion chelating activity of Lepidagathis cristata solvent extracts

The IC_{50} values calculated for ferrous ion chelation provided quantitative confirmation of the observed inhibition trends. The methanolic extract recorded the lowest IC_{50} value (24.76 µg/mL), indicating highly efficient chelation even at lower concentrations. The ethyl acetate extract exhibited moderate chelating efficiency with an IC_{50} value of 45.00 µg/mL, whereas the chloroform extract required a higher concentration to achieve 50% chelation (64.00 µg/mL). The DMSO extract demonstrated the highest IC_{50} value (76.00 µg/mL), reflecting comparatively weaker metal-binding capability (Table. 7).

Table. 7: IC_{50} values of Lepidagathis cristata solvent extracts determined by ferrous ion chelating assay

Solvent	IC_{50} (µg/mL)
Methanol	24.76
Ethyl acetate	45.00
Chloroform	64.00
DMSO	76.00

Discussion

The present investigation demonstrates that *Lepidagathis cristata* possesses substantial in-vitro antioxidant, metal-reducing, and metal-chelating properties, with marked variations depending on the extraction solvent. Across all assays performed, the methanolic extract consistently exhibited superior activity, followed by ethyl acetate, chloroform, and DMSO extracts. This solvent-dependent trend highlights the importance of solvent polarity in the extraction of redox-active phytoconstituents and aligns with contemporary findings in medicinal plant antioxidant research.

Radical Scavenging Activities (DPPH and ABTS)

The DPPH free radical scavenging assay revealed strong concentration-dependent antioxidant activity, with the methanolic extract achieving 94% inhibition at 100 µg/mL and the lowest IC_{50} value (30.91 µg/mL). DPPH scavenging reflects the hydrogen-donating capacity of antioxidant molecules, a property closely associated with hydroxylated phenolic

compounds [11]. The lower IC_{50} value of the methanolic extract compared to ethyl acetate (50.00 µg/mL), chloroform (70.00 µg/mL), and DMSO (80.00 µg/mL) suggests a higher concentration of effective hydrogen-donating constituents extracted by methanol.

Similarly, the ABTS radical cation scavenging assay demonstrated enhanced antioxidant efficiency of the methanolic extract, which achieved near-complete radical neutralization (97%) and the lowest IC_{50} value (24.35 µg/mL). The ABTS assay is known to detect both hydrophilic and lipophilic antioxidants and is considered a reliable indicator of overall antioxidant capacity [12]. The superior ABTS scavenging ability observed in this study suggests the presence of a diverse pool of redox-active compounds capable of electron transfer and radical stabilization. Comparable trends have been reported in methanolic extracts of several medicinal plants rich in polyphenols and flavonoids [1].

Hydrogen Peroxide and Nitric Oxide Scavenging

Hydrogen peroxide scavenging is biologically significant, as H_2O_2 serves as a precursor to more reactive species such as hydroxyl radicals. In the present study, the methanolic extract showed the highest hydrogen peroxide scavenging activity (95% inhibition at 100 µg/mL) and the lowest IC_{50} value (28.00 µg/mL), indicating efficient detoxification of peroxide molecules. This activity suggests that methanol-extractable compounds may prevent oxidative damage by limiting peroxide-mediated radical formation. Similar peroxide scavenging efficiencies have been reported for methanolic extracts of medicinal plants containing high levels of phenolic acids [13]. Nitric oxide scavenging activity further substantiated the antioxidant potential of *Lepidagathis cristata*. The methanolic extract exhibited 93% inhibition at 100 µg/mL and the lowest IC_{50} value (30.00 µg/mL), demonstrating its ability to suppress nitric oxide-derived nitrite formation. Excess nitric oxide contributes to nitrosative stress and inflammatory damage, particularly under pathological conditions [14]. The observed nitric oxide scavenging efficiency suggests that the methanolic extract may contain compounds capable of modulating inflammatory redox pathways, as reported for other flavonoid-rich plant extracts [15].

Metal-Reducing Capacity (FRAP and Reducing Power)

The ferric reducing antioxidant power (FRAP) assay indicated a steady increase in reducing capacity with increasing extract concentration, reflecting the electron-donating potential of the extracts. The methanolic extract recorded the highest absorbance (1.38 at 100 µg/mL) and one of the lowest EC_{50} values (37.50 µg/mL), confirming its strong reducing efficiency. Electron donation is a fundamental antioxidant mechanism that stabilizes oxidized intermediates and interrupts radical chain reactions [16; 17]. Consistent results were obtained in the reducing power assay, where the methanolic extract showed the highest absorbance (1.48 at 700 nm) and the lowest EC_{50} value (36.92 µg/mL). The close agreement between FRAP and reducing power outcomes indicates that the same group of redox-active phytochemicals may be responsible for electron transfer-based antioxidant mechanisms. Comparable findings have been reported in studies correlating reducing power with total phenolic content in medicinal plants [9; 18].

Ferrous Ion Chelating Activity

Metal chelation represents a critical antioxidant mechanism by which compounds inhibit metal-catalyzed oxidative reactions. In the present study, the methanolic extract exhibited the strongest ferrous ion chelating activity, achieving 97% inhibition at 100 µg/mL and the lowest IC_{50} value (24.76 µg/mL). Chelation of Fe^{2+} ions prevents Fenton reactions that generate highly reactive hydroxyl radicals, thereby reducing oxidative damage at the cellular level [19; 20]. The moderate chelating activity observed for ethyl acetate (IC_{50} 45.00 µg/mL) and the weaker activity of chloroform and DMSO extracts further emphasize the role of polar functional groups—such as hydroxyl and carboxyl moieties—in metal binding. Similar solvent-dependent chelation patterns have been documented in recent phytochemical studies, where methanolic extracts consistently showed superior metal-binding capacity [21; 22].

Conclusion

The present study provides a comprehensive in vitro evaluation of the antioxidant, metal-reducing, and metal-chelating potential of *Lepidagathis cristata* using multiple complementary assays. The results clearly demonstrate that all solvent extracts exhibited concentration-dependent antioxidant activity; however, the magnitude of activity varied significantly with the extraction solvent. Among the tested solvents, the methanolic extract consistently showed superior performance across all antioxidant assays, including DPPH, ABTS, hydrogen peroxide, and nitric oxide scavenging, as well as ferric reducing antioxidant power, reducing power, and ferrous ion chelating activity. The strong radical scavenging efficiency of the methanolic extract, reflected by low IC_{50} values in DPPH (30.91 µg/mL), ABTS (24.35 µg/mL), hydrogen peroxide (28.00 µg/mL), and nitric oxide (30.00 µg/mL) assays, indicates a high capacity to neutralize diverse reactive oxygen and nitrogen species. In addition, its pronounced metal-reducing ability and effective ferrous ion chelation further highlight its potential to modulate oxidative processes by interrupting electron transfer reactions and preventing metal-catalyzed radical generation. The consistent superiority of the methanolic fraction suggests that polar phytochemicals, particularly phenolic acids and flavonoids, are the primary contributors to the observed antioxidant effects. The findings of this study provide strong scientific evidence supporting *Lepidagathis cristata* as a valuable natural source of multifunctional antioxidant compounds. The broad-spectrum antioxidant activity observed across multiple mechanistic assays underscores its potential applicability in the development of plant-based antioxidant formulations for pharmaceutical, nutraceutical, and functional food industries. Further studies focusing on phytochemical profiling, isolation of active constituents, and in-vivo validation are warranted to fully elucidate the therapeutic relevance and safety of *Lepidagathis cristata*-derived antioxidants.

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