

Comparative Evaluation of the Antioxidant Potential of *Dolichandrone falcata* Bark Extracts Using DPPH and FRAP Assay

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Abstract

The present study evaluated the antioxidant potential of *Dolichandrone falcata* bark extracts using DPPH radical scavenging and ferric reducing antioxidant power (FRAP) assays. The bark was subjected to successive Soxhlet extraction using petroleum ether, chloroform, and methanol, yielding *D. falcata* petroleum ether extract (DFPE), chloroform extract (DFCE), and methanolic extract (DFME), respectively. The total phenolic and flavonoid contents of the extracts were determined, with DFME exhibiting the highest phenolic content (163.22 ± 0.17 mg GAE/g) and flavonoid content (95.98 ± 0.15 mg QE/g), followed by DFCE and DFPE. Antioxidant activity was assessed at different concentrations using FRAP and DPPH assays, with ascorbic acid used as the standard antioxidant. All three extracts demonstrated concentration-dependent ferric reducing activity, with DFME showing the highest reducing power, reaching an absorbance of 1.22 at 1000 μ g/mL. Similarly, the DPPH radical scavenging activity increased with increasing extract concentration. DFME exhibited the highest scavenging activity, reaching 94.07% inhibition at 1000 μ g/mL, followed by DFCE (78.45%) and DFPE (58.68%). The IC₅₀ values for DFME, DFCE, and DFPE were 429, 545, and 905 μ g/mL, respectively, indicating greater antioxidant potency of the methanolic extract. The findings demonstrate that *D. falcata* bark possesses considerable antioxidant potential, particularly in the methanolic extract, which may be associated with its higher phenolic and flavonoid contents. These results support the potential of *D. falcata* bark as a promising natural source of antioxidant compounds.

Keywords: *Dolichandrone falcata*; bark extract; antioxidant activity; DPPH; FRAP; phenolic compounds; flavonoids; free radical scavenging.

Introduction

Medicinal plants have served as an indispensable source of therapeutic agents since ancient times and continue to play a pivotal role in contemporary drug discovery. Their importance is particularly pronounced in developing countries, where traditional systems of medicine remain the primary mode of healthcare for a large segment of the population. According to the World Health Organization, nearly 80% of the global population relies on herbal medicines for

primary healthcare, highlighting the urgent need for systematic scientific evaluation of medicinal plants (WHO, 2013; Ekor, 2014). Furthermore, the rapid emergence of drug-resistant microbial strains, coupled with the adverse effects associated with many synthetic drugs, has intensified the global search for safer, effective, and naturally derived therapeutic agents (Newman & Cragg, 2020). Plants synthesize a diverse array of secondary metabolites, including alkaloids, phenolic compounds, flavonoids, terpenoids, steroids, fatty acids, and glycosides, which play vital roles in plant defense mechanisms and exhibit a broad spectrum of pharmacological activities in humans (Harborne, 1998; Wink, 2015). These phytochemicals are responsible for numerous biological effects such as antimicrobial, anti-inflammatory, antioxidant, antidiabetic, and anticancer activities (Cowan, 1999; Pandey & Rizvi, 2009; Atanasov et al., 2015). Therefore, phytochemical investigations aimed at the identification, isolation, and characterization of these bioactive constituents are essential not only for validating traditional medicinal claims but also for discovering novel lead compounds for modern pharmaceutical development (Gurib-Fakim, 2006; Newman & Cragg, 2020).

Over the past few decades, extensive screening of medicinal and food plants for antioxidant properties has been conducted in the hope of identifying effective therapeutic agents for modern diseases and delaying aging-related disorders (Halliwell, 2008). Additionally, there is an increasing demand for natural antioxidants in the food industry to replace synthetic preservatives used to prevent lipid rancidity and color degradation. Although the mammalian body possesses intrinsic defense mechanisms to mitigate oxidative damage, epidemiological evidence suggests that consumption of antioxidant-rich food, particularly those containing flavonoids and other polyphenols, offers significant health benefits (Amarowicz et al., 2004). The potent antioxidant effects of plant-based foods attributed to the additive and synergistic interactions of these bio-active compounds (Peres et al., 2006). Antioxidants, even at low concentrations relative to oxidizable substrates, significantly delay or inhibit oxidation processes (Halliwell and Gutteridge, 1989). Beyond their physiological importance, antioxidants are widely incorporated into food systems to prevent oxidative deterioration initiated by environmental factors such as air, light, and temperature (Hras et al., 2000). Currently, most commercially used antioxidants are synthetic, however, their potential adverse effects in vivo remain a major concern (Chen, 1992). Secondary metabolites derived from medicinal plants act as low-molecular-weight antioxidants with diverse mechanisms of action, depending on their chemical structure and surrounding environment. These mechanisms include direct free radical scavenging, interruption of radical chain propagation, metal ion chelation, and inhibition of ROS-producing enzymes such as xanthine oxidase and inducible nitric oxide synthase. In vivo antioxidant effects may also involve enhancement of endogenous defense systems, such as up-regulation of super-oxide dismutase activity (Halliwell, 2008). Consequently, growing interest has emerged in exploring the biological activities of Ayurveda medicinal plants due to their natural origin, cost-effectiveness, and minimal side effects (Naik et al., 2003).

Dolichandrone falcata, belonging to the family Bignoniaceae, is a medicinally important plant widely distributed in tropical and subtropical regions of India. The plant has been traditionally used in various indigenous systems of medicine for the treatment of inflammatory conditions,

infections, diabetes, and other ailments. Different parts of the plant, particularly the bark, have been reported to possess antimicrobial, anti-inflammatory, antioxidant, and therapeutic properties. Despite its extensive ethnomedicinal use, systematic phytochemical investigations of *Dolichandrone falcata* bark remain limited, particularly with respect to comprehensive chemical profiling using advanced analytical techniques.

Materials and Methods

Collection of Plant Material

The *Dolichandrone falcata* plant was collected from the Gulbarga University campus, Kalaburagi, Karnataka, India. The taxonomic identity of the plant material was authenticated by the Department of Botany, Government College (Autonomous), Kalaburagi. A voucher specimen was deposited in the departmental herbarium (Voucher specimen No. GCAK-117).

Extraction of *Dolichandrone falcata* Bark

The shade-dried bark was powdered and sieved to obtain a uniform particle size of 22 mesh. The powdered material was subjected to successive sequential Soxhlet extraction using solvents of varying polarity, ranging from polar to non-polar, namely petroleum ether, chloroform, and methanol (40–60 °C). The extraction process was continued until the solvent became colorless. The obtained extracts were concentrated under reduced pressure and dried in vacuum desiccators. The dried extracts were subsequently subjected to phytochemical analysis for the detection of various secondary metabolites (Evans, 1996; Harborne, 1992; Indian Herbal Pharmacopoeia, 1996).

The different solvent extracts were designated as follows: *Dolichandrone falcata* petroleum ether extract (DFPE), *Dolichandrone falcata* chloroform extract (DFCE), and *Dolichandrone falcata* methanol extract (DFME).

Determination of Total Phenolic Content (TPC)

The total phenolic content of plant extracts is commonly determined using the Folin–Ciocalteu (F–C) colorimetric method, which is based on the reduction of the phosphomolybdic–phosphotungstic acid complexes present in the Folin–Ciocalteu reagent by phenolic compounds under alkaline conditions, resulting in the formation of a blue-colored complex. The intensity of the blue color is directly proportional to the concentration of phenolic compounds present in the plant extract (Singleton, Orthofer & Lamuela-Raventós, 1999). An aliquot (0.5 ml) of the plant extract was mixed with 2.5 mL of 10% Folin–Ciocalteu reagent. The mixture was allowed to stand for 5 minutes at room temperature. Then, 2.0 mL of 7.5% sodium carbonate solution was added to the mixture. The reaction mixture was incubated for 30 minutes in the dark at room temperature. The absorbance was measured at 765 nm using a UV–Visible spectrophotometer against a reagent blank. A calibration curve was constructed using gallic acid at different concentrations, and the total phenolic content of the extract was calculated from the standard curve.

The total phenolic content (TPC) was expressed as milligrams of gallic acid equivalents (GAE) per gram of dry extract.

Determination of Total Flavonoid Content (TFC)

Flavonoids form stable complexes with aluminum chloride and aluminum nitrate in the presence of potassium acetate under alkaline conditions. This reaction produces a yellow-colored complex whose intensity is directly proportional to the flavonoid concentration in the sample. The absorbance of this complex is measured at **415 nm** and compared with a standard flavonoid (quercetin) to determine total flavonoid content (Moreno et al., 2000). The total flavonoid content of *Dolichandrone falcata* extracts was expressed as mg quercetin equivalents (QE) per gram of extract (mg QE/g).

One milligram of each plant extract was dissolved in 1 mL of 80% ethanol. From this, **0.5 mL** of extract solution was transferred into test tubes. To each tube, 0.1 mL of 10% aluminum nitrate, 0.1 mL of 1 M potassium acetate, and 4.3 mL of 80% ethanol were added. The reaction mixtures were incubated at room temperature for **40 minutes** to allow complete complex formation. After incubation, the absorbance of the supernatant was measured at **415 nm** against a reagent blank using a UV–Visible spectrophotometer. The total flavonoid content was calculated using the quercetin standard calibration curve.

Antioxidant Activity

The antioxidant activity of *Dolichandrone falcata* bark extracts was evaluated using **Ferric Reducing Antioxidant Power (FRAP) assay and DPPH radical scavenging assay** with **ascorbic acid** as the standard antioxidant.

Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay measures the ability of antioxidants in plant extracts to reduce ferric ions (Fe^{3+}) to ferrous absorbance indicates greater reducing power (Barros et al., 2007). Different concentrations of plant extracts (62.5, 125, 250, 500, 1000 $\mu\text{g/ml}$) were mixed with **2.5 mL of 200 mM sodium phosphate buffer (pH 6.6)** and **2.5 mL of 1% potassium ferricyanide**. The mixture was incubated at **50°C for 20 minutes** to allow reduction of ferricyanide. Then, **2.5 mL of 10% trichloroacetic acid** was added to terminate the reaction, followed by centrifugation at **1000 rpm for 8 minutes**. The upper layer was collected and mixed with **5 mL of deionized water** and **1 mL of 0.1% ferric chloride**. The absorbance was measured at **700 nm**. Higher absorbance indicated stronger reducing (antioxidant) power. This assay reflects the **electron-donating capacity** of antioxidants, which is a key mechanism in neutralizing free radicals and preventing oxidative damage.

DPPH Radical Scavenging Activity

DPPH (1,1-diphenyl-2-picrylhydrazyl) is a stable free radical that exhibits a deep violet color. When an antioxidant donates a hydrogen atom or electron to DPPH, it becomes reduced, leading to discoloration. The decrease in absorbance at **517 nm** reflects the free radical scavenging ability of the extract (Barros et al., 2007). Different concentrations (62.5, 125, 250, 500, 1000 $\mu\text{g/ml}$) of plant extracts and the standard antioxidant Ascorbic acid (10 $\mu\text{g/ml}$) were prepared and made up to **100 μL** with methanol. To each tube, **5 mL of 0.1 mM DPPH methanolic solution** was added. The mixtures were shaken vigorously and

incubated in the dark for **30 minutes**. A control was prepared containing DPPH without extract. Absorbance was measured at **517 nm**, and radical scavenging activity was calculated using:

$$\% \text{ RSA} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

The intensity of the yellow color formed was measured spectrophotometrically at **517 nm** against a reagent blank. Lower absorbance values indicate higher hydroxyl radical scavenging activity of the extract (Singh et al., 2002).

Data / Statistical Analysis

GC–MS data acquisition and processing were performed using **ChemStation software** with automated peak integration. The total ion chromatograms (TICs) were analyzed to determine retention times, peak areas, and compound distribution in each extract.

All experiments in antioxidant activity were performed in **triplicate**, and results were expressed as **mean ± standard error (SEM)**. The **IC₅₀ values** were calculated from dose–response curves to compare antioxidant potency. Data were analyzed using **one-way ANOVA followed by Tukey’s post-hoc test**. Statistical analysis was carried out using **GraphPad Prism/SPSS software**.

Results and Discussion

The total phenolic and flavonoid contents expressed as gallic acid and quercetin equivalents are represented in Fig 3.4 and 3.5 respectively. The methanolic extract (DFME) exhibited the **highest total phenolic content** (163.22 ± 0.17 mg GAE/g), followed by the chloroform extract (DFCE, 51.63 ± 0.14 mg GAE/g), while the petroleum ether extract (DFPE) showed the **lowest phenolic content** (25.36 ± 0.23 mg GAE/g) (Table 3.4).

Similarly methanolic extract (DFME) exhibited the highest flavonoid content (95.98 ± 0.15 mg QE/g), followed by the chloroform extract (DFCE, 55.05 ± 0.11 mg QE/g), while the petroleum ether extract (DFPE) showed the lowest flavonoid content (21.62 ± 0.12 mg QE/g). This pattern is consistent with the polarity of the extraction solvents.

The high phenolic and flavonoid content of DFME directly explains its superior biological activity observed in the antioxidant and antimicrobial assays. Phenolic compounds are known for their strong free-radical scavenging ability, metal-chelating properties, and capacity to inhibit oxidative enzymes, while flavonoids are widely recognized for their antimicrobial, anti-inflammatory, and cytoprotective effects (Cowan, 1999; Pandey & Rizvi, 2009; Atanasov et al., 2015). These compounds can disrupt microbial cell membranes, inhibit nucleic acid synthesis, and neutralize reactive oxygen species, thereby contributing to both antimicrobial and antioxidant activities.

Table 3.5 represents FRAP of *Dolichandrone falcata*. All three extracts showed a **concentration-dependent increase** in FRAP absorbance. Among the extracts, the **methanolic extract exhibited the highest reducing power**, with absorbance values increasing from **0.18 at 62.5 µg/mL to 1.22 at 1000 µg/mL**, exceeding the methanolic ascorbic acid standard (**0.65**). The **chloroform extract** showed moderate reducing

Table 3.4 Total phenolic and flavonoids content of *Dolichandrone falcata*

Extracts	Total Phenols (mg/g) $\pm$ SEM	Total flavonoids (mg/g) $\pm$ SEM
DFPE	25.36 $\pm$ 0.23	21.62 $\pm$ 0.12
DFCE	51.63 $\pm$ 0.14	55.05 $\pm$ 0.11
DFME	163.22 $\pm$ 0.17	95.98 $\pm$ 0.15

Values expressed as Mean $\pm$ SEM

DFPE – *Dolichandrone falcata* Petroleum Ether Extract

DFCE -*Dolichandrone falcata* Chloroform Extract

DFME-*Dolichandrone falcata* Methanolic extract

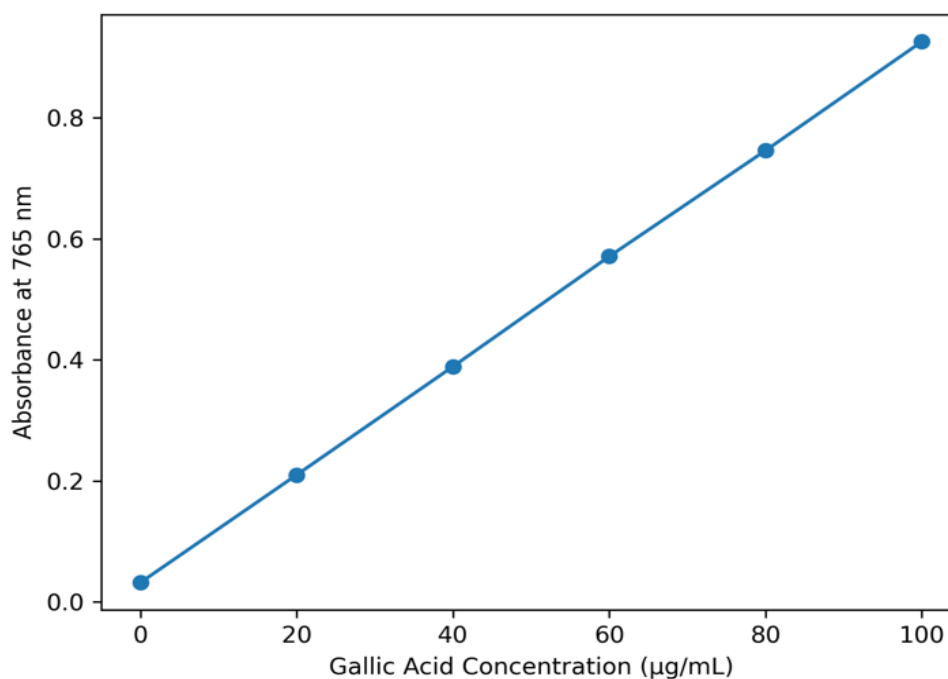


Fig 3.4 Calibration Curve of Gallic Acid

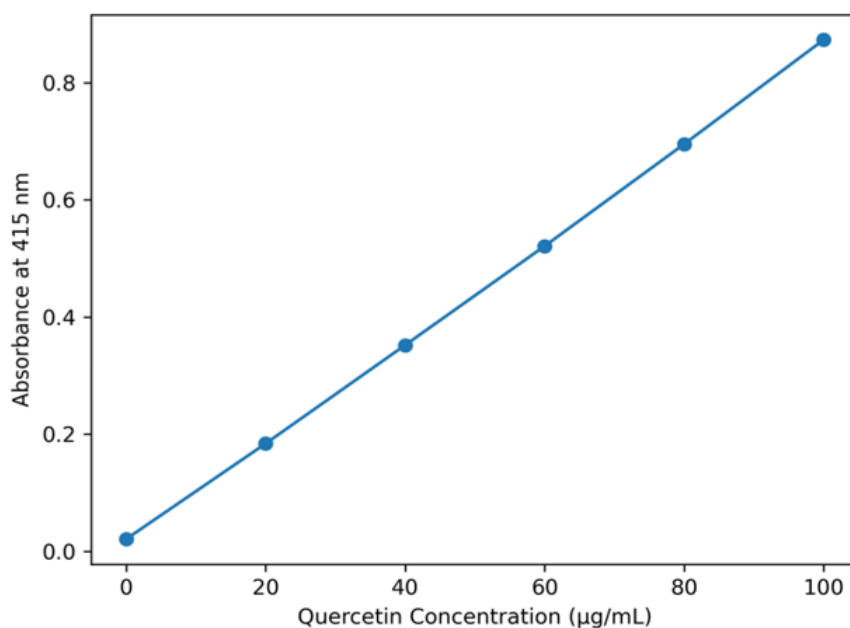


Fig 3.5 Calibration Curve of Quercetin

Table 3.5: Ferric Reducing Antioxidant Power (FRAP) of *Dolichandrone falcata*

Concentration (µg/ml)	Absorbance of Pet Ether extract	Absorbance of Chloroform extract	Absorbance of Methanol extract
DPPH alone	0	0	0
Ascorbic acid(10µg/ml)	0.33	0.48	0.65
62.5	0.07	0.12	0.18
125	0.14	0.20	0.29
250	0.25	0.33	0.44
500	0.42	0.54	0.72
1000	0.72	0.92	1.22

The values are an average of triplicate determination. ability, increasing from **0.12 to 0.92**, which also surpassed its corresponding ascorbic acid reference (**0.48**) at higher concentrations. The **petroleum ether extract** displayed the lowest reducing power, ranging from **0.07 to 0.72**, though it still exceeded its ascorbic acid reference (**0.33**) at higher concentrations. The methanolic extract of *Dolichandrone falcata* showed FRAP values comparable to or exceeding the methanolic ascorbic acid standard at higher concentrations, indicating strong reducing power. Fig 3.6 illustrates the ferric reducing antioxidant power of different solvent extracts of *Dolichandrone falcata* in comparison with the standard antioxidant Ascorbic acid. This indicates that compounds with strong **electron-donating properties** are more abundant in the methanolic extract. The ferric reducing activity increased in a **dose-dependent manner** for all extracts, indicating that higher concentrations of the extracts exhibited greater electron-donating ability. Among the tested extracts, **DFME showed the highest reducing power**,

followed by DFCE, while DFPE exhibited the lowest activity. The higher ferric reducing activity of DFME may be attributed to its higher content of **phenolic compounds and flavonoids**, which are known to act as strong electron donors capable of reducing ferric (Fe^{3+}) ions to ferrous (Fe^{2+}) ions.

Tables 3.6 and Figure 3.7 represents DPPH scavenging of *Dolichandrone falcata*. All three extracts exhibited a **concentration-dependent increase** in DPPH scavenging activity, indicating the presence of hydrogen-donating antioxidant compounds. The **methanolic extract showed the strongest activity**, increasing from **17.04% at 62.5 $\mu\text{g/mL}$ to 94.07% at 1000 $\mu\text{g/mL}$** , which was markedly higher than the ascorbic acid standard (44.85% at 10 $\mu\text{g/mL}$). This demonstrates that the methanolic fraction contains a high concentration of potent free radical scavengers. The chloroform extract also showed substantial activity, increasing from **15.55% to**

Table 3.6: DPPH Radical Scavenging Activity of *Dolichandrone falcata*

Concentration ($\mu\text{g/ml}$)	% of DPPH inhibition of pet ether extract	% of DPPH inhibition of chloroform extract	% of DPPH inhibition of Methanol
DPPH alone	0	0	0
Ascorbic acid	56.55766621	47.6548881	44.8534424
62.5	7.666214383	15.54770318	17.04158146
125	9.430122117	23.32155477	22.35855487
250	13.43283582	32.09658422	34.01499659
500	20.82767978	49.05771496	65.03067485
1000	58.68385346	78.44522968	94.06952965

The values are an average of triplicate determination.

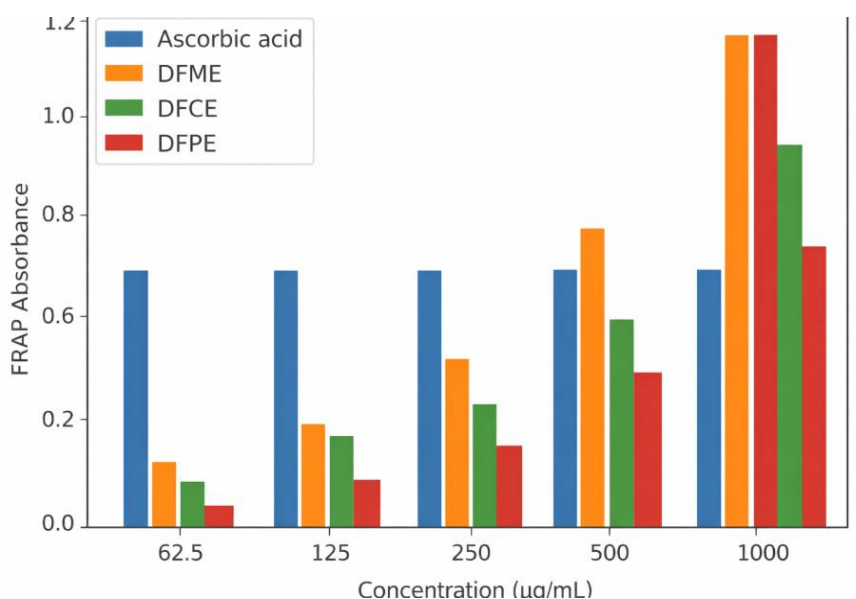


Fig. 3.6 Ferric reducing activity of *Dolichandrone falcata*

DFPE-Dolichandrone falcata petether extract

DFCE-Dolichandrone falcata chloroform extract

DFME-Dolichandrone falcata methanol extract

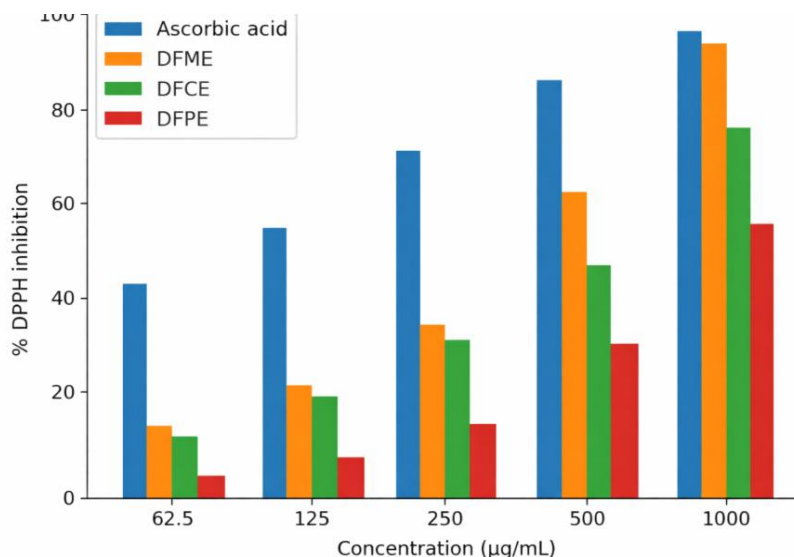


Fig 3.7 DPPH Radical Inhibition of *Dolichandrone falcata*

DFPE-Dolichandrone falcata petether extract

DFCE-Dolichandrone falcata chloroform extract

DFME-Dolichandrone falcata methanol extract

78.45%, whereas the pet ether extract exhibited comparatively lower scavenging capacity, ranging from 7.66% to 58.68%. The overall trend of antioxidant activity was:

Methanol extract > Petroleum ether extract > Chloroform extract

Previous phytochemical investigations on *Dolichandrone falcata* have revealed the presence of several bioactive compounds (Harborne, 1998; Cowan, 1999), these compounds are capable of neutralizing free radicals, inhibiting lipid peroxidation, and protecting cellular components from oxidative damage. In addition, flavonoids and phenolic acids present in the bark can modulate endogenous antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, thereby enhancing cellular defense mechanisms against oxidative stress (Valko et al., 2007).

The reduction capability of DPPH radical is determined by the decrease in absorbance at 517nm and IC₅₀ values. Fig 3.8 represents IC₅₀ of a compound is inversely related to its antioxidant capacity as it is expressing the amount of antioxidant required to decrease the DPPH concentration by 50%. Further, plants seem to have strong antioxidants if their IC₅₀ values are lower than 50mg/ml. The IC₅₀ values found in present study were 429 µg/ml, 545 µg/ml and 905 µg/ml for methanol, chloroform and pet ether extracts respectively.

The DPPH free radical scavenging assay is widely used to assess antioxidant activity in plant extracts. In *Dolichandrone falcata*, the methanolic bark extract showed substantial

antioxidant capacity with an IC_{50} value of approximately **429 $\mu\text{g/mL}$** , compared to other solvent fractions (Author et al., 2022). Similar solvent-dependent antioxidant trends have been reported in other medicinal plants: for example, the methanolic leaf extract of *Ocimum sanctum* exhibited an IC_{50} of **~45 $\mu\text{g/mL}$** , indicating strong free radical scavenging potential (Singh et al., 2014). The root extract of *Withania somnifera* demonstrated an IC_{50} of **~82 $\mu\text{g/mL}$** in the DPPH assay, further highlighting the influence of plant part and extraction solvent on antioxidant efficacy (Kumari et al., 2017). Additionally, *Tinospora cordifolia* stem methanolic extract showed an IC_{50} value of **~120 $\mu\text{g/mL}$** , reinforcing that polar extracts generally yield better antioxidant activity than non-polar fractions (Patel & Mishra, 2015). These comparative values suggest that while *Dolichandrone falcata* possesses antioxidant properties, other medicinal plants with strong phenolic contents may exhibit more potent free radical scavenging activity in DPPH assays.

Conclusion

. The bark of *Dolichandrone falcata* also exhibited strong antioxidant potential, as demonstrated by both ferric reducing antioxidant power (FRAP) assay and DPPH radical scavenging assays, with the methanolic extract showing the highest activity. The remarkable antioxidant effect of the methanolic extract may be attributed to its higher content of phenolic compounds and flavonoids, which are well known for their free radical scavenging and reducing properties.

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