JBEP

Journal of Biosciences and Experimental Pharmacology
ISSN-2960-284X

Original Article

HPLC-Analysis of Polyphenolic Compounds in *Crateva nurvala* and Evaluation of *In vitro* Antioxidant Activities of its Flash Column Chromatographic Fraction

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Citation: Sadman, A. S. M.; Samanta, N. M.; Eva, S. A.; Alimullah, M.; Sultana, S.; Ghosh, H. C. Jahan, N.; Mandal, S.; Banik, R.; Ahmed, K. S.; Hossain, H.; Subhan, N., Alam, M. A. HPLC-Analysis of Polyphenolic Compounds in *Crateva nurvala* and Evaluation of *In Vitro* Antioxidant Activities of its Flash Column Chromatographic Fraction. *J. Bio. Exp. Pharm*. 2024, **2(2), 71-85**
https://doi.org/10.62624/JBEP00.019

Academic Editor: Dr. Sazid Md. Sarker

Received: August 17, 2024
Accepted: November 29, 2024
Published: December 27, 2024

Publisher's Note: JBEP stays neutral regarding jurisdictional claims in published maps and institutional affiliations.

Abstract: *Crateva nurvala* (Barun), a medicinal plant extensively used in traditional medicine, is rich in phenolic compounds that exert antioxidant effects. The escalating demand for safer, natural antioxidants has renewed interest in medicinal plants. This study investigates the antioxidant potential of *Crateva nurvala* bark extract and its flash column chromatographic fractions, with emphasis on polyphenolic composition and *in vitro* radical scavenging activity. Ethanolic crude extract of *Crateva nurvala* bark was fractionated using gradient flash column chromatography into five major groups. Polyphenolic compounds were identified and quantified via HPLC-DAD. Antioxidant potential was evaluated using DPPH, nitric oxide (NO), hydroxyl radical (•OH) scavenging, ferric reducing antioxidant power (FRAP), and total antioxidant capacity (TAC) assays. IC_{50} values were calculated from dose-response curves. HPLC profiling revealed three major phenolics: p-coumaric acid (203.09 ± 0.67 mg/100g), (-)epicatechin (39.77 ± 0.46 mg/100g), and myricetin (37.35 ± 0.32 mg/100g). In the DPPH assay, IC_{50} values were lowest for the *Crateva nurvala* crude extract (3.276 µg/mL) and methanol fraction (3.436 µg/mL), compared to ascorbic acid (0.8579 µg/mL). NO scavenging results followed a similar trend: crude extract (IC_{50} = 7.865 µg/mL), methanol (8.371 µg/mL). For hydroxyl radicals, the crude extract showed IC_{50} = 156.5 µg/mL, approaching ascorbic acid (105.8 µg/mL). FRAP and TAC assays indicated the methanol fraction had the highest reducing potential and antioxidant capacity, respectively. The potent antioxidant activity of *Crateva nurvala*, supported by its rich polyphenolic content, underscores its value as a promising natural source of therapeutic antioxidants. Low IC_{50} values in multiple assays validate its potential for further development into nutraceutical or pharmaceutical applications.

Keywords: *Crateva nurvala*; Barun; *In vitro* assays; oxidative stress; antioxidant; polyphenols; total antioxidant capacity

1. Introduction

Oxidative stress is a pathological condition that arises due to an imbalance between the production of reactive oxygen species (ROS) and the body's ability to detoxify them through its antioxidant defenses [1]. ROS, including

free radicals like superoxide anion, hydroxyl radical, and hydrogen peroxide, are generated as natural byproducts of cellular metabolism. However, excessive ROS accumulation can lead to oxidative damage to lipids, proteins, and DNA, contributing to various chronic diseases such as cancer, neurodegenerative disorders, and cardiovascular diseases [2], and metabolic syndromes [3]. The detrimental effects of oxidative stress highlight the necessity of maintaining redox homeostasis to prevent cellular dysfunction and disease progression [4].

The body employs enzymatic and non-enzymatic antioxidant systems that neutralize ROS and repair oxidative damage to counteract oxidative stress. Antioxidants are classified into endogenous (produced within the body) and exogenous (obtained from diet or supplements). The primary enzymatic antioxidants include superoxide dismutase (SOD), catalase, and glutathione peroxidase, which catalyze the breakdown of harmful ROS [5]. Non-enzymatic antioxidants, such as vitamins C and E, polyphenols, and flavonoids, scavenge free radicals and mitigate oxidative damage. Plant-derived antioxidants are particularly significant due to their high bioavailability and minimal side effects [6]. Research has consistently demonstrated their ability to mitigate oxidative damage while exhibiting anti-inflammatory [7], anti-allergy, and anti-cancer [8] properties. These findings underscore the value of polyphenols as key contributors to human health and highlight their potential as therapeutic agents [9]. Various *in vitro* assays, such as the DPPH radical scavenging assay, ferric reducing antioxidant power (FRAP) assay, and nitric oxide scavenging assay, are commonly employed to assess antioxidant potential[10].

Among dietary antioxidants, polyphenols- abundant in fruits, vegetables, and plant-based foods- have gained prominence due to their potent antioxidant effects and additional health benefits [10]. Synthetic antioxidants, like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), have been associated with toxicological concerns and carcinogenic risks [11]. Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have been extensively utilized for their free radical-scavenging abilities, however, multiple studies have raised concerns about their possible carcinogenicity and adverse metabolic effects [12, 13]. Toxicological studies have indicated that BHT and BHA, when administered at high doses (typically 0.1–0.5% in food), can induce liver toxicity, promote tumorigenesis, and disrupt endocrine signaling pathways [13]. These limitations highlight the urgent need for natural, biocompatible alternatives that not only combat oxidative stress effectively but also provide additional health benefits.

In contrast, plant-derived phytochemicals, including polyphenols, flavonoids, tannins, and terpenoids, have demonstrated significant antioxidant activity and health-promoting effects [10]. Other essential antioxidants, including vitamins C and E, beta-carotene, selenium, and zinc, play fundamental roles in sustaining oxidative balance and overall health. The increasing recognition of natural antioxidants highlights their potential in therapeutic applications, reinforcing their importance in maintaining cellular resilience against oxidative damage.

The Barun tree, or *Crateva nurvala*, is an important medicinal plant that has long been valued in traditional medical systems throughout many countries [14]. *Crateva nurvala* is frequently utilized in several Ayurvedic and Unani medicine formularies and has been characterized in numerous noteworthy qualitative studies as having a variety of therapeutic actions [15]. According to reports, *Crateva nurvala's* bark, leaves, and roots contain antioxidant compounds like phenolics (linoleic acid, oleic acid, stearic acid, lauric acid, rutin, quercetin, γ-taraxasterol, lupeol, β-sitosterol acetate, β-sitosterol, and β-epilupeol) and flavonoids (L-stachydrine, rutin, quercetin-3-O-α-D-glucoside, quercetin, isoquercetin, methyl pentacosanoate, kaempferol-O-α-D-glucoside, and dodecanoic anhydride) [16]. Alkaloids, flavonoid glycosides, glucosinolates, triterpenes, tannins, and phytosterols are all abundant in the plant [17]. This plant is also used by practitioners of traditional medicine to treat rheumatic fever, vomiting, gastrointestinal discomfort, kidney and bladder stones, and more [18]. In our recent study, in 2K1C rats, *Crateva nurvala* bark extract enhanced antioxidant capacity and decreased fibrosis, inflammation, cardiovascular problems, and renal impairment [19]. This study aims to bridge this knowledge gap by evaluating the antioxidant potential of Barun bark extracts and their fractions by column chromatography. By analyzing their ability to mitigate oxidative stress, this research seeks to provide a scientific basis for the potential therapeutic applications of Barun bark-derived antioxidants.

2. Materials and Methods

2.1 Plant Material Collection and Extraction

The bark of the *Crateva nurvala* plant was gathered from Mymensingh, Bangladesh's local marketplaces. The National Herbarium in Mirpur, Bangladesh, provided the initial identification and authentication of the plant. An accession number (DACB-78798) was deposited for future use. They sliced the bark into little pieces. All of the bark bits were then pulverised into a powder. After that, 200 gm of powder was immersed in 80% ethanol for seven days. After that, the solvent was filtered via filter paper and decanted. A rotary evaporator was used to condense the extract at 40°C. The result was a sticky, crude extract. The yield as a percentage was computed. The plant extract's final percentage yield from the soaked powder was 33.25% (w/w).

2.2 Different fraction preparation

2.2.1 Column Chromatography Preparation of reagent:

The crude extract was fractionated via column chromatography on silica gel, employing a gradient solvent system (hexane, ethyl acetate, and methanol). Initially, 1 gram of crude extract was processed through the column. Subsequently, a total of 8 grams of crude extract was fractionated in successive runs using the solvent gradient system. This process yielded 13 sub-fractions, which were later grouped into 5 major fractions based on their thin-layer chromatography (TLC) profiles and stored at 4°C for further analysis.

Solvent gradient system: The gradient solvent ratio to be applied in column chromatography is summarized in the following table.

Table 1: Gradient solvent system to be used in column chromatography

Solvent system	Ratio	Volume	Fraction No.
Hexane	100%	100 ml	1
Hexane: Ethyl acetate	90:10	100 ml	2
Hexane: Ethyl acetate	80:20	100 ml	3
Hexane: Ethyl acetate	70:30	100 ml	4
Hexane: Ethyl acetate	60:40	100 ml	5
Hexane: Ethyl acetate	50:50	100 ml	6
Hexane: Ethyl acetate	40:60	100 ml	7
Hexane: Ethyl acetate	30:70	100 ml	8
Hexane: Ethyl acetate	20:80	100 ml	9
Hexane: Ethyl acetate	10:90	100 ml	10
Ethyl acetate	100%	100 ml	11
Ethyl acetate: Methanol	90:10	100 ml	12
Ethyl acetate: Methanol	80:20	100 ml	13
Ethyl acetate: Methanol	70:30	100 ml	14
Ethyl acetate: Methanol	60:40	100 ml	15
Ethyl acetate: Methanol	50:50	100 ml	16
Ethyl acetate: Methanol	40:60	100 ml	17
Ethyl acetate: Methanol	30:70	100 ml	18
Ethyl acetate: Methanol	20:80	100 ml	19
Ethyl acetate: Methanol	10:90	100 ml	20
Methanol	100%	100 ml	21

2.3 Quantitative and Qualitative Analysis through HPLC

2.3.1 Chemicals

Gallic acid, 3,4-dihydroxybenzoic acid, catechin-hydrate, catechol, (-) epicatechin, caffeic acid, vanillic acid, syringic acid, rutin hydrate, p-coumaric acid, trans-ferulic acid, rosmarinic acid, myricetin, quercetin, trans-cinnamic acid, and kaempferol were purchased from Sigma–Aldrich (St. Louis, MO, USA). Acetonitrile (HPLC), methanol (HPLC), acetic acid (HPLC), and ethanol were obtained from Merck (Darmstadt, Germany). α-amylase, 1, 1-diphenyl-2-picrylhydrazyl (DPPH), and naphthyl ethylenediamine dihydrochloride were obtained from Sigma-Aldrich Chemical Co. (USA). Starch soluble (extra pure) was obtained from J.T. Baker Inc., Phillipsburg, USA. Other chemicals and reagents used were of analytical grade.

2.3.2 High-performance liquid chromatography (HPLC) Analysis

Detection and quantification of polyphenolic compounds in the *Crateva nurvala* bark extracts were determined by HPLC-DAD analysis as described by Ahmed et al. (2021) with some modifications [20]. HPLC analysis was performed on a LC-20A (Shimadzu, Kyoto, Japan) equipped with a binary solvent delivery pump (LC-20AT), an auto sampler (SIL-20A HT), column oven (CTO-20A) and a photodiode array detector (SPD-M20A) and controlled by the LC solution software (Lab Solution Separation was performed using Luna C18 (5µm) Phenomenex column (4.6 x 250 mm) at 33ºC. The mobile phase composed of A (1% acetic acid in acetonitrile) and B (1% acetic acid in water) with gradient elution: 0.01-20 min (5-25% A), 20-30 min (25-40% A), 30-35 min (40-60% A), 35-40 min (60-30% A), 40–45 min (30–5% A), and 45–50 min (5% A) was used in this study. The sample injection volume was 20µL, and the flow rate was set at 0.5 mL/min. The UV detector was set at 270 nm and applied for validation of the method and analysis. The mobile phase was filtered through a 0.45 µm nylon 6, 6 membrane filter (India) and degassed under vacuum. For the preparation of calibration curve, a standard stock solution was prepared in methanol containing Gallic acid (20 µg/ml); 3,4-dihydroxybenzoic acid (15 µg/ml); catechin hydrate (50 µg/ml); catechol, (-) epicatechin, rosmarinic acid (30 µg/ml each); caffeic acid, vanillic acid, syringic acid, rutin hydrate, p-coumaric acid, trans-ferulic acid, quercetin (10 µg/ml each); myricetin, kaempferol (8 µg/ml each); trans-cinnamic acid (4 µg/ml).

2.4 Total Antioxidant Capacity Assay

The phosphomolybdenum method, as described by Prieto and his colleagues, was used to assess the extract's antioxidant activity [34]. As the extract reduces Mo (VI) to Mo (V), a green phosphate/Mo(V) complex is formed at acidic pH, which serves as the basis for the assay. A reagent solution consisting of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate was mixed with 0.3 ml of extract. For ninety minutes, the reaction solution-containing tubes were incubated at 95°C. After the solution had cooled to room temperature, its absorbance at 695 nm was measured with a spectrophotometer against a blank. As a blank, 0.3 ml of methanol was used in place of the extract. The number of equivalents of ascorbic acid is used to express antioxidant activity.

2.5 In vitro Antioxidant Assays

2.5.1 DPPH (1, 1-Diphenyl-2- Picrylhydrazyl) Radical Scavenging Assay

In HPLC grade methanol, a DPPH solution(0.004% w/v) was prepared [21]. To make the stock solution (500 µg/mL), each of the five fractions (A, B, C, D & E) and the *Crateva nurvala* crude extract was combined individually with Milli-Q water 1mL extracts were taken to the test tubes of serially diluted different concentrations (12.5 µg/mL to 500 µg/mL), and then a freshly made 1mL DPPH solution (0.004% w/v) was added. So, the total volume was 2 mL, and after 10 minutes of incubation in a dark place, the absorbance was measured at 515 nm using a spectrophotometer (HACH 4000 DU UV-visible spectrophotometer). As a reference standard, ascorbic acid was dissolved in Milli-Q water to create a stock solution with the same strength (500 mg/mL). The percent scavenging of the DPPH free radical activity was measured by using the following equation:

% of inhibition = [(absorbance of the control – absorbance of the test sample) / absorbance of the control] X 100

Lower absorbance of the reaction mixture indicates higher free radical-scavenging activity. All the tests were performed in triplicate.

2.5.2 Nitric Oxide (NO) Radical Scavenging Assay

For the NO radical scavenging assay, a previously described method was used [22]. At a physiological pH, sodium nitroprusside solution produced nitric oxide (NO) radicals. In phosphate buffer, 1 ml of sodium nitroprusside (10 mM) was combined with 1 ml of ethanolic extract and fractions of various concentrations (12.5 - 150 g/ml) (pH 7.4). 150 minutes were spent incubating the mixture at 25°C. Griess' reagent (1% sulfanilamide, 2% o-phosphoric acid, and 0.1% naphthyl ethylene diamine dihydrochloride) was added to 1 ml of the incubated solution. The absorbance was measured at 546 nm, and the formula for % inhibition was applied are mentioned below:

% of inhibition = [(absorbance of the control – absorbance of the test sample) / absorbance of the control] X 100

2.5.3 Ferric Reducing Power Assay

The method outlined by Oyaizu was used to determine the reducing power of *Crateva nurvala* extract and fractions [23]. The reducing power of the fractions was evaluated by mixing 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1%) with 1 mL of each fraction. The mixture was incubated at 50°C for 20 minutes, followed by the addition of 2.5 mL of trichloroacetic acid (10%). After centrifugation at 3000 rpm for 10 minutes, 2.5 mL of the supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of ferric chloride (0.1%). The absorbance was measured at 700 nm. Ascorbic acid was used as the standard. A phosphate buffer (pH 6.6) was used as the blank solution.

3. Results and Discussion:

Reactive nitrogen and oxygen species (RNS/ROS) are double-edged biological entities that exert both beneficial and detrimental effects depending on their concentration. At physiological levels, they contribute to critical processes such as cell signaling, redox homeostasis, mitogenic responses, and immune modulation. However, under pathological conditions, excessive ROS and RNS production lead to oxidative and nitrosative stress, resulting in the oxidation of proteins, lipids, and nucleic acids, ultimately impairing cellular function and promoting disease progression [25, 26].

3.1 HPLC-Detected Polyphenolics

High-performance liquid chromatography (HPLC-DAD) analysis of *Crateva nurvala* bark extract revealed the presence of several potent phenolic compounds. The most abundant was p-coumaric acid (2)(203.09 ± 0.67 mg/100g) [27, 28], followed by (-) epicatechin (1) (39.77 ± 0.46 mg/100g)[29, 30] and myricetin (3) (37.35 ± 0.32 mg/100g) [31, 32]. These compounds are recognized for their strong antioxidant properties. p-Coumaric acid (2) has demonstrated the ability to directly scavenge reactive oxygen species (ROS) and upregulate endogenous antioxidant enzymes. It also exhibits anti-inflammatory and cardioprotective effects. Epicatechin (1) and myricetin (3), two well-known flavonoids (**Figure 1**), contribute to radical scavenging through their hydroxyl-rich structure and redox potential [10]. Their documented activities include inhibition of lipid peroxidation, ROS neutralization, and enhancement of cellular antioxidant defenses. The high concentrations of these compounds likely underpin the strong antioxidant performance seen across all assays in this study.

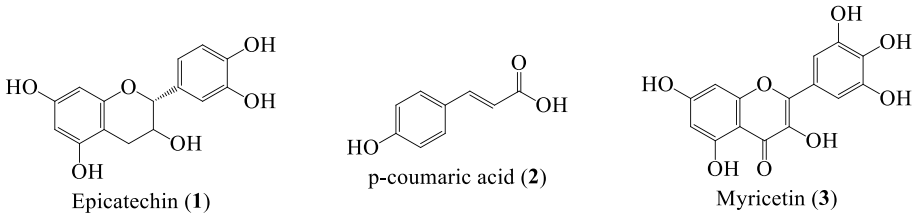
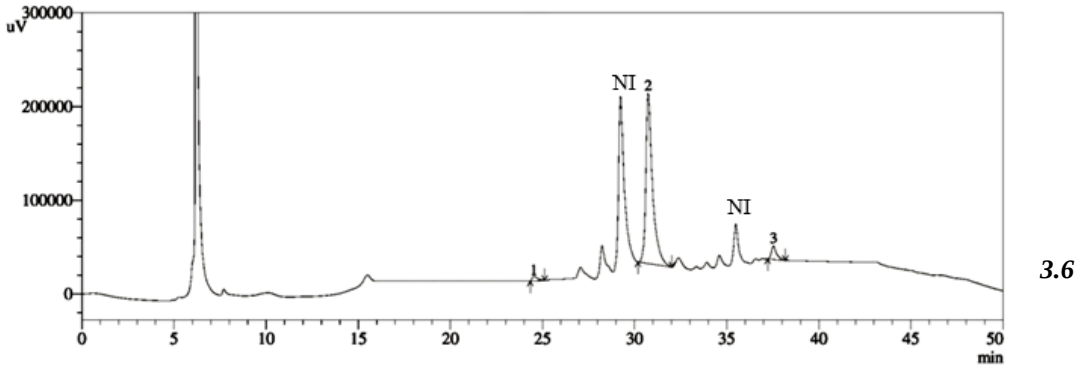


Figure 1: Identified phenolic acids and flavonoids in the ethanolic extract of *Crateva nurvala*

The HPLC - DAD chromatogram of *Crateva nurvala* extract is presented in **Figure 2**. Several bioactive antioxidants such as epicatechin, p-coumaric acid, and myricetin were detected in the sample. Among them, p-coumaric acid (203 mg/100 g dry extract) was found in high amount in *Crateva nurvala* extract.

Table 2: HPLC data of available standard compounds found in *Crateva nurvala* bark extract

Peak no.	Name of standard compounds	Retention time (min)	*Crateva nurvala* (mg/100g dry extract)
1	Gallic acid	ND	ND
2	3,4-Dihydroxybenzoic acid	ND	ND
3	Catechin hydrate	ND	ND
4	Catechol	ND	ND
5	(-) Epicatechin	24.56	39.77±0.46
6	Caffeic acid	ND	ND
7	Vanillic acid	ND	ND
8	Syringic acid	ND	ND
9	Rutin hydrate	ND	ND
10	p-Coumaric acid	30.75	203.09±0.67
11	trans-Ferulic acid	ND	ND
12	Rosmarinic acid	ND	ND
13	Myricetin	37.55	37.35±0.32
14	Quercetin	ND	ND
15	trans-Cinnamic acid	ND	ND
16	Kaempferol	ND	ND



3.6

Figure 2: HPLC chromatogram of *Crateva nurvala* bark extract showing detected phenolic compounds- p-coumaric acid, (-) epicatechin, and myricetin, ND = Not detected.

3.2 Total antioxidant capacity of Crateva nurvala bark extract and its fractions with ascorbic acid (standard)

The total antioxidant capacity of the crude ethanol extract of *Crateva nurvala* bark and its fractions with ascorbic acid is presented in **Figure 3**. The extract and sub-fractions showed increased total antioxidant capacity in a concentration-dependent manner compared to the ascorbic acid.

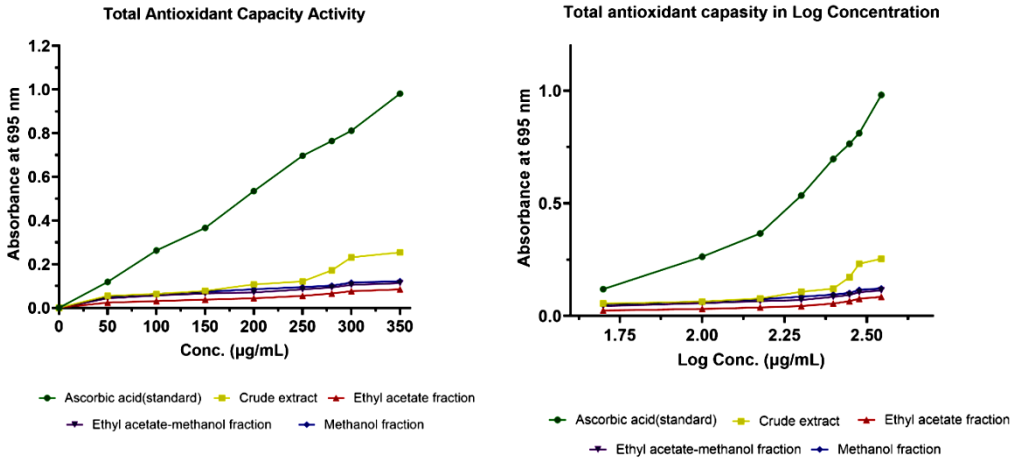


Figure 3: Total antioxidant capacity of *Crateva nurvala* bark crude ethanol extract and its fractions with ascorbic acid (standard)

The total antioxidant capacity, assessed using the phosphomolybdenum method, reflects the overall reducing capacity of all antioxidants in the sample. The assay revealed that the methanol fraction showed the highest TAC, followed by the crude extract and the ethyl acetate: methanol fraction. Results were expressed in µg/mL of ascorbic acid equivalent (AAE) per mg of extract, with values indicating strong reducing potential across all fractions. The TAC results complement the radical scavenging and FRAP data, suggesting that *Crateva nurvala* bark consists of several strong and complex phenolic compounds; for this, the antioxidant activity of the extract and its fractions is considerably high. This includes both high-molecular-weight polyphenols and low-molecular-weight secondary metabolites, many of which have been characterized in the HPLC chromatogram. The results are further supported by studies on other polyphenol-rich Bangladeshi medicinal plants, where TAC values showed similar or lower ranges [9, 20].

The antioxidant potential of *Crateva nurvala* bark extract and its chromatographic fractions was evaluated using multiple *in vitro* assays, and results indicated promising activity across all tested parameters. Each assay offers insight into different mechanisms of antioxidant defense, and when considered collectively alongside the HPLC-detected polyphenolics, these data support the extract's therapeutic potential against oxidative stress.

3.3 DPPH Radical Scavenging Activity of Crateva nurvala bark crude extract and its fractions with ascorbic acid (standard)

Currently, interest has vigorously increased in the naturally occurring antioxidants from natural sources due to their safety margin in the reduction of human oxidative stress and free radical scavenging DPPH method is an easy, rapid, and sensitive way to determine the antioxidant activity of a specific compound or plant extracts [24]. DPPH radical scavenging activity of the ethanolic extract of *Crateva nurvala* and its fraction was found to increase with increasing concentration, as shown in **Figure 4**. Crude extract showed the maximum DPPH scavenging activity of 85.46% at a higher concentration of 250 µg/mL **Figure 4** but the other fractions also showed a considerable quantity of DPPH scavenging activity. There was a respectable correlation revealed between the DPPH scavenging assay and the total antioxidant activity result **Figure 4**.

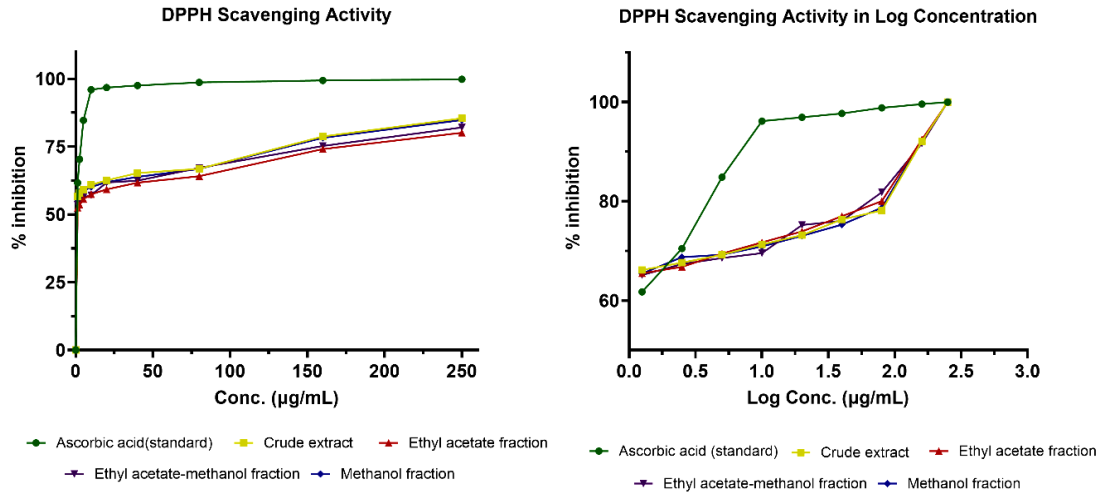


Figure 4: DPPH scavenging activity of *Crateva nurvala* bark crude ethanol extract and its fractions with ascorbic acid (standard).

The DPPH assay showed that all tested samples had significant radical scavenging capacity. The IC_{50} value for ascorbic acid was 0.8579 µg/mL, indicating high antioxidant potential. Among the test samples, the *Crateva nurvala* crude extract exhibited an IC_{50} of 3.276 µg/mL, followed closely by the methanol fraction (3.436 µg/mL). The ethyl acetate: methanol (4.291 µg/mL) and ethyl acetate (4.841 µg/mL) fractions also showed notable activity. Compared to previous reports, triterpenes from *Crateva nurvala* showed DPPH IC_{50} values near 95 µg/mL, the current results are remarkably potent. These low IC_{50} values suggest that the extract and its fractions possess excellent hydrogen-donating abilities, likely due to the presence of abundant phenolics identified in the HPLC analysis [16]. The antioxidant efficacy can be attributed to the presence of bioactive polyphenols, particularly (-) epicatechin (1) and myricetin (3), both of which are well-documented DPPH scavengers [33].

The comparison between total antioxidant capacity (TAC) vs DPPH radical scavenging activity are presented in **Figure 5**. Compare to ascorbic acid the extract and subfractions showed promising radical scavenging potential.

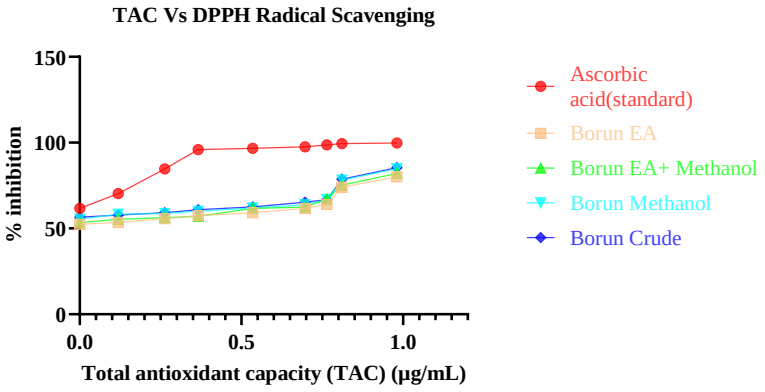


Figure 5: Comparison between Total antioxidant capacity (TAC) and DPPH free radical scavenging activity of the ethanolic bark extract of *Crateva nurvala* and its different fractions.

3.4 NO Scavenging Activity of Crateva nurvala bark crude ethanol extract and its fractions with ascorbic acid (standard).

The Nitric Oxide (NO) scavenging activity of *Crateva nurvala* bark crude ethanol extract and its fractions with ascorbic acid (standard). The extract showed the strong NO scavenging activity compared to the ascorbic acid. The IC_{50} value for ascorbic acid was found 2.979 µg/mL while for the *Crateva nurvala* extract possesses IC_{50} value of 7.865 µg/mL (**Table 2**)

In the NO radical scavenging assay, ascorbic acid showed an IC_{50} of 2.979 µg/mL. Among the test samples, the crude extract exhibited an IC_{50} of 7.865 µg/mL, with slightly reduced activity in the methanol (8.371 µg/mL), ethyl acetate: methanol (9.413 µg/mL), and ethyl acetate (10.05 µg/mL) fractions. This high level of NO inhibition indicates a substantial ability to neutralize reactive nitrogen species. Earlier studies reported IC_{50} values >20 µg/mL for *Crateva nurvala* leaf extracts [34]. The observed efficacy is further supported by the presence of myricetin (3) and (-) epicatechin (1), both of which are capable of neutralizing reactive nitrogen species (RNS) and preventing peroxynitrite formation [35].

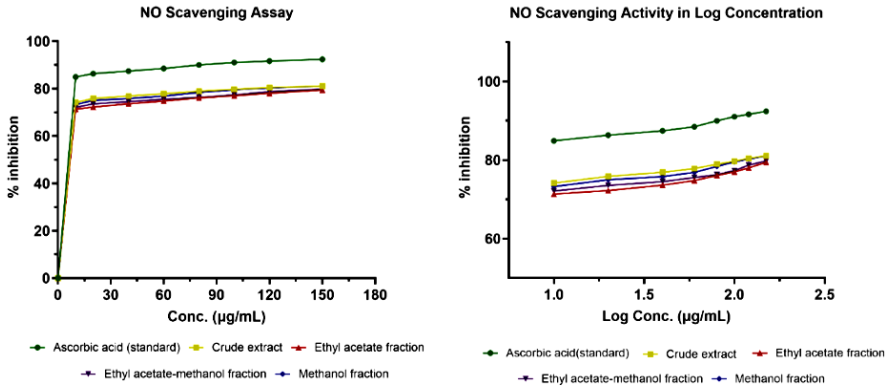


Figure 6: NO scavenging activity of *Crateva nurvala* bark crude ethanol extract and its fractions with ascorbic acid (standard)

3.5 Hydroxyl radical scavenging activity

Hydroxyl radical scavenging was the least potent among all assays, with ascorbic acid showing an IC_{50} of 105.8 µg/mL. The *Crateva nurvala* crude extract (IC_{50} = 156.5 µg/mL) was slightly more effective than the methanol fraction (157.5 µg/mL) and ethyl acetate: methanol fraction (171.6 µg/mL). The ethyl acetate fraction (190.8 µg/mL) showed the least activity. The hydroxyl radical (•OH) scavenging activity indicated moderate-to-strong inhibition. The hydroxyl radical is among the most reactive and damaging ROS, and its neutralization is a key indicator of antioxidant strength. Studies on similar polyphenols show that epicatechin and myricetin effectively inhibit •OH-mediated damage, with IC_{50} values of 12.3 µM and 15.8 µM, respectively [9]. These findings corroborate the effectiveness of the tested extract in mitigating oxidative insults associated with hydroxyl radicals.

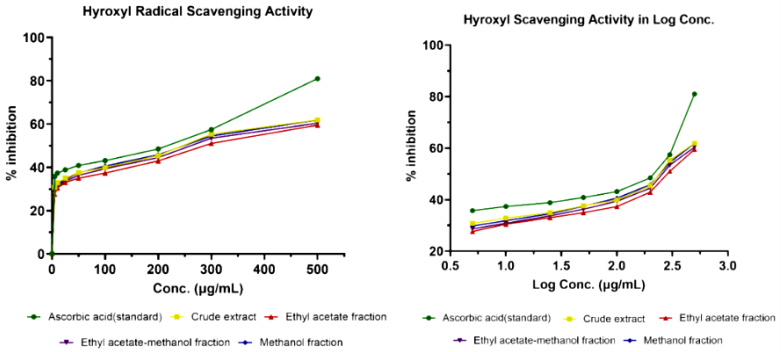


Figure 7: Hydroxyl radical scavenging activity of *Crateva nurvala* crude extract and its fractions with ascorbic acid (standard)

3.6 Ferric Reducing Activity Assay

The ferric reducing power assay evaluates the electron donating capacity of antioxidants, reflecting their potential to reduce Fe^{3+} to Fe^{2+} [36]. The increasing absorbance at 700 nm with sample concentration indicates a dose-dependent enhancement of reducing capacity. Among the tested fractions, the methanol and crude extracts exhibited the highest absorbance values, suggesting a stronger reducing ability. The mechanism behind this activity is typically linked to the presence of phenolic hydroxyl groups that can donate electrons to oxidized intermediates, thus terminating free radical chain reactions. Given the presence of (-) epicatechin (2) and myricetin (3), which have strong redox potentials, the observed FRAP activity aligns well with the HPLC findings. This result is also in agreement with previous studies, where high phenolic content was associated with superior ferric reducing ability in medicinal plant extracts [37]. These findings reinforce the role of *Crateva nurvala* fractions as effective electron donors capable of stabilizing oxidative intermediates.

Collectively, the results from all antioxidant assays and HPLC profiling confirm the strong antioxidant capacity of *Crateva nurvala* bark extract, with activities mediated primarily by phenolic compounds such as p-coumaric acid (2), (-) epicatechin (1), and myricetin (3). Based on IC_{50} values across all antioxidant assays, the general order of radical scavenging activity for the tested fractions was:

Ascorbic acid > *Crateva nurvala* crude extract > Methanol fraction > Ethyl acetate: methanol fraction > Ethyl acetate fraction.

All the fractions showed considerable antioxidant activity, because the ethyl acetate and methanol fractions can retain phenolic compounds due to their higher polarity and better solubility for phenolic components present in plant materials [38]. This trend was consistent for DPPH, nitric oxide, and hydroxyl radical assays, highlighting the superior efficacy of the crude and methanol fractions, likely due to their higher concentration of bioactive phenolics such as epicatechin (1) and myricetin (3). These findings substantiate the therapeutic relevance of *Crateva nurvala* in mitigating oxidative stress and validate its traditional use as a medicinal plant.

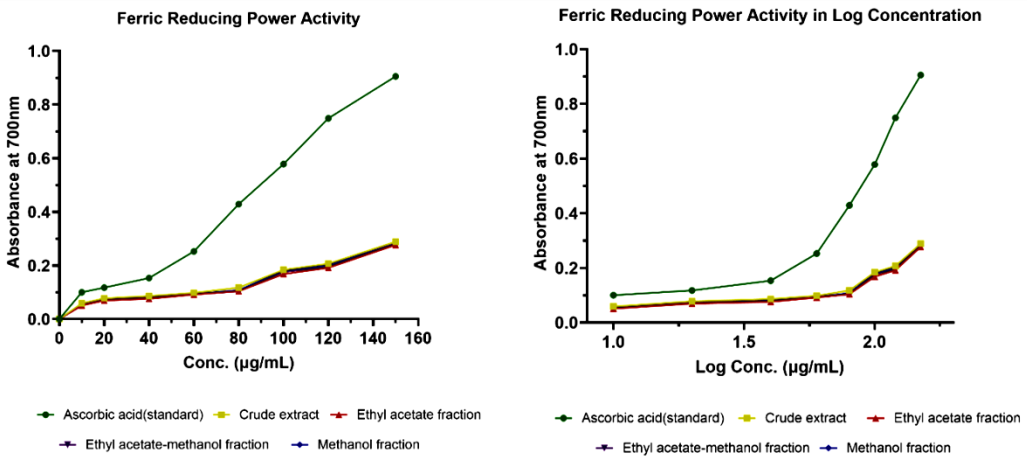


Figure 8: Ferric reducing power activity of *Crateva nurvala* crude extract and its fractions with ascorbic acid (standard)

3.7 IC_{50} values of Crateva nurvala bark extract and its fractions in different antioxidant assays

Table 2: IC_{50} values of *Crateva nurvala* bark extract and its fractions in different antioxidant assays,

Sample	DPPH Scavenging Method (µg/mL)	NO Scavenging Method (µg/mL)	H_3O Scavenging Activity (µg/mL)
Ascorbic Acid	0.8579	2.979	105.8
Crateva nurvala Crude	3.276	7.865	156.5
Ethyl acetate fraction	4.841	10.05	190.8
Ethyl acetate: methanol fraction	4.291	9.413	171.6
Methanol fraction	3.436	8.371	157.5

4. Conclusion:

The growing concerns over their long-term toxicity and the rising cost of synthetic pharmaceuticals have intensified global interest in traditional, plant-based medicine. The profuse growth of *Crateva nurvala* ensures the availability of this plant in this subcontinent region and its use as an herbal complementary medicine. *Crateva nurvala*, long valued in ethnomedicine, offers a promising natural alternative with its rich reservoir of bioactive phytochemicals. In this study, comprehensive HPLC-DAD analysis identified key antioxidant compounds- p-coumaric acid (2), (-) epicatechin (1), and myricetin (3)- which demonstrated strong radical scavenging and reducing power across multiple in vitro assays. Among the tested samples, the methanol fraction and crude extract exhibited the most potent antioxidant effects, with low IC_{50} values and high total antioxidant capacity. These results not only validate the traditional therapeutic claims of *Crateva nurvala* but also address a critical scientific gap by providing quantified biochemical evidence of its efficacy. Given their potential for low toxicity, affordability, and therapeutic effectiveness, plant-derived antioxidants like those in *Crateva nurvala* are increasingly vital in modern healthcare. This study underscores the importance of bioprospecting and phytopharmacological evaluation in discovering safe, natural agents for managing oxidative stress and related metabolic disorders. Thus, *Crateva nurvala* fractions may hold promise as cost-effective nutraceuticals or complementary therapies in the prevention of chronic diseases.

Author Contributions: Conceptualization, MA and NS; methodology, MA, SS, and HCG; software, MA and SS; validation, MA, NS, and HH; formal analysis, MA, MAA, and NS; investigation, ASMS, NMS, SAE, SM and NJ; resources, NJ and RB; data curation, MA and KSA; writing—original draft preparation, SS, HCG; writing—review and editing, MA; visualization, MA, NS; supervision, MA and NS project administration, MA; funding acquisition, MA. All authors have read and agreed to the published version of the manuscript.

Funding: This research did not receive any internal or external funding from profit and non-profit organization.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Data used in this study will be available upon reasonable request from the corresponding author.

Acknowledgments: Authors are gratefully acknowledging the logistic support from the department of Pharmaceutical Sciences, North South University.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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