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Original Arrticle

Evaluation of Preliminary Phytochemical Screening, Estimation of Total Phenolic Content, and Antioxidant Potential of *Amoora rohituka* Roxb

Manjunatha Pai Chennaithody, Rajesh Kaverikana Shankar*

NITTE (Deemed to be University), NGSM Institute of Pharmaceutical Sciences (NGSMIPS), Department of Pharmacology, Mangalore, Karnataka, INDIA.

ABSTRACT

Introduction: The present study aimed to evaluate the antioxidant activity of *Amoora rohituka* Roxb by assessing its phytochemical profile, total phenolic content, and DPPH radical scavenging potential. **Materials and Methods:** Aqueous extract from the leaves of *A. rohituka* was prepared by cold maceration and fractionated using different solvents i.e, petroleum ether, chloroform, n-butanol, methanol, and water fractions according to the increasing order of polarity. All these fractions were then subjected to the qualitative phytochemical tests, phenolic content estimation, and DPPH radical scavenging antioxidant assay. **Results:** Qualitative chemical tests revealed the presence of phenolics, tannins, terpenoids, saponins, and phytosterols in different proportions across the fractions. Among the fractions of *A. rohituka* leaf extract, the order of best total phenolic content and antioxidant capacity was found as follows: petroleum ether fraction, chloroform fraction, methanol fraction, n-buanol fraction, aqueous fraction. Among the various fractions of *A. rohituka* leaf extract, the petroleum ether fraction showed maximum phenolic content 657±0.33 GAE/mg (Gallic acid equivalent) and antioxidant capacity with the lowest IC_{50} value (36.08±0.44 µg/mL). **Conclusion**: A significant and linear relationship was found between the antioxidant activity and phenolic content among the fractions of *A. rohituka* leaf extract, indicating that phenolic compounds could be major contributors to the antioxidant activity. Since oxidative stress is one of the contributing factors for the pathophysiology of various diseases it can be concluded that the medicinal herb *A. rohituka* could be a potential source of evidence to evaluate the diseases related to oxidative stress.

Keywords: *A. rohituka*, Antioxidant, DPPH, Fractions, Phenolic Content.

Correspondence:
Dr. Rajesh Kaverikana Shankar
Assistant Professor, NITTE (Deemed to be University), NGSM Institute of Pharmaceutical Sciences (NGSMIPS), Department of Pharmacology, Mangalore-575018, Karnataka, INDIA.
Email: rajeshks@nitte.edu.in

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INTRODUCTION

Oxidation, while often thought of as harmful, is actually critical for energy production and countless other biochemical tasks that keep cells alive. At the same time, the body constantly churns out free radicals and other Reactive Oxygen Species (ROS), and when too many build up they can shred cell membranes and kill tissue. Scientists now link this oxidative stress to a long list of ailments-cancer, diabetes, heart disease, even the very process of aging.[1] Antioxidants, substances that mop up these aggressive molecules, are therefore essential for guarding the body against such damage. In particular, researchers are looking closely at natural antioxidants like the polyphenols found in many therapeutic herbs along with everyday fruits and vegetables, hoping they can reduce or even block oxidative harm. The human system already runs a powerful in-house defense, composed of both enzymatic tools and small non-enzymatic metabolites that continuously scavenge ROS.[2] On mild days that internal shield is usually enough, yet it can be overwhelmed by heavy or prolonged stress. For that reason, we must regularly supply some extra, outside antioxidants-studies show doing so helps tip the balance back toward health. Although modern derivatives like Butylated Hydroxyl Anisole (BHA) and Butylated Hydroxyl Toluene (BHT) are widely praised in nutraceutical field for their strength, safety concerns remind us that they can carry unwanted side effects and toxicity for people. Therefore, substances-specially those drawn from plants-that help shield cells from harm caused by reactive oxygen species could be useful for both disease prevention and therapy. The phenolic compounds found in many herbs serve this role, because their favorable redox chemistry enables them to donate hydrogen, reduce oxidized species, and trap free radicals.[3]

A. rohituka, a tree in the meliaceae family, grows widely across India and Southeast Asia and has a long history of medicinal use. In Ayurvedic practice, healers apply its bark and leaves to treat

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liver ailments, swollen spleen, tumors, and various inflammatory conditions. This traditional knowledge has spurred detailed phytochemical studies, which confirm that the plant contains flavonoids, limonoids, triterpenoids, steroids, and coumarins. Laboratory trials show that extracts of *A. rohituka* can kill breast cancer cells and trigger programmed cell death in other tumor lines, pointing to its anticancer potential. Chemical assays confirm that *A. rohituka* is abundant in secondary metabolites like terpenoids and limonoids, each linked to diverse bioactivity. Among them, phenolic substances stand out for its ability to mop up free radicals and shield cells from oxidative damage. The presence of these phytochemicals is frequently linked to antioxidant activity, which has a crucial role in reducing the pathogenesis of chronic ailments such as cancer, heart disease, and conditions that affect the nervous system Research into *A. rohituka* has identified a broad spectrum of pharmacological effects, including reductions in inflammation, anticancer action, protection of liver cells, antimicrobial activity, and modulation of immune responses. Investigators believe these roles arise mainly from the plants robust antioxidant system and its elevated levels of phenolic compounds. Nevertheless, systematic measurements of total phenolics and direct comparisons with antioxidant capacity remain sparse in the literature.[4]

In light of this knowledge gap, the current project quantitatively assesses the antioxidant potential and the total phenolic content of the various fractions from the aqueous leaf extracts of *A. rohituka*. A strong correlation between these two parameters would strengthen the plants therapeutic profile and open the door to its application as a natural source of dietary antioxidants.

MATERIALS AND METHODS

Chemicals

Chemical reagents 2,2-diphenyl-1-picryl hydrazyl (DPPH) (SRL Pvt. Ltd.), gallic acid and ascorbic acid (standard), F.C reagent, anhydrous sodium carbonate, petroleum ether, chloroform, n-butanol, methanol (Loba Chemie Pvt. Ltd.). All the reagents and chemicals used were of analytical quality.

Plant material

Leaves of *Amoora rohituka* were collected from Pilikula Botanical Garden, Mangaluru, India, and identified by Ms. Swathi, Department of Applied Botany, Mangalore University. A voucher specimen (Voucher No: SBMU011) has been deposited in the herbarium of the Department of Applied Botany, Mangalore University.

Preparation of the aqueous extract

The leaves of *A. rohituka* were cleaned thoroughly with tap water and then dried in the shade for a week and ground into coarse powder. The powder (around 250 g) was then subjected to cold maceration for 3 days using water as an extracting solvent, squeezed through muslin cloth, and filtered through a vacuum filter. The marc is remacerated after drying and filtered again by a vacuum filter. The obtained filtrate was distilled using a rotary flash evaporator under reduced pressure and then concentrated in a clean petri dish and stored in a dessicator to protect it from moisture.[5]

Fractionation of the aqueous leaf extract of *A. rohituka*

The aqueous extract was subjected to successive solvent fractionation. The process of fractionation was carried out based on the increasing order of polarity among the chosen solvents, i.e, petroleum ether, chloroform, n-butanol, ethanol, and methanol. 50 mL of 5% suspension of the aqueous leaf extract of *A. rohituka* was dissolved in water by sonication for 30 min. The solution was added to a separating funnel, and 50 mL of petroleum ether was added. The mixture was shaken vigorously for 30 min. The two layers of solvent are formed, i.e, petroleum ether layer at the top and water layer at the bottom. The petroleum ether layer has been collected. The same process was repeated for two times by the addition of fresh petroleum ether. The successively collected petroleum ether portion was kept aside for some minutes. Chloroform was then added into the funnel in a quantity equal to the solution of water extract. The same process was followed with successive additions of fresh chloroform. The successively collected portions of chloroform are set aside. The same procedure was applied for n-butanol. Once the n-butanol layer and aqueous layer are separated, the n-butanol layer was dried to obtain the n-butanol fraction, while the aqueous layer was dried to yield the aqueous residue. The aqueous residue was then dissolved in methanol, with the methanol-soluble portion considered as the methanol fraction. The methanol-insoluble portions are dried and collected as the aqueous fraction. The solution of petroleum ether, chloroform, n-butanol, ethanol, and methanol fractions obtained was distilled through a flash rotary evaporator and concentrated in an electric water bath below 50°C. The dried fractions are stored in the desiccator to protect it from moisture (Figures 1 and 2).[6]

Preliminary phytochemical screening

Procedure

The petroleum ether, chloroform, n-butanol, methanol, and aqueous fractions of *A. rohituka* leaf extract were each separately dissolved in water and analysed through preliminary phytochemical screening to analyse the various classes of phytoconstituents.

Procedure

Detection of Alkaloids

a) **Mayer's Test:** The sample solution was tested with Mayer's reagent. The formation of a white precipitate confirms the presence of alkaloids.[7]

b) **Wagner's Test:** On addition of Wagner's reagent to the sample solution, the formation of a reddish-brown precipitate confirms the alkaloids.

c) **Hager's Test:** The sample was mixed with Hager's reagent. A yellow precipitate suggests the presence of alkaloids.

d) **Dragendorff's Test:** Treatment of the sample with Dragendorff's reagent results in a red precipitate if alkaloids are present.

Detection of Flavonoids

a) **Alkaline Reagent Test:** Addition of sodium hydroxide to the sample produces a yellow color, confirming flavonoids.[8]

b) **Lead Acetate Test:** When the sample was added with lead acetate solution, a yellow coloration indicates the presence of flavonoids.

c) **Shinoda Test:** The sample was tested with a magnesium ribbon followed by a few drops of concentrated hydrochloric acid. The formation of a pink to red color signifies flavonoids.

Detection of Tannins

a) **Gelatin Test:** The sample was combined with 1% gelatin solution and 10% sodium chloride. The formation of a white precipitate indicates the tannins.[9]

b) **Ferric Chloride Test:** Upon treating the sample with aqueous ferric chloride solution, a greenish-brown color confirms tannins.

c) **Lead Acetate Test:** Addition of 10% lead acetate to the sample results in a white precipitate, indicating tannins.

d) **Vanillin-HCl Test:** When treated with a few drops of vanillin-HCl, the sample developing a pink color indicates the tannins.

Detection of Glycosides

a) **Borntrager's Test:** The sample was hydrolyzed with dilute hydrochloric acid, heated in a water bath for 10 min, filtered, and the filtrate extracted with benzene. The benzene layer was treated with ammonia and shaken. A pink color indicates glycosides.[10]

b) **Modified Borntrager's Test:** The sample was boiled with dilute HCl and 5% ferric chloride for 10 min, cooled, and filtered. The filtrate is extracted with benzene and treated with ammonia. A pink coloration confirms glycosides.

c) **Legal's Test:** The sample was mixed with pyridine and sodium nitroprusside, followed by 20% sodium hydroxide. A deep red color indicates the glycosides.

Detection of Phenolic Compounds

a) **Ferric Chloride Test:** The addition of ferric chloride to the sample yields a bluish-black color, indicating phenolics.[10]

b) **Lead Acetate Test:** A white precipitate formed upon adding 10% lead acetate solution indicates phenolic compounds.

Detection of Phytosterols

a) **Salkowski Test:** The sample was treated with concentrated sulfuric acid and allowed to stand. The development of a blood red color confirms phytosterols.[11]

b) **Liebermann-Burchard's Test:** The sample was reacted with acetic anhydride, heated, cooled, and then treated with concentrated sulfuric acid. A bluish-green color denotes phytosterols.

Detection of Saponins

The sample was dissolved in distilled water, filtered, and the filtrate is used for the following tests:

a) **Foam Test:** The filtrate was diluted and shaken in a graduated cylinder for 15 min. Formation of stable foam (about 1 cm in height) indicates saponins.[12]

b) **Haemolysis Test:** Fresh sheep blood was collected in an anticoagulant-treated tube and centrifuged at 3000 rpm for 10 min to separate the plasma. RBCs are washed multiple times with saline and resuspended to make a 2% suspension. To this, 2 mL of the test solution was added, mixed, and incubated at 37°C for 1 hr. The appearance of a clear red solution with sedimentation of RBCs indicates saponin-induced haemolysis.

Detection of Diterpenoids

a) **Copper Acetate Test:** The sample was treated with copper acetate solution. An emerald green color confirms the presence of diterpenoids.[13]

Detection of Triterpenoids

a) **Chloroform-Sulfuric Acid Test:** The sample was mixed with chloroform, followed by concentrated sulfuric acid. The appearance of a reddish-brown or yellow color at the interface indicates triterpenoids.[14]

b) **Vanillin-Sulfuric Acid Test:** The sample was treated with vanillin solution and concentrated sulfuric acid. A reddish or pink coloration denotes triterpenoids.

Estimation of Total Phenolic Content

A stock solution (Stock A) was prepared by dissolving gallic acid and each fraction from *Amoora rohituka* leaves in Millipore water at a concentration of 1 mg/mL. From this stock, 0.1 to 0.5 mL of gallic acid and 100 to 200 µL of each leaf fraction were pipetted into separate clean test tubes. To each tube, 1 mL of freshly prepared Folin-Ciocalteu reagent (diluted 1:1) was added and allowed to react for 5 min. Subsequently, 1 mL of 8% sodium carbonate solution was introduced into each tube. The volume in each tube was then adjusted to 5 mL using Millipore water. All tubes were incubated in the dark for 1 hr, after which the absorbance was measured at 765 nm.[15,16]

DPPH radical scavenging antioxidant assay

Stock solutions of ascorbic acid and each fraction from *Amoora rohituka* were prepared in Millipore water at a concentration of 1 mg/mL (referred to as Stock A). From these solutions, volumes ranging from 0.1 to 0.5 mL were transferred into clean test tubes,

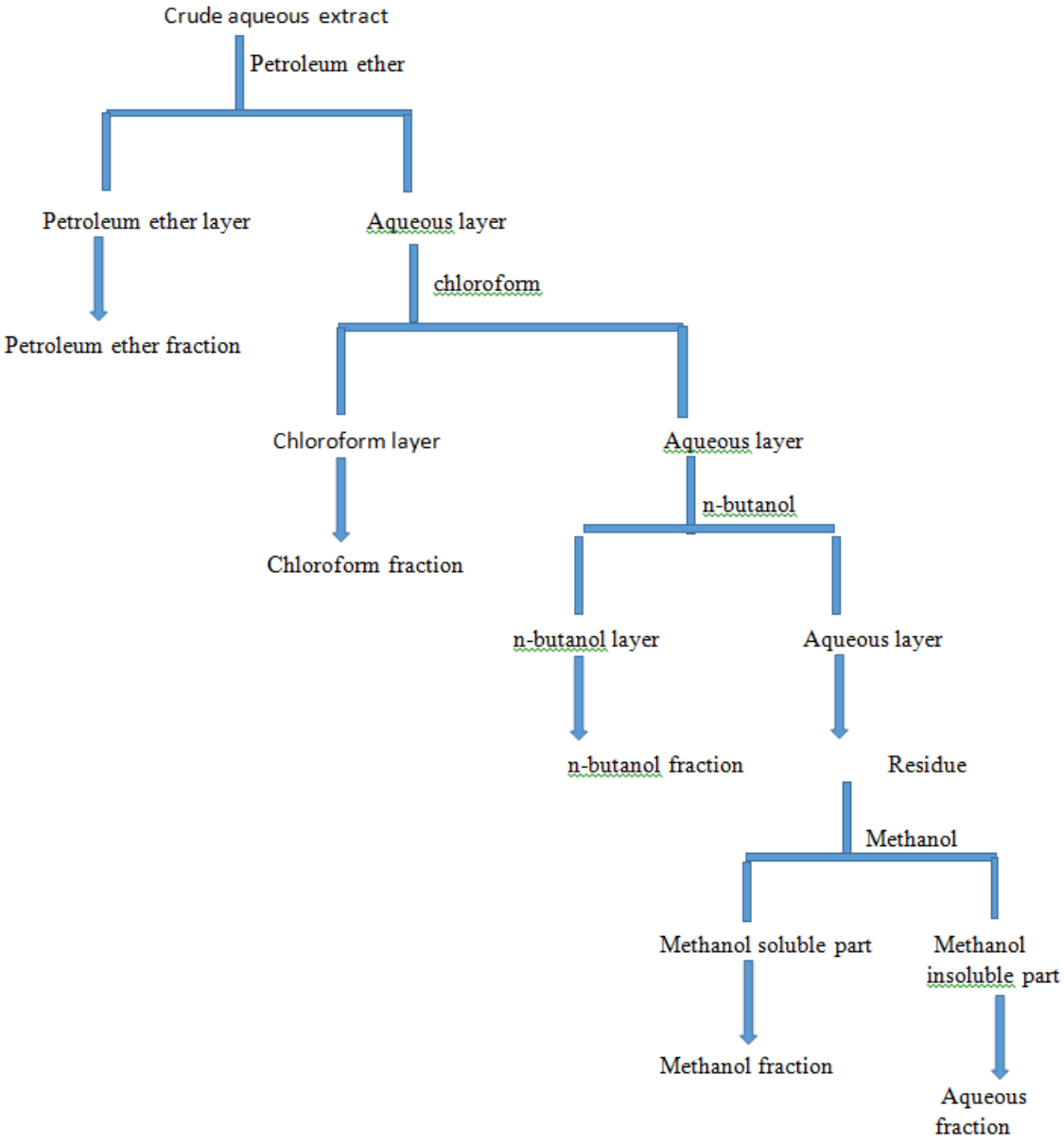

Figure 1: Fractionation of the aqueous leaf extract of *A. rohituka*.

Table 1: Preliminary phytochemical screening of the fractions of *A. rohituka*.

Samples	Pet. Ether fraction	Chloroform Fraction	n-butanol fraction	Methanol fraction	Aqueous fraction
Alkaloids	-	-	-	-	-
Flavonoids	-	-	-	-	-
Glycosides	-	-	-	-	-
Tannins	-	-	+	+	+
Phenolics	+	+	+	+	+
Phytosterols	+	+	-	+	-
Saponins	-	-	+	+	+
Di and tri-terpenoids	+	+	-	-	+

and the volume in each tube was adjusted to 1 mL using methanol. A control solution was prepared by mixing 3 mL of DPPH solution with 1 mL of methanol, adjusting the absorbance to fall between 0.8 and 1. Then, 3 mL of the same DPPH solution was added to each test tube containing either the sample or the standard. After 20 min of incubation, the absorbance of all tubes was measured at 517 nm using a UV-visible spectrophotometer.[17-19]

RESULTS

Percentage extractive yield of aqueous leaf extract of *A. rohituka* was found to be- 6% (15 g)

Yield of fractions obtained by successive solvent fractionation process from 1 g of aqueous leaf extract of A. rohituka is as follows

Petroleum ether fraction- 480 mg (48%),

Chloroform fraction- 210 mg (21%),

N-butanol fraction- 146 mg (14.6%),

Methanolic fraction- 94 mg (9.4%),

Aqueous fraction- 55 mg (5.5%).

Preliminary phytochemical screening the fractions of the *A. rohituka* leaf extract

The results of the phytochemical analysis are represented in Table 1. It revealed the presence of key secondary metabolites including phenolics, tannins, terpenoids, saponins, and phytosterols in varying concentrations across the fractions.

Total phenolic content of the fractions of *A. rohituka*

The results of the total phenolic content estimation are shown in the Table 2. Gallic acid equivalent per milligram of the fractions found in the different fractions of *A. rohituka* aqueous leaf extract such as P.E fraction, chloroform fraction, n-butanol fraction, methanol fractions, aqueous fractions were as follows-657.33±0.33, 589.87±0.41, 451.451±0.56, 495.79±0.53, 270.73±0.61.

Table 2: Gallic acid equivalent/mg (GAE/mg) of the PEFAR, CFAR, BFAR, MFAR, AFAR.

Sl. No.	Sample name	TPC (GAE/Mg)
1	Concentration	25 µg
2	PEFAR	657.33±0.33
3	CFAR	589.87±0.41
4	MFAR	495.79±0.53
5	BFAR	451.41±0.56
6	AFAR	270.73±0.61

Values are given as Mean±S.D.

DPPH radical scavenging antioxidant assay

The results of the percentage radical scavenging of the fractions of *A. rohituka* are indicated in Table 3, and the IC_{50} values are graphically represented in Figure 2. The IC_{50} values obtained for the different fractions of *A. rohituka* aqueous leaf extract such as P.E fraction, chloroform fraction, n-butanol fraction, methanol fractions, aqueous fractions were as follows-36.08±0.63, 48.26±0.19, 90.91±, 53.15±0.73, 106.8±0.6. The IC_{50} value of the standard ascorbic acid were found as 26.13±0.61.

DISCUSSION

Among the fractions of the *A. rohituka* leaf extract, non-polar fractions like petroleum ether fraction, chloroform fractions have shown positive results for phytosterols, di and tri-terpenoids and phenolics.

Mid polar and polar fractions like n-butanolic, methanolic and aqueous fractions has shown a positive results for tannins, phenolics, saponins. Importantly, it was noticed that phenolics were present in all the fractions and terpenoids were also present in aqueous fraction along with non-polar fractions.

The total phenolic content of the *Amoora rohituka* leaf extract fractions was denoted as Gallic Acid Equivalents (GAE) per mg of fraction. Across three different concentrations tested (25 µg, 50 µg, and 75 µg), each fraction displayed relatively consistent results. Among the various fractions, the total phenolic content

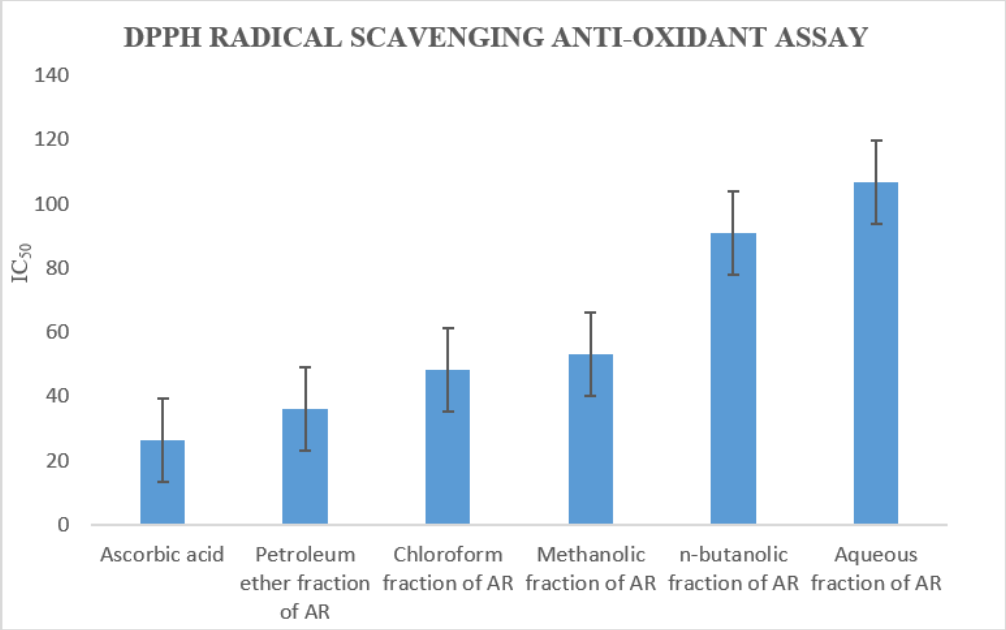

Figure 2: Graphical representation of IC_{50} values of the fractions of *A. rohituka*.

Table 3: Percentage radical scavenging of the fractions of AR leaf extract.

Sl. No.	Concentration (µg/mL)	Standard (ascorbic acid)	CFAR	BFAR	AFAR	PEFAR	MFAR
1	12.5	20.16±0.068	15.64±0.33	18.31±0.56	18.88±1.80	45.14±0.30	13.54±0.27
2	25	29.07±0.253	47.01±0.34	23.21±0.55	20.71±2.71	47.21±0.16	46.02±0.36
3	37.5	34.55±0.574	56.39±0.69	37.45±0.84	24.69±3.21	50.27±0.51	56.76±0.51
4	50	42.87±0.475	61.56±0.37	42.58±0.74	34.97±0.30	53.89±0.40	59.30±0.24
5	62.5	52.31±0.292	63.58±0.25	46.87±0.13	44.54±0.30	58.41±0.14	62.88±0.40
6	75	65.65±0.624	68.50±0.33	53.96±0.61	50.26±0.27	63.84±0.64	64.33±0.40
7	100	69.03±0.554	71.74±0.86	59.76±0.20	54.01±0.29	73.46±0.45	69.49±0.29
8	125	72.27±0.849	74.79±0.32	67.17±0.54	61.75±0.33	79.14±0.40	73.40±0.25

Values are given as Mean±S.D.PEFAR=petroleum ether fraction of *A. rohituka*, CFAR=chloroform fraction of *A. rohituka*, BFAR=n-butanol fraction of *A. rohituka*, MFAR=methanol fraction of *A. rohituka* AFAR= aqueous fraction of *A. rohituka*.

(in terms of GAE/mg) was observed in the following descending order: petroleum ether fraction, chloroform fraction, methanol fraction, n-butanol fraction, and aqueous fraction. Notably, the petroleum ether fraction exhibited the highest phenolic content, recorded at 683.81±0.32 GAE/mg.

Phenolic compounds are vital phytochemicals in plants, primarily due to their free radical scavenging ability attributed because of their hydroxyl groups, which may contribute significantly to antioxidant activity. Based on its strong results in qualitative phytochemical screening and its highest phenolic content, the petroleum ether fraction of *A. rohituka* was expected to exhibit the most potent antioxidant activity among all tested fractions.

Based on the prior knowledge of phenolic content estimation of the fractions of *A. rohituka* this particular antioxidant model was performed by taking ascorbic acid as standard. The results were expressed by percentage inhibition and IC_{50} values. Among the fractions of the *A. rohituka* leaf extract, order of best antioxidant activity was found as follows: Pet ether fraction, chloroform fraction, methanol fraction, n-butanol fraction, aqueous fraction. Hence among the various fractions of *A. rohituka* leaf extract, petroleum ether fraction showed maximum antioxidant capacity with a IC_{50} value of 36.08±0.44 compared to the ascorbic acid (standard) 6.774±0.07. Hence the same relationship was observed among the fractions of AR for the antioxidant activity and phenolic estimation.

CONCLUSION

It is a known factor that free radicals are of significant part in the development of various neurological disorders. The petroleum ether fraction of *Amoora rohituka* leaf extract demonstrated a

high phenolic content along with strong antioxidant activity. Phenolic compounds, widely found in plants, are known to be key contributors to their antioxidant potential. In this study, a clear and significant linear correlation was observed between phenolic content and antioxidant activity, suggesting that phenolic constituents are likely the primary contributors to the observed antioxidant effect. Based on these findings, the petroleum ether fraction holds promise for further investigation in the management of diseases related to oxidative stress. Additionally, *A. rohituka* may serve as a valuable and accessible natural source of antioxidants, offering potential therapeutic benefits.

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ABBREVIATIONS

A. rohituka: *Amoora rohituka*; **P.E ether:** Petroleum Ether; **GAE:** Gallic Acid Equivalent; **ROS:** Reactive Oxygen Species; **DPPH:** 2,2-Diphenyl-1-Picryl Hydrazyl; **PEFAR:** Petroleum Ether Fraction of *A. rohituka*; **CFAR:** Chloroform Fraction of *A. rohituka*; **BFAR:** N-Butanol Fraction of *A. rohituka*; **MFAR:** Methanol Fraction of *A. rohituka*; **AFAR:** Aqueous Fraction of *A. rohituka*.

CONFLICT OF INTEREST

The authors declare that there are no conflict of interest.

SUMMARY

The study evaluated the antioxidant potential of *Amoora rohituka* leaf extracts by phytochemical screening, total phenolic content estimation, and DPPH radical scavenging assay. Various solvent fractions were tested, with the petroleum ether fraction showing the highest phenolic content (657.33±0.33 GAE/mg) and strongest antioxidant activity (IC_{50} 033 GAE±0.44 µg/mL). A strong correlation was observed between phenolic content and antioxidant activity, suggesting phenolics as major contributors. The findings highlight *A. rohituka* as a promising natural antioxidant source for managing oxidative stress-related diseases.

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