Evaluation of Antioxidant Activity of Ricinus Communis Bark Fractions

Shahzadi Bano[1], Abdul Rahman Khan[1], Farogh Ahsan[2], Jamal Akhtar Ansari[1*]

[1]Department of Chemistry, Integral University, Dasauli, Kursi Road, Lucknow (India)-226026

[2]Department of Pharmacy, Integral University, Dasauli, Kursi Road, Lucknow (India)-226026

***Corresponding author:**

Dr. Jamal Akhtar Ansari
Assistant Professor,
Department of Chemistry, Integral University, Lucknow
jamalakahtarindia@gmail.com, jamalakhtar@iul.ac.in

Abstract

Antioxidants are substances that protect cells from damaged caused by free radicals, which are unstable molecules produced during normal metabolism. *Ricinus communis* bark is known for its antimicrobial activity due to presence of diverse group of phytochemicals. The present study aimed to extract and fractionate the bark of *Ricinus communis*, and evaluate the antioxidant activity of different polarity based fractions.

The extraction was performed using methanol, followed by liquid-liquid fractionation with solvents n-hexane, ethyl acetate, chloroform, and methanol. Antioxidant activity was evaluated using DPPH, ABTS and FRAP assays. Among the different fractions, the methanolic fraction yielded the highest amount, followed by hexane, chloroform and ethyl acetate fraction. Among the different fractions, the ethyl acetate fraction exhibited highest antioxidant activity as revealed by lowest IC_{50} values. The overall study demonstrated the antioxidant potential of *R. communis* bark.

Keywords: *Ricinus communis*, Extraction, Fraction, Antioxidants activity.

Introduction

Free radical like reactive oxygen species (ROS), refers to highly reactive oxygen containing molecule or free radicals, are generated during normal cellular metabolism, which can damage cell membrane and DNA. A free radical is an atom or molecule with one or more unpaired electrons, making it unstable, short-lived, and highly reactive. Free radicals have a dual role in the organism, acting as both harmful and beneficial species [1]. Reactive oxygen species (ROS) or Free radicals played a role in normal physiological functions at low to

usual concentrations, serving as essential cellular signalling molecules. Nevertheless, an overabundance of free radicals or a reduction in antioxidant levels can result in oxidative stress. Elevated oxidative stress has been linked to the development of certain age-related chronic diseases [2]. While there is substantial evidence backing the importance of dietary antioxidants in reducing the risk and impact of cancer, cardiovascular issues, and neurodegenerative conditions [3]. Antioxidants are those chemical compounds which have capability to inhibits these free radicals [4]. It is crucial to emphasize that antioxidants ingested through the diet along with other components may not have the same effects as the pure compounds typically utilized in clinical studies. Enzymatic and nonenzymatic are the two primary categories of antioxidants. Enzymatic antioxidants such as dismutase, glutathione peroxidase, and superoxide catalase are internally generated [5]. The non-enzymatic antioxidants found in our diet consist of phytochemicals such as tocopherols, carotenoids, ascorbic acid, flavonoids, and tannins. These antioxidants are primarily sourced from natural plant-based foods and are typically consumed in combination. While epidemiological research suggests that the consumption of medicinal plants can reduce the likelihood of developing various chronic diseases, it remains uncertain whether this outcome is attributed to the antioxidants present, other compounds within the plants, or overall healthy lifestyle habits commonly linked to their consumption [6].

Ricinus communis, a perennial plant from the Euphorbiaceae family, is mainly found in Southeast Asia, India, China, Egypt, Japan, and different regions of Africa. It has been ethnologically used in Indian medicine to treat various health issues such as jaundice, diabetes, hemorrhoids, dysentery, headaches, ringworm infections, and leprosy. Over 50 chemical compounds have been discovered within the plant, including proteins, flavonoids, anthraquinones, fatty acids, volatile components, saponin triterpenes, and other phytoconstituents. Both crude extracts and isolated compounds from *R. Communis* have displayed a wide range of medicinal activities, including antidiabetic, hepatoprotective, antiulcer, anticancer, antihyperlipidemic, analgesic, antioxidant, CNS depressant, immunomodulatory, anti-inflammatory and antimicrobial effects. Although preclinical research has established the plant's safety for human consumption, extensive clinical trials are still required. More research is essential to move from anecdotal evidence in traditional medicine to evidence-based practices. Assessing the plant's pharmacological activities with specific biomarkers will help in understanding the mechanisms behind its therapeutic effects [7].

The extraction method used was customized to align with the polarity of the specific compound being targeted. In accordance with the principle of similar dissolving similar, a solvent will dissolve compounds with matching polarity levels [8]. Flavonoids, which resemble polyphenol compounds found in plants, can be allocated into two groups: aglycon flavonoids and glycoside flavonoids. Aglycon flavonoids do not contain sugar in their structure, while glycoside flavonoids do. The existence of sugar in the glycoside flavonoids structure makes them polar compounds, whereas aglycon flavonoids are less polar [9].

Flavonoids are typically extracted using polar and nonpolar solvents such as chloroform, ethyl acetate, methanol, acetone, hexane, and ethanol. The natural origin substances possess the capacity to counteract obesity through various mechanisms such as regulating lipid absorption, energy intake and expenditure, enhancing lipolysis, reducing lipogenesis, and controlling the differentiation and proliferation of preadipocytes [10]. This study was designed to evaluate the antiradical property of *R. communis* bark different fractions. The fractionation process was carried out through liquid-liquid partition using n-hexane, ethyl acetate, methanol, and chloroform solvents. The antiradical's activity was carried out by DPPH, ABTS and FRAP Assays.

Materials and Methods

Plant materials

Dried mature barks of *Ricinus communis* were collected from the local farmer's field of village Dasauli, Lucknow.

Identification/Authentication

The specimen of collected *Ricinus communis* bark was submitted for identification in the Department of Pharmacognosy, Faculty of Pharmacy, Integral University, Lucknow. The bark was identified by Taxonomist Dr. Mohammad Arif, Associate Professor, Department of Pharmacognosy & Phytochemistry, with a reference number (IU/PHAR/HRB/24/19).

Preparation of *Ricinus communis* bark powder

The collected bark of bark of *R. Communis* were washed, air-dried in shade and powdered with the help of grinder. The resulting *R. Communis* bark powder was stored and labelled.

Extraction and fractionation

The powdered ground bark of *R. Communis* (1 kg) was extracted with 95% methanol. The combined filtrate was concentrated under vacuum using a rotary evaporator and controlled temperature and pressure. The crude extract obtained from the methanol extraction was fractionated based on polarity. The crude extract was first macerated with hexane, followed by chloroform, ethyl acetate, and lastly with methanol. The fractions were completely dried under high vacuum on a rotatory

evaporator, labelled, and kept in a desiccator for further studies.

Antioxidant activity

The antioxidant activity was assessed through the free radical scavenging DPPH assay, ABTS assay, and FRAP assay.

DPPH assay

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity was evaluated based on method given by [11]. It was measured a decrease in absorbance at 517 nm of a solution of coloured DPPH in methanol brought about by the sample. A stock solution of 0.0052 mg/100ml of DPPH was prepared. Ascorbic acid was used as reference material and a stock solution of 1.0 mg/mL in methanol of standard was prepared. Fractions were dissolved in methanol and prepared a concentration of 1.0 mg/mL. Series of reference standard of different concentrations were prepared; 1.0 µg/mL, 2.0 µg/mL, 3.0 µg/mL, 4.0 µg/mL and 5.0 µg/mL. Similarly, fractions were prepared of 100 µg/mL, 200 µg/mL, 400 µg/mL, 800 µg/mL and 1000 µg/mL. Freshly prepared DPPH stock solution was added to each of the above sample tube and absorbance were recorded at 517 nm. DPPH radical scavenging activity was calculated as follows:

% inhibition = [Abs sample- Abs control)/ (Abs of control)] X 100

Control = 1.0 mL Methanol + 1.0 mL DPPH

FRAP assay

Ferric reducing antioxidant power (FRAP) assay was carried out according to the method of [12]. The FRAP mixture was created using acetate buffer (1.6g sodium acetate and 8 ml acetic acid make up to 100mL) (pH 3.6), 10 mM 2,4,6-Tripyridyl-s-Triazine (TPTZ) solution in 40 mM HCl along with 20 mM ferric chloride solution in proportion of 10:1:1 (v/v) respectively. The FRAP reagent was freshly prepared and warmed to 37ºC in an oven before use. 100µL *R. Communis* fractions were added to 3 mL of the FRAP reagent and thoroughly mixed. The absorbance was noted at 593 nm using a spectrophotometer at 0 min and after 4 min. A standard ascorbic acid (0.1%) solution was made ready by dissolving ascorbic acid

(1 mg) in 1 mL of water. The FRAP reagent was used as a blank for both standard and samples.

The FRAP value was calculated using a specific formula:

$$\%\ Inhibition = \frac{Change\ in\ absorbance\ of\ sample\ from\ 0\ to\ 4\ mins}{Change\ in\ absorbance\ of\ standard\ from\ 0\ to\ 4\ mins}\ X\ 1000uM$$

ABTS radical scavenging assay

The ABTS assay was obtained using the method outlined by [13], with stock solutions consisting of 7 mM ABTS solution and 2.4 mM potassium persulfate/ammonium persulfate solution. A working solution was created by combining equal amounts of the two stock solutions and allowing them to react for 12 hours at 30ºC in the absence of light. This

solution was then diluted by mixing 1 mL ABTS solution with 60 mL methanol to achieve an OD at 734 nm using a spectrophotometer. *R. Communis* fractions (1 mL) were mixed with 1 mL of the ABTS solution, and the OD was noted at 734 nm after 7 minutes using the spectrophotometer. All measurements were taken in triplicate. Trolox served as the standard, and the ABTS scavenging capacity of the extract was determined accordingly:

$$ABTS\ radical\ scavenging\ activity\ (\%) = \frac{ABTScontrol - ABTSsample}{ABTS\ Control} X\ 100$$

ABTS control is the absorbance of ABTS radical + methanol; ABTS sample is the absorbance of ABTS radical + sample extract/standard.

Statistical analysis

The statistical analysis was conducted using GraphPad Prism version 8.0 for Windows. Data is presented as the mean±S.D., and group differences were evaluated through one-way analysis of variance (ANOVA). Significance levels were denoted as *P < 0.05, **P < 0.01, and ***P < 0.001.

Results

Extraction and fractionation

The % yield of methanolic crude extract and different fraction have been summarised in **Table 1.** The results show that highest percentage yield was obtained for methanol fraction followed by ethyl acetate which indicated that the *R. Communis* bark holds molecule polar to polar compound on the basis of dissolution properties.

Table 1: % Yields of extracts and fractions of selected plant material

Plant material	Extract	% Yields	Fractions	% Yields
Ricinus communis bark (RC)	Methanolic (MERC)	10.38	Hexane (HFRC)	25.61
			Chloroform (CFRC)	9.33
			Ethyl acetate (EARC)	23.08
			Methanol (MFRC)	39.73

Antioxidant Activity

DPPH Assay

The antioxidant potential of bark extracts was investigated by assessing their ability to reduce DPPH. This process involves the transfer of a hydrogen atom or electron to the DPPH radical, resulting in the formation of 1, 1-diphenyl-2-picrylhydrazine [14]. The reduction of DPPH radicals leads to a color change

from purple to pale yellow, indicating scavenging activity [15]. The antioxidant activity of four different fractions and ascorbic acid against the DPPH assay was evaluated at concentrations ranging from 12.5 to 1000 µg/ml, as depicted in **Figure 1**. The HFRC, CFRC, EFRC, and MFRC extracts exhibited significant concentration-dependent free radical scavenging activity, ranging from 96.73% to 31.09%, 99.24% to 21.49 %, 96.02% to 39.06%, and 94.17% to 23.16%, respectively, in comparison to the standard Ascorbic acid (99.57% to 72.11%). The IC_{50} values of the DPPH assay for the HFRC, CFRC, EFRC, and MFRC were 65.41µg/ml, 61.60µg/ml, 19.23µg/ml, and 36.65µg/ml, respectively, while the standard antioxidant had an IC_{50} of 6.14µg/ml. Lower IC_{50} values are indicative of higher antioxidant activity Table 2 [16].

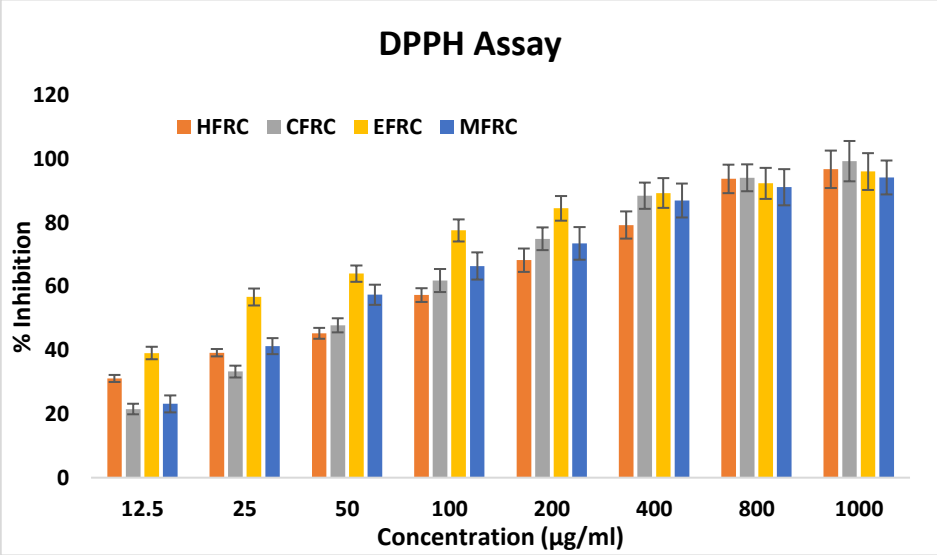

Figure 1: Percent inhibition of DPPH free radicals, after treatment with methanol, ethyl acetate, hexane, and chloroform fractions of *R. Communis bark*

ABTS Assay

The ABTS oxidation by potassium persulfate results in the formation of the ABTS radical cation, which is subsequently reduced by hydrogen-donating antioxidants. This reaction leads to the loss of color in the blue ABTS radical cation [17, 18]. The ABTS foraging activity of the four fractions is presented in **Figure 2**. The graph above illustrates that as the fractions concentration rises, the percentage of inhibition also rises. The HFRC, CFRC, EFRC, and MFRC extracts exhibited significant concentration-dependent free radical scavenging activity, ranging from 94.15% to 37.64%, 72.56% to 22.13 %, 92.71% to 36.26%, and 74.16% to 25.04%, respectively. The standard antioxidant exhibited an IC_{50} value of 16.09µg/ml.

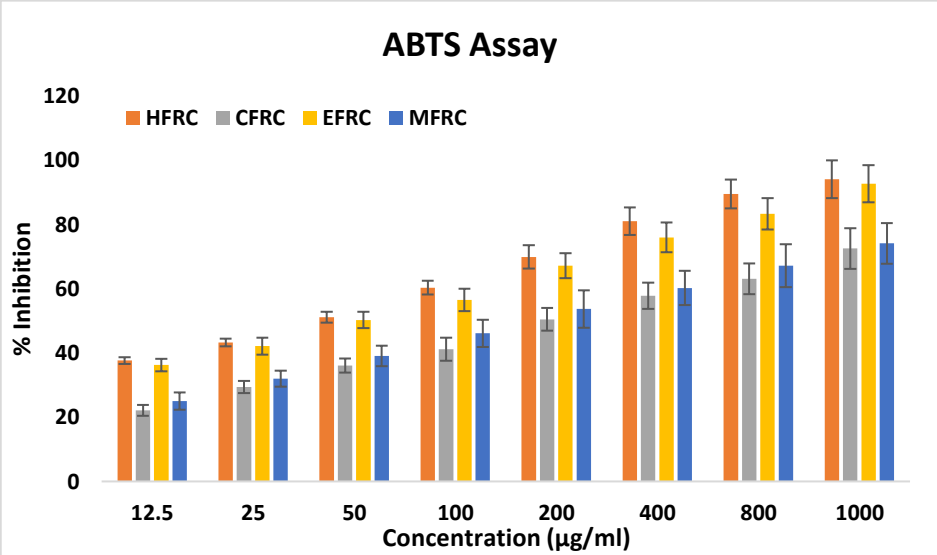

Figure 2: Percent inhibition of ABTS activity after treatment with methanol, ethyl acetate, hexane and chloroform fractions of *R. Communis* bark

FRAP Assay

The fractions were evaluated for their antioxidant activity in scavenging FRAP assay, and the results were compared to ascorbic acid. The maximum antioxidant scavenging activity among all fractions was observed at a concentration of 1000 µg/mL. The HFRC, CFRC, HFRC, and MFRC extracts exhibited moderate FRAP radical scavenging activity, with values ranging from 89.11 to 21.09, 91.52 to 26.54, 97.18 to 32.52, and 92.22 to 35.06, respectively, when compared to ascorbic acid (99.57 to 68.11) (Figure 3). The standard antioxidant exhibited an IC_{50} value of 23.49µg/ml.

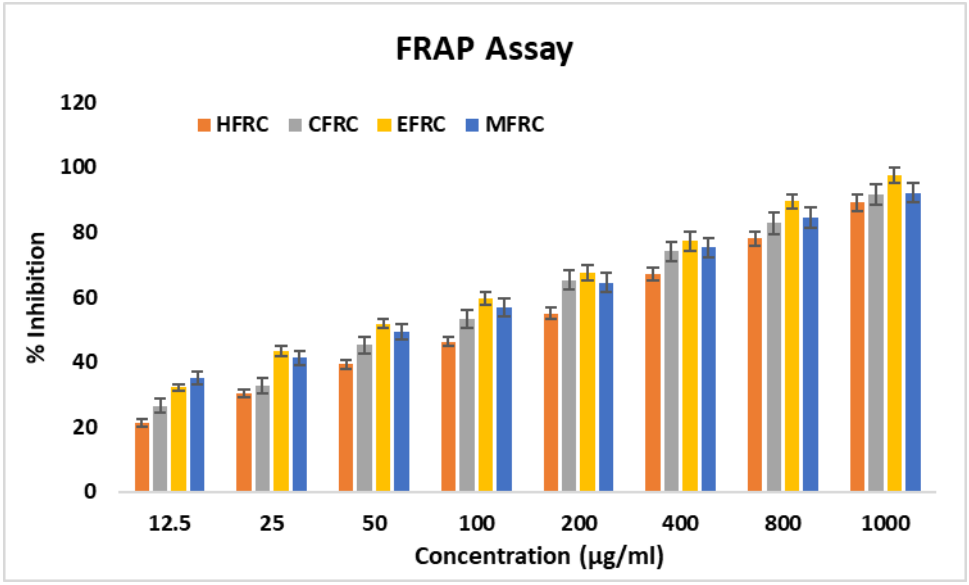

Figure 3: Percent inhibition of FRAP Analysis after treatment with methanol, ethyl acetate, hexane, and chloroform fractions of *R. Communis* bark

Table 2: IC_{50} value of different fractions of *R. Communis* and standard

Fractions	IC_{50} (µg/ml)		
	DPPH	**ABTS**	**FRAP**
HFRC	65.41	36.48	92.6
CFRC	61.6	51.11	58.8
EFRC	19.23	34.43	40.25
MFRC	36.65	40.2	37.58
Ascorbic acid (Standard)	6.14	-	23.49
Trolex Solution	-	16.09	-

Discussion

The extensive variety within the plant kingdom includes large plants with miscellaneous therapeutic properties [19]. One such plant is *R. Communis*, which is recognized for its composition containing numerous bioactive chemical compounds such as phenolic acids, flavonoids, tannins, terpenoids, saponins, and polyphenols. These compounds are accountable for a range of activities, including antioxidant, antifungal, and antimicrobial effects as evidenced in prior studies.

Our initial phytochemical screening of bark also confirmed the presence of these compounds. Hence, it is conceivable that the antioxidant properties of the fractionated extract could also be linked to the presence of additional antioxidants. Flavonoids were found in the ethyl acetate, hexane, methanol, and chloroform fractions. This is due to the solvent's polarity level, which determines its ability to dissolve flavonoids and the compound being extracted.

The DPPH radical scavenging assay is a commonly utilized technique for assessing the antioxidant properties of natural compounds and plant extracts. The extent of color change reflects the scavenging ability of the antioxidant extract, which is attributed to its ability to donate hydrogen atoms [20]. The experimental outcomes verified that all four *R. communis* fractions exhibited the capability to scavenge free radicals, showing a correlation with the dosage in DPPH radical scavenging activity.

The evaluation of antioxidant properties in

methanolic, ethyl acetate, chloroform, and methanol fractions was conducted. The DPPH radical scavenging method was employed for this purpose due to its simplicity, ease of use, and requirement of only a small sample size [21]. Furthermore, this approach eliminates the need for a substrate as free radicals are readily accessible to take the place of the substrate. The DPPH molecule acts as a free radical due to the electron delocalization surrounding the molecule. The appearance of antioxidant properties in the sample led to the fading of the DPPH solution in methanol [22]. This approach utilizes IC_{50} as a parameter for assessing the concentration of antioxidant compounds capable of inhibiting 50% of free radicals. As per [23] and various textbooks, antioxidant effectiveness is classified as very potent if IC_{50} <50 µg/mL, strong for IC_{50} values ranging from 50-100 µg/mL, moderate at 101-250 µg/mL, weak for IC_{50} values of 250-500 µg/mL, and inactive if IC_{50} exceeds 500 µg/mL. The total flavonoid content and total phenolic content can be linked to the overall antioxidant activity, as indicated by the IC_{50} of DPPH. Among ethyl acetate filtrate, methanolic hexane, chloroform fraction, and ascorbic acid as a positive control, ethyl acetate filtrate exhibits the highest antioxidant activity. However, based on IC_{50} results, the ethyl acetate fraction is considered to have a very potent antioxidant activity due to its IC_{50} value falling below 50µg/mL. The antioxidant potential of flavonoids and phenolic compounds primarily stems from their ability to act as hydrogen or electron donors, where a hydrogen atom from an aromatic hydroxyl (OH) group can be donated to a free radical [24]. The above DPPH activity was also supported by FRAP and ABTS assay.

Conclusion

The current study's findings revealed that percentage yields of four fractions. Among these fractions, the ethyl acetate fraction of *R. Communis* exhibited the highest antioxidant properties. Bioactive compounds found in the plants could potentially hinder enzyme activity, highlighting the necessity for further exploration to identify and characterize these constituents.

Declaration

Ethics Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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Conflict of Interest

No conflicts of interest.

Authors Contribution

SB: Help in experimentation; JAA: Validation of results and Reviewing the manuscript; FA: Visualization; ARK: Conceptualization

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