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Original Research Article

Antioxidant Activity, Phenolic and Flavonoid Contents of Abrus precatorius Leaf in four Different Extracts

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Abstract: This research aimed to assess the antioxidant capabilities against DPPH, as well as determine the overall phenolic and flavonoid contents in extracts of abrus precatorius leaves. Different extracts of abrus precatorius leaves were successively prepared using methanol, ethanol, petroleum ether, and distilled water. The DPPH antioxidant capacity of methanol extracts was highest with a low IC_{50} value of 62.86±0.68µg/mL. Ethanol, water, and petroleum ether extracts also showed significant antioxidant activity with IC_{50} values of 89.66±0.51, 78.67±0.35, 142±4.26 µg/mL. We observed a significantly higher total phenolic content of 34.67± 2.52 gallic acid equivalent / g dry extract and total flavonoid content of 27.55±1.84 mg quercetin equivalent/g dry extract for methanolic leaves extract than others. The phenolic content of ethanol and water extract is close enough (22.19±1.45, 26.35±1.65 gallic acid equivalent / g dry extract). The flavonoid content of ethanol, water, and petroleum ether extract was found as 14.43±1.35, 20.84±1.97, 1.6±0.41 mg quercetin equivalent/g dry extract. These findings will aid in future studies attempting to explore medicinal agents from the abrus precatorius plants of Bangladesh.

Keywords: Abrus precatorius, phytochemicals, DPPH antioxidant activity, phenolic, flavonoid.

1. INTRODUCTION

Free radicals are harmful because they can "steal" electrons from other molecules in the body, leading to a chain reaction of oxidative damage to DNA, proteins, and lipids. This damage, known as oxidative stress, is implicated in various diseases, including cancer, liver cirrhosis, cardiovascular diseases, asthma, arthritis, neurodegenerative disorders, Parkinson's disease and aging [1-5]. To counteract the harmful effects of free radicals, the body has built-in antioxidant defense systems that neutralize them. Our body's natural antioxidant defenses are usually adequate to keep free radicals in check. Antioxidants act as scavengers, eliminating free radicals to shield cells from harm, ultimately benefiting human health. The body's innate antioxidant defense systems act as shields, safeguarding against the detrimental effects of oxidative harm [6, 7]. But in certain situations, such as when we're exposed to excessive free radicals (e.g., through pollution or smoking) or when our bodies are under stress, our natural defenses can become overwhelmed. This is why it's important to get antioxidants from our diet. Many natural products, particularly those from plants, are rich in antioxidants [8]. These compounds play a pivotal role in combating oxidative stress by neutralizing harmful free radicals and preventing cell and tissue damage by delaying, intercepting, or preventing chemical reactions caused by free radicals [9]. Among the diverse sources of antioxidants, plant extracts have become a focus of extensive research, primarily due to their richness in phenolic and flavonoid compounds [10-12].

Abrus precatorius belongs to the Fabaceae family and is a deciduous dextrose climber with slender flexible branches [13-16]. Locally in Bangladesh, it is known as kunch, ratti, sarakao(marma). Medicinal plants represent an important component of traditional medicine to combat various disease conditions [17]. The leaves bear a resemblance to tamarind leaves. The leaves are typically munched on or delicately savored to experience their pleasant, natural sweetness [18]. Abrus precatorius extract possesses potent antibacterial and

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antifungal properties [19] and also functions as an effective insect repellent [20]. They prove beneficial to alleviate allergic reactions and act as antiasthmatic agents [21], supporting ocular health by countering cataract formation [22]. Their cytotoxic potential enables them to induce cell death in specific contexts [23]. Some studies show that they exhibit anticancer potential [24], and can inhibit tumor growth [25]. Their analgesic properties provide relief from pain [26], and they show promise in regulating blood sugar levels [27, 28]. Other pharmacological activities exhibited include antimalarial [29, 30], antispasmodic [31], antiepileptic [32], antiserotonergic [33], antimigraine [34], antiarthritic [35] abortifacient [36], antihelminthic [37], nephroprotective [38-40] & neuroprotective [41]. Abrus precatorius extracts have demonstrated efficacy in countering antifertility [42-48], decreased sperm motility, and altered sperm morphology in epididymis, antiestrogenic [49, 50]. Other application antioxidative [51], immunostimulatory [52-53], antidepression [54]. According to phytochemical screening tests, the leaf extract of A. precatorius principally contains phenolics, flavonoids, carbohydrates, alkaloids, glycosides, steroids, terpenoids, tannins, quinones, fatty acids and coumarins. The current study was designed to assess the antioxidative potential, total phenolic content, and total flavonoid content in aqueous, methanol, ethanol and petroleum ether extracts of A. precatorius leaves using the DPPH scavenging assay, Folin-Ciocalteau reagent, and aluminum chloride techniques.

2. METHODS AND MATERIALS

2.1 Chemicals and reagents

2,2-dipphenyl-1-picrylhydrazyl (DPPH) was procured from Sigma-Aldrich Chemicals Co. (Germany). All other chemicals like Folin-Ciocalteau reagent, ascorbic acid, quercetin, methanol, ethanol, and petroleum ether used were of analytical grade.

2.2 Abrus precatorius leafs

Fresh leaf of abrus precatorius was collected from Chattogram region, Bangladesh. It was taxonomically authenticated by the Industrial Botany Research Division of BCSIR Chattogram Laboratories, Bangladesh Council of Scientific and Industrial Research (BCSIR).

2.3 Preparation of extracts

The leaves after collection were cleaned under running tap water and dried under sunlight for a few days in the laboratory. After grinding the materials into fine powder using a mixing grinder, the powders were then soaked for 15 days at room temperature in four different solvents (water, methanol, ethanol, petroleum ether). The next step involved filtering each of the extracts through the Whatman No. 1 filter paper. The solvents were evaporated using a rotating vacuum evaporator (Rotavapor R- 300, Buchi, Switzerland). The dried extracts were used for phytochemical screening, antioxidant activity evaluation, and total phenolic & flavonoid content determination.

2.4 Phytochemical screening

The plant extract test solution was prepared by diluting the dry extracts in the same solvent for the phytochemical test.

Detection of alkaloids: Dragen-Dorff's reagent was allowed to mix with 1mL of each extract test solution. The presence of alkaloids was confirmed by the formation of bright orange precipitates.

Detection of sugars: 1 mL of 10 % methanolic α - naphthol solution and 1 mL of each test solution were mixed, and then 4 to 5 drops of concentrated H_2SO_4 were added along the side of the test tube. Observation for violet ring indicates the presence of glycoside or sugar.

Detection of protein and amino acid: Adding 4 % sodium hydroxide followed by a few drops of 15 % copper sulfate to a drop of extract test solution. A pink color indicates a positive result.

Detection of sterol: 2 mL of into 1 mL of test solution was taken in a test tube and 2 mL concentrated H_2SO_4 was added along the side of the test tube, forming two layers and developing of red color confirming the presence of sterol.

Test for cardiac glycosides: 1 mL of extract was treated with 0.4 ml glacial acetic acid, ferric chloride solution, and conc. H_2SO_4. Brown color ring formation indicates positive results.

Test for coumarin: 2 mL of extract was shaken well for 5 minutes with 10% NaOH and observed for yellow color.

Detection of terpenoids: 0.5 mL of the extract was taken with 2 mL of chloroform and conc. sulphuric acid. The formation of a red-brown color at the interface confirms the positive results of terpenoids.

Detection of tannins: Addition of 2 mL of a 5% ferric chloride solution to 1 mL of the extract. The appearance of a dark blue or greenish-black color indicates the presence of tannins.

Detection of saponins: 2 mL of distilled water was shaken with 2 mL extract for 15 mins. The formation of a layer of foam indicates the presence of saponins.

Detection of quinones: To 1 mL of concentrated sulphuric acid, 1 mL of each extract test solution was added. The red color indicates the presence of quinones.

2.5 DPPH Antioxidant Assay

The free radical scavenging activity of each crude extract was calculated with minor modifications by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay [55]. A 0.004%(w/v) freshly prepared DPPH methanolic solution was incubated in the dark for 2 hours before examination. Dissolution of each crude extract was carried out using the solvent used for its extraction. For each extract 5000µg/mL stock solution was prepared and serial dilution to different concentrations (500, 250, 125, 62.5, 31.25, & 15.62 µg/mL) was done from the stock solution. Ascorbic acid(0.002%w/v) was used as a reference. The final solution was prepared in a test tube by taking 2mL extract solution of different concentrations/ascorbic acid followed by the addition of 3mL of DPPH.3mL DPPH and 2mL solvent was used as control. After an incubation time of half an hour in the dark, the absorbance was measured at 517 nm using a UV spectrophotometer. The scavenging activity of DPPH free radical in percent was calculated as:

$$\text{Scavenging ability}(\%) = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100$$

IC_{50} was calculated using the above equation. Here, the IC_{50} value is the concentration at which the tested compound effectively scavenges 50% of the DPPH radicals in the reaction mixture, demonstrating its ability to counteract oxidative stress.

2.6 Total Phenolic Content:

The total phenolics of the four extracts were determined using the Folin-Ciocalteu reagent described by Singleton *et al.*, [56]. The extracts(1mL of different concentration) was mixed with 10% 5mL Folin Ciocalteu reagent and 75% 4 mL of sodium carbonate in a test tube. Then, the mixture in tubes was vortexed for about 15 seconds. Then it was allowed to stand for 2 hours at 40°C for the development of color. Using the same approach, a calibration curve for quercetin was created at concentrations ranging from 5 to 100 µg/mL. Absorbance was measured in a spectrophotometer at 765nm. A calibration curve was prepared for standard gallic acid & total phenolic content of the extracts is expressed as mg GAE / gm of dw.

2.7 Total Flavonoid Content:

The flavonoids can be estimated by the aluminum chloride technique [57]. 2mL of 2% $AlCl_3$ ethanolic solution was added to 2ml of the sample and allowed to stand for one hour at room temperature. Absorbance was taken at 420nm after one hour. Using the same approach, a calibration curve for quercetin was created at concentrations ranging from 5 to 100 µg/mL. Total flavonoid content is then calculated as mg of quercetin equivalent per g of dw, using the calibration curve equation obtained.

3. RESULTS

3.1 Phytochemical screening

The qualitative phytochemical screening tests revealed the presence of various secondary metabolites alkaloids, flavonoids, carbohydrates, phenols, glycosides, saponins, steroids, terpenoids, tannins, quinones, fatty acids, and coumarins in the extracts (Table 1). The phytochemical screening of methanolic leaf extract reported the presence of phytochemicals with the presence of alkaloids, sugar, protein, terpenoid, sterol, cardiac glycoside, coumarin, tannin, saponin & quinone. Ethanol extract of Abrus precatorius leaves revealed the presence of alkaloids, sugar, protein, terpenoid, coumarin, tannin, & saponin as phytoconstituents. This result of ethanol extract is consistent with that found by. Boye *et al*., and Taur *et al.*, [58, 21]. Quinones, saponin, protein, glycosides, and coumarin are not present in the aqueous extract. However, the results of aqueous extract in a previous study by Solanki *et al.* found the presence of saponin in phytochemical tests which may be due to differences in extraction method [59]. Alkaloids, proteins, tannins, steroids, and glycosides are present in petroleum ether extract.

Table 1: Phytochemical Screening of the extracts

Tests name / Leaves Extracts	Alkaloid	Sugar	Protein	Terpenoid	Sterol	Cardiac glycoside	Coumarin	Tannin	Saponin	Quinone
Methanol	+	+	+	+	+	+	+	+	+	+
Ethanol	+	+	+	+	_	_	+	+	+	_
Water	+	+	_	+	+	_	_	+	_	_
Pet. ether	+	+	+	_	+	+	_	+	_	_

3.2 Antioxidant

The DPPH antioxidant test assesses the antioxidant capacity of a sample. It is based on the principle that antioxidants can donate electrons to neutralize the stable DPPH free radical, causing a color change from purple to yellow. The extent of this color change is a direct measure of the sample's antioxidant activity, with greater color change indicating higher antioxidant potential.Significant antioxidant efficacy was observed in all four extracts and this efficacy exhibited a proportional rise with increasing concentration. (Figure 1).

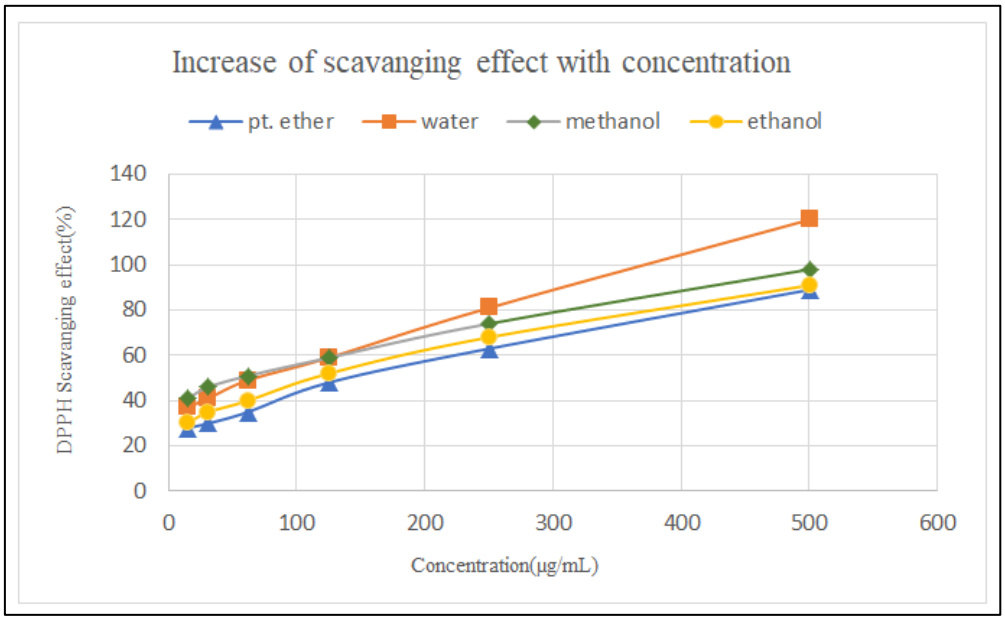

Figure 1: Scavenging activities of Abrus precatorius leaf extracts at varying concentrations

The IC_{50} values of different solvent extracts of the samples are depicted in Figure 2 and Table 2. The IC_{50} value of ascorbic acid was 8.38 ±0.97 µg/mL. A lower IC_{50} value signifies a higher level of antioxidant activity. The IC_{50} value of methanol leaves extract was (62.86±0.68 µg/ml) which was lower than other extracts and different from that found in another report 59.39 ± 1.58 % inhibition at 50 µg/mL.[60]. The IC_{50} values of ethanol and water extracts were 89.66±0.51µg/ml and 78.67±0.35µg/ml, which are different from that of the previous report [61]. Whereas, petroleum ether extracts showed the least DPPH radical scavenging among the four with an IC_{50} value of 142.45±4.26 µg/ml.

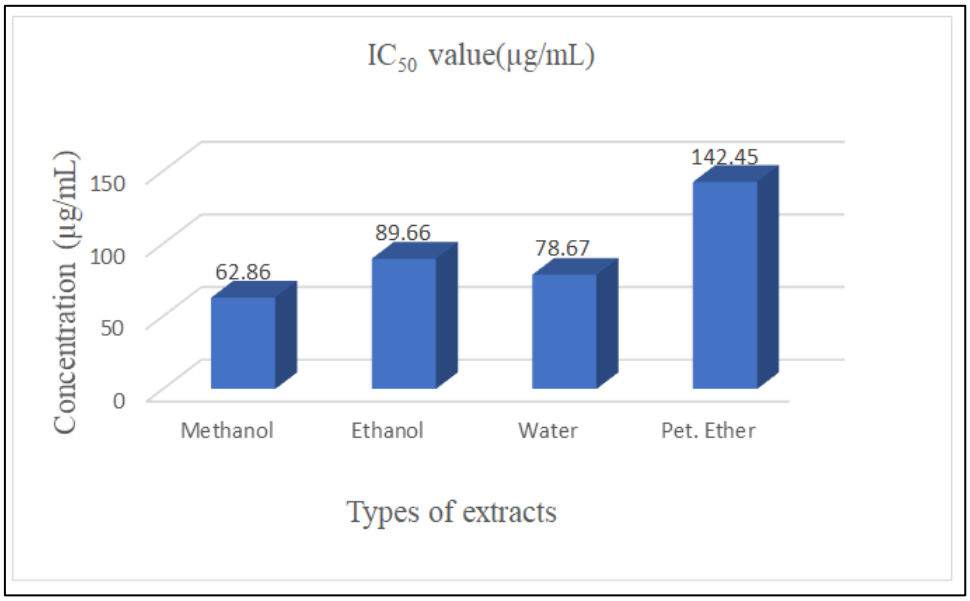

Figure 2: IC_{50} value of the test samples

The following table presents findings of quantitative assessment of antioxidant, phenolic and flavonoid contents.

Table 2: Quantitative assessment of antioxidant activities, phenolic and flavonoid content in various abrus precatorius extracts. The data is presented as the average value ± standard deviation (n = 3)

Extracts of Abrus precatorius	IC_{50} value(µg/mL)	Total Phenolic content(GAE mg/g)	Total Flavonoid content (quercetin mg/g)
Methanolic	62.86±0.68	34.67±2.52	27.55±1.84
Ethanolic	89.66±0.51	22.19±1.45	14.43±1.35
Water	78.67±0.35	26.35±1.65	20.84±1.97
Petroleum ether	142.45±4.26	8.57±0.71	1.6±0.41

3.3 Phenolics

The outcomes of total phenolic content in leaf extracts of abrus precatorius assessed by the Folin-Ciocalteu method are presented in Figure 3. And Table 2. The more phenolic content in extracts, the more the shifting of electrons from phenolic compounds to the phosphomolybdic acid in the reagent. Variations in the total polyphenolic content among extracts were observed due to the specific solvents used during the extraction. The findings showed that methanolic extract possessed a significant amount of phenolics amounting to 34.67 ± 2.52 mg GAE/g dry extract, while the GAE value of ethanolic extract was 22.19 ± 1.45 mg/g dry extract. The total phenolic content in the water extract was found to be 26.57±1.65mg GAE g of dried extract. The result of the petroleum ether extract showed less amount of flavonoid which is 8.57± 0.71 mg GAE /g dry extract. The phenolic content values for water, methanol, and ethanol extracts were found to be quite different and higher than the previous report where the values reported were as 3.33±0.8, 4.59±0.1, 4.65±0.1 mg GAE/g [62].

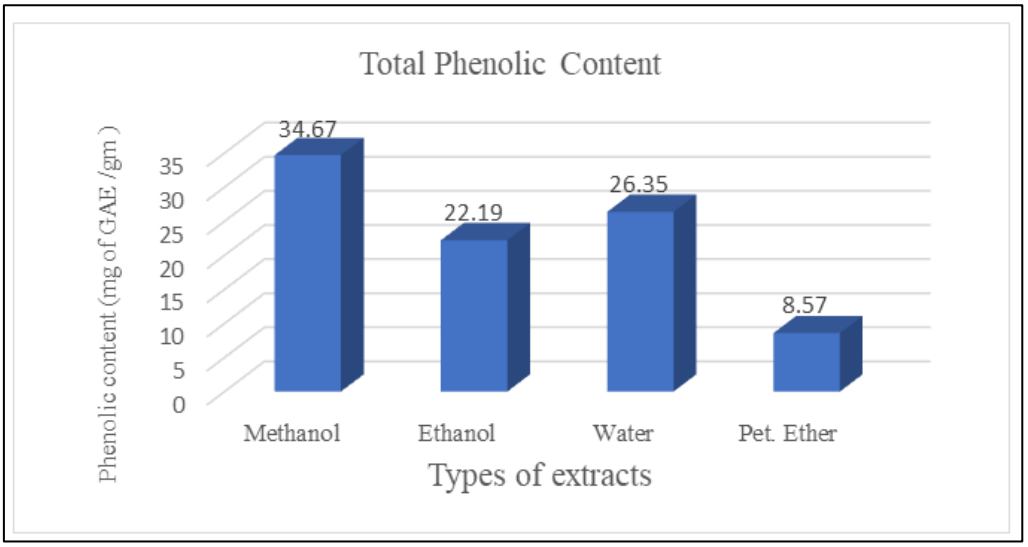

Figure 3: Total phenolics in extracts

3.4 Flavonoids

Total flavonoid content (TFC) represents the amount of flavonoids in extract, which are the largest class of plant phenolic. The flavonoid quantities in the extracts, expressed as quercetin equivalents, ranged from 1.6 ± 0.41 to 27.55 ± 1.84 mg/g of dry weight. The methanolic extract exhibited higher flavonoid content (27.55 ± 1.84 mg/g dw) in comparison to the other three extracts. The flavonoid content found in the water, ethanol, and petroleum ether extracts was 20.84±1.97,14.43±1.35,1.67 ±0.41 mg quercetin/ g of dried extract. The amount of flavonoid content found in ethanol extract in this study is almost similar to that found by Guz *et al.,* which was 17.16 ± 1.04 mg /g dw while the value of water extract differs a lot which was 10.70 ± 0.56 mg/g dw [61].

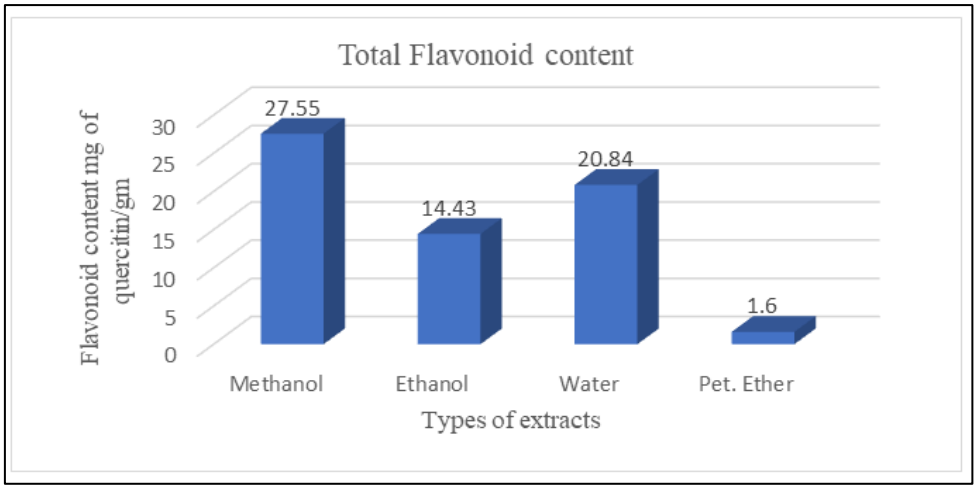

Figure 4: Total Flavonoid content in leaf extracts

4. CONCLUSION

The current study's findings provide experimental proof that A. precatorius leaves contain substantial quantities of polyphenols and flavonoids, displaying noteworthy antioxidant properties across all tested solvents (ethanol, petroleum ether, water, and methanol). Among these, the methanolic solvent was best for extracting the primary phytochemicals due to its ability to dissolve a wide range of polar compounds. Consequently, it was observed that the methanolic extract of A. precatorius leaves possesses notable levels of total phenolic and flavonoid content, exhibiting more antioxidant capabilities compared to extracts obtained using ethanol, water, and petroleum ether. Further investigations are required to extract and identify the potent bioactive constituents from this plant. This presents opportunities for creating natural pharmaceuticals, utilizing the isolated antioxidant compounds from A. precatorius for enhancing food preservation and as nutraceuticals against degenerative diseases.

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