In Vitro Antioxidant Activity and Phytochemical Assessment of Achyranthes Aspera (L.) Leaf Extracts

Ku. Archana G. Kate[1], Dr. Ravindra S. Matte[2]
[1]*Bhiwaji Warbhe Vidyalaya (Bothali), Chimur, Chandrapur- 442 906*
[2]*Head and Associate professor, Department of Botany, Lokmanya Tilak Mahavidyalaya, Wani, Yavatmal- 445 304*
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***Abstract*—Achyranthes aspera L. is a medicinally important plant widely used in traditional healthcare systems. The present study was carried out to evaluate the extractive values, phytochemical composition, and antioxidant potential of A. aspera leaves. Extractive values were determined using six solvents of different polarities, namely methanol, ethanol, acetone, ethyl acetate, chloroform, and petroleum ether. Among the solvents tested, methanol showed the highest extractive value (3.090 ± 0.135%), followed by ethanol (2.910 ± 0.258%), whereas the lowest extractive yield was observed in chloroform (1.102 ± 0.023%) and petroleum ether (1.230 ± 0.351%). Quantitative phytochemical analysis revealed significant variation in total phenolic and flavonoid contents across different solvent extracts. The highest total phenolic content was observed in the ethyl acetate extract (198 ± 0.158 mg/g gallic acid equivalents), followed by methanol (176 ± 0.110 mg/g GAE), while the lowest phenolic content was recorded in petroleum ether extract (33 ± 0.158 mg/g GAE). Total flavonoid content was found to be maximum in the methanolic extract (48 ± 0.210 mg/g quercetin equivalents), followed by ethyl acetate (17.59 ± 0.174 mg/g QE), acetone (11.32 ± 0.154 mg/g QE), and petroleum ether (7 ± 0.256 mg/g QE).**

The antioxidant potential of the methanolic leaf extract was evaluated using various in vitro antioxidant assays. The extract exhibited moderate free radical scavenging activity with IC_{50} values of 47.40 ± 0.112 µg/mL for DPPH, 68.19 ± 0.300 µg/mL for ABTS, and 71.12 ± 0.510 µg/mL for DMPD assays. The ferric reducing antioxidant power (FRAP) was found to be 0.519 ± 0.012, while the total antioxidant activity (TAA) was recorded as 0.396 ± 0.005. Ascorbic acid, BHT and BHA were used as the standard antioxidant in all assays. The findings suggest that Achyranthes aspera leaves are a promising source of natural antioxidants, attributed to their phenolic and flavonoid constituents.

***Index Terms*—Achyranthes aspera, Extractive value, DPPH, ABTS and DMPD, Phytochemical analysis, Phenolic compounds, Flavonoids, Ascorbic acid, etc.**

I. INTRODUCTION

Plants have served as a primary source of medicine since prehistoric times, forming the foundation of traditional healing systems that have been transmitted across generations (Gurib-Fakim, 2006; Jamshidi-Kia et al., 2017). Even today, it is estimated that nearly 80% of the global population depends on plant-based remedies for their primary healthcare needs, particularly in developing countries (Solomons, 2000; Majeed, 2017). The growing global interest in herbal medicine has intensified scientific exploration of medicinal plants, especially those used in indigenous healthcare systems, with the aim of validating traditional claims and discovering novel therapeutic agents (Sen et al., 2011; Saggar et al., 2022; Manisha et al., 2025).

India, owing to its remarkable cultural and biological diversity, represents a rich reservoir of ethnobotanical knowledge. Numerous ethnic communities inhabiting diverse ecological regions rely extensively on native plant species for the treatment of various ailments and have preserved valuable traditional knowledge related to herbal therapeutics (Anyinam, 1995; Sharma et al., 2021; Dean, 2024). Documentation and scientific evaluation of such indigenous knowledge are crucial, as they not only facilitate drug discovery but also contribute to biodiversity conservation and the sustainable utilization of medicinal plants (King et al., 1996; Oguamanam, 2006; Davis & Choisy, 2024).

Achyranthes aspera (L.) (Amaranthaceae) is a widely distributed medicinal plant found across Asia, Africa, and South America. In Indian traditional medicine, A.

aspera is extensively used for the treatment of fever, inflammation, rheumatism, gastrointestinal disorders, respiratory ailments, diarrhea, dysentery, asthma, renal disorders, and throat infections (Srivastav et al., 2011; Akbar, 2020; Chaudhary, 2025). In other traditional medicinal systems, the plant is also recognized for its diuretic, hepatoprotective, emmenagogue, antihypertensive, antidiabetic, and antimalarial properties (Verma et al., 2021; Jain et al., 2024).

Oxidative stress, resulting from an imbalance between the generation of reactive oxygen species (ROS) and the antioxidant defense system, plays a pivotal role in the pathogenesis of numerous chronic and degenerative diseases, including cancer, diabetes mellitus, cardiovascular disorders, neurodegenerative diseases, atherosclerosis, and inflammatory conditions (Ozougwu, 2016; Ansari et al., 2025). Reactive oxygen species such as superoxide anion, hydrogen peroxide, hydroxyl radicals, and nitric oxide are continuously produced during normal cellular metabolism and can damage nucleic acids, proteins, lipids, and cellular organelles when not adequately neutralized (Kumbhare, et al., 2023; Ukey & Gogle, 2024). Although endogenous antioxidant mechanisms exist, their efficiency can be compromised by aging, poor nutrition, and disease conditions, thereby necessitating external antioxidant supplementation (Conti et al., 2016; Tan et al., 2018).

Medicinal plants constitute a valuable source of natural antioxidants, particularly phenolic compounds, flavonoids, and alkaloids, which exhibit strong free radical scavenging, metal chelating, and redox-modulating properties (Talib et al., 2022; Bansal, 2023; Ukey & Gogle, 2024). Unlike synthetic antioxidants, plant-derived antioxidants are generally considered safer and less toxic, making them attractive candidates for therapeutic and nutraceutical applications (Stevenson & Lowe, 2009; Pereira et al., 2025). Furthermore, natural antioxidants play a significant role in preventing oxidative damage associated with metabolic disorders such as diabetes and may offer protective benefits with minimal adverse effects (Ceriello et al., 2016; Dal & Sigrist, 2016; Muscolo et al., 2024).

In this context, scientific evaluation of the antioxidant potential and phytochemical composition of traditionally used medicinal plants is essential to substantiate their therapeutic relevance. Therefore, the present study aims to investigate the in vitro antioxidant activity and phytochemical profile of Achyranthes aspera (L.) leaf extracts, thereby providing experimental evidence to support its traditional use and exploring its potential as a natural source of bioactive antioxidant compounds.

II. MATERIALS AND METHODS

Collection and drying

Fresh leaves of Achyranthes aspera (L.) were collected from nearby area from Wani district. The leaves were washed thoroughly three times with sterile distilled water. The materials were dried in open under the fan for 4-5 days and then under hot air oven at 45°C for 3 hours and powdered in a mechanical grinder. The powdered samples were sealed in separate polythene bags until the time of the extraction.

Extract preparation

Fifty grams of powdered sample was extracted successively with several organic solvents such as n-hexane, methanol, petroleum ether, acetone, ethyl acetate, and ethanol. All the solvents were used depending upon their polarity from non-polar solvent to semi polar to polar solvent. For this purpose, 250mL each of solvents were put in Soxhlet extractor for serial fraction until the extract was clear. The extracts were filtered through Whatman No.1 (40) filter paper and then for further use kept in a refrigerator.

Extractive value

The dry powdered plant material of Achyranthes aspera (L.) was extracted with methanol, ethanol, acetone, chloroform, ethyl acetate, and petroleum ether using a maceration process. 1gm of the coarsely powdered plant material was weighed in a weighing pan and transferred into a dry 250mL conical flask. Then the flask was filled with different solvents (15mL) separately. The flasks were covered with aluminium foil and kept aside for 24hrs at room temperature, shaking frequently. The mixtures were filtered through Whatmann No. 1 filter paper into a 50mL conical flask. After the filtrate has obtained, it was then transferred into a weighed petry plates. The obtained extracts were concentrated to dryness by keeping filtrate for complete evaporation of solvent (Pawar and Jadhav, 2016; Ukey and Gogle, 2024).

The extractive value in percentage was calculated by using following formula and recorded.

Extractive value (%) = Weight of dried extract / Weight of plant material X 100

Preliminary Phytochemical Analysis

Preliminary phytochemical tests were performed as per the standardized procedures (Harborne 1998; Evans, 2009; Shaikh JR & Patil, 2020):

Test for Phenols

Ferric chloride test:

Take 2mL of crude extract of plants. In that add 3-4 drops of 5% $FeCl_3$ solution. Bluish black colour confirms the presence of phenols.

Test for Flavonoids

Lead acetate test

In 1-2 mL of plant extract, add 1mL of 10% lead acetate solution. Blue colour confirms the presence of flavonoids.

Alkaline reagent test

Take 1-2mL of aqueous extract of plants. To this add 2mL of 2% NaOH, it will give intense yellow colour. To this solution add 3mL of 5% HCl, if it turns reaction mixture colourless indicates presence of flavonoids.

Test for Alkaloids

Wagner's test:

Take 2mL of plant extract and in this add1mL of Wagner's reagent. Reddish brown precipitate confirms presence of alkaloids.

Test for Steroids

Salkowski test:

In 2mL of plant extract, add 2mL chloroform and 1-2mL concentrated sulphuric acid, the reddish-brown colour at the junction of aqueous and chloroform layer indicates presence of steroids.

Test for Tannins

Bramer's Test:

In plant extract (diluted) add 2-3 drops of 5% $FeCl_3$ solution. Green or bluish black precipitate indicates presence of tannins.

Gelatin Test:

1mL of 1% gelatin solution in 10% NaCl was prepared and added to 2mL of extract. Formation of white precipitate indicates presence of tannins.

Test for Saponins

Foam test:

5mL of aqueous extract or 500mg of dry extract was heated and shaken with 5mL distilled water. Foam produced persisted for 10 minutes indicates presence of saponins.

Olive oil test:

In 5mL of extract a few drops of olive oil was added and the solution was shaken vigorously. Formation of emulsion confirms presence of saponins.

Test for Glycosides

Keller-Kiliani test:

To the 2mL of plant extract 1mL of glacial acetic acid was added followed by addition of a few drops of $FeCl_3$ and at the end 1mL of H_2SO_4 added slowly and the solution allowed settling. A reddish-brown colour ring appears at the junction of two layers and the upper layer turns bluish green. These results suggest the presence of cardiac steroidal glycosides (aglycon).

Liebermann's test:

2mL of extract was mixed with 2mL of acetic anhydride. Solution was heated then after cooling a few drops of concentrated H_2SO_4 was added from the sides of the test tube. Appearance of the blue or green colour precipitate indicates presence of glycosides.

Test for Terpenoids

Chloroform test:

In this test, to the 2mL of plant extract 2mL chloroform was added and the solution was evaporated in a water bath to make its concentrate. Later 3mL H_2SO_4 was added and the solution was boiled. The grey colour will appear when the terpenoids are present.

Determination of Total Phenol and Flavonoid Content:

Initially for the experimentation six solvents were selected depending upon their polarity. On the basis of results obtained from extractive value and preliminary phytochemical analysis only four solvents selected viz., methanol, acetone, ethyl acetate and petroleum ether for the phenol and flavonoid estimation and for in-vitro antioxidant assays only methanol extract was used.

Total Phenol Content (TPC):
The total phenolic content of the plant extracts was estimated using the Folin–Ciocalteu colorimetric assay, a widely accepted method for the quantification of phenolic compounds, with minor modifications (Baba and Malik, 2015). Plant extracts prepared using different solvents, namely methanol, acetone, petroleum ether, and ethyl acetate, as described in the sample preparation section, were employed for the analysis. For the assay, 500 µg of extract was mixed with 2.5 mL of 10% Folin–Ciocalteu reagent and 2 mL of 7.5% sodium carbonate solution. The reaction mixture was incubated at 45°C for 45 min, leading to the formation of a blue-colored phosphomolybdic–phosphotungstic acid complex. The absorbance was recorded at 760 nm, and the total phenolic content was calculated using a gallic acid calibration curve and expressed as mg gallic acid equivalents (GAE) per g of extract.

Total Flavonoid Content (TFC):
The total flavonoid content of the plant extracts was quantified using the aluminium chloride colorimetric assay, which is based on the formation of a stable flavonoid–aluminium complex measurable by spectrophotometry (Baba and Malik, 2015). The same extracts employed for total phenolic content estimation were used for TFC analysis. In brief, 200 µg of the extract was mixed with 200 µL of 5% sodium nitrite solution and incubated for 5 min. Subsequently, 300 µL of 10% aluminium chloride was added, followed by a further incubation of 5 min. The reaction was terminated by the addition of 2 mL of 1 M sodium hydroxide, resulting in the formation of an orange-red chromophore. The absorbance was recorded at 510 nm, and the flavonoid content was calculated using a quercetin standard curve and expressed as mg quercetin equivalents (QE) per g of extract.

In-vitro Antioxidant Assays:
DPPH (2, 2-Diphenyl-1-picrylhydrazyl radical) Scavenging Assay: The antioxidant potential of the crude plant extracts and isolated compounds was evaluated using the DPPH free radical scavenging assay. The assay was performed according to the method described by Tuba and Gülçin (2008), with minor modifications as suggested by Kedare and Singh (2011). A methanolic $DPPH^{\bullet}$ solution was freshly prepared to obtain an absorbance of 0.950 ± 0.025 at 517 nm. For the assay, varying concentrations of the plant extract (4, 8, 12, 16, and 20 µg) were mixed with 3 mL of methanol, followed by the addition of 1 mL of $DPPH^{\bullet}$ solution. The reaction mixtures were incubated in the dark at room temperature for 30 min. The decrease in absorbance was recorded at 517 nm against a methanol blank. Ascorbic acid, butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) were used as reference standards, and the half-maximal inhibitory concentration (IC_{50}) values of the samples were calculated accordingly.

$ABTS^{\bullet+}$(2, 2-azinobis (3-ethylbenzothiazoline-6-Sulfonic Acid) Radical Scavenging Assay: The ABTS radical cation ($ABTS^{\bullet+}$) scavenging activity of the plant extracts was evaluated following the method described by Mandade et al. (2011). The $ABTS^{\bullet+}$ solution was generated by reacting 7 mM ABTS with 2.45 mM potassium persulfate in deionized water and allowing the mixture to stand in the dark at room temperature for 12–16 h. Prior to analysis, the absorbance of the $ABTS^{\bullet+}$ solution was adjusted to 0.750 ± 0.005 at 734 nm. For the assay, varying concentrations of the plant extracts (2, 4, 6, 8, and 10 µg) were mixed with 3 mL of methanol and 1 mL of the $ABTS^{\bullet+}$ solution. After incubation at room temperature for 10 min, the reduction in absorbance due to radical scavenging was measured at 734 nm against a methanol blank. Ascorbic acid, butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) were used as reference standards, and IC_{50} values were calculated for all samples.

N, N-dimethyl-p-phenylenediamine dihydrochloride radicle ($DMPD^{\bullet+}$) scavenging assay: The DMPD cation radical ($DMPD^{\bullet+}$) scavenging activity of the plant extracts was determined according to the method described by Fogliano et al. (1999). The $DMPD^{\bullet+}$ radical was generated by reacting DMPD with ferric chloride in acetate buffer. Briefly, 500 µL of 100 mM DMPD solution was added to 50 mL of 0.1 M acetate buffer (pH 5.3), followed by the addition of 100 µL of ferric chloride to initiate radical formation. The absorbance of the resulting $DMPD^{\bullet+}$ solution was adjusted to 0.900 ± 0.100 at 505 nm using acetate buffer or ferric chloride as required. For the assay, 2 mL of the DMPD•$^{+}$ solution was mixed with different volumes of the plant extract (10, 20, 30, 40, and 50 µL) and incubated at room temperature for 10 min. The decrease in absorbance, indicating radical scavenging activity, was measured at 505 nm against an acetate buffer blank. Ascorbic acid, butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) were used as reference standards, and IC_{50} values were calculated for all samples.

FRAP (Ferric Ion Reducing ($Fe^{3+} \rightarrow Fe^{2+}$) Antioxidant Power) Assay: The ferric reducing antioxidant power of the plant extracts was evaluated based on their ability to reduce ferric (Fe^{3+}) ions to ferrous (Fe^{2+}) ions, resulting in the formation of an intense Perl's Prussian blue–colored Fe^{2+}–ferricyanide complex (Tuba and Gülçin, 2008). Briefly, varying concentrations of the plant extracts (5, 10, 20, 30, 40, and 60 µg) were mixed with 2.5 mL of 1% potassium ferricyanide prepared in 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6). The reaction mixtures were incubated at 50°C for 20 min, followed by the addition of 2.5 mL of 10% trichloroacetic acid to terminate the reaction. An aliquot of 2.5 mL of the resulting mixture was then diluted with 2.5 mL of distilled water, and 0.5 mL of 0.1% ferric chloride was added. The absorbance of the developed Prussian blue complex was measured at 700 nm (Rastogi et al., 2018).

Phosphomolybdenum Method for Total Antioxidant Activity (TAA): The total antioxidant capacity of the plant extracts was determined using the phosphomolybdenum method as described by Prieto et al. (1999). Briefly, different concentrations of the plant extracts (20, 40, 60, 80, and 100 µg) were mixed with 5.4 mL of phosphomolybdenum reagent comprising 28 mM sodium phosphate, 4 mM ammonium molybdate, and 0.6 M sulfuric acid. The reaction mixtures were incubated at 95°C for 90 min to facilitate the reduction of Mo (VI) to Mo(V). After cooling to room temperature, the formation of the green phosphate/Mo(V) complex was quantified by measuring the absorbance at 695 nm.

III. STATISTICAL ANALYSIS

All the analyses were performed in triplicate experiments (n=3). The results of EV, TPC, TFC, TAA and FRAP were calculated as mean of observations ± SD. Whereas for DPPH, ABTS, and DMPD scavenging activities, the means of IC_{50}±SD was calculated.

IV. RESULTS

Extractive value

The figure 1 illustrates the percent extractive values of Achyranthes aspera L. obtained using different solvents, namely chloroform, petroleum ether, ethyl acetate, acetone, ethanol, and methanol. Extractive value is an important parameter that reflects the efficiency of a solvent in extracting bioactive constituents from plant material. Among the solvents tested, methanol showed the highest extractive value (approximately 3.1%), followed closely by ethanol (around 2.9%). This indicates that polar solvents are more effective in extracting phytoconstituents from A. aspera, suggesting that the plant is rich in polar compounds such as phenolics, flavonoids, glycosides, and other secondary metabolites.

Ethyl acetate and acetone exhibited moderate extractive values (approximately 2.4% and 2.2%, respectively), indicating the presence of moderately polar constituents. In contrast, petroleum ether and chloroform showed the lowest extractive values (approximately 1.2% and 1.1%, respectively), suggesting a comparatively lower abundance of non-polar compounds such as lipids and waxes in the plant material. The error bars represent the standard deviation, indicating good reproducibility of the extraction process. Overall, the results demonstrate that solvent polarity plays a crucial role in extraction efficiency, and methanol is the most suitable solvent for further phytochemical and antioxidant analyses of A. aspera L.

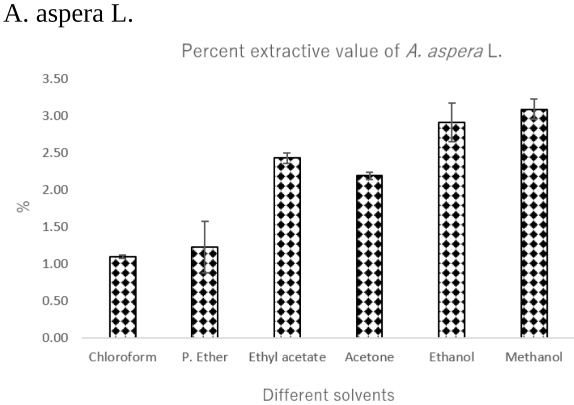

Fig 1: Percent extractive value of A. aspera L.

Preliminary phytochemical screening

Qualitative phytochemical screening of plant extracts prepared using solvents of different polarities revealed the presence of diverse secondary metabolites. Methanol and ethanol extracts showed the richest phytochemical profile, with strong positive reactions for flavonoids, phenols, tannins, glycosides, saponins, alkaloids, steroids, and terpenoids. Acetone and ethyl acetate extracts exhibited moderate phytochemical diversity, while petroleum ether and chloroform extracts showed comparatively fewer constituents. The consistent presence of glycosides, saponins, alkaloids, and terpenoids across all extracts suggests their abundance and wide solvent solubility. Overall, the results demonstrate that solvent polarity significantly influences the extraction of phytoconstituents, with polar solvents being more effective in extracting bioactive compounds. The results of phytochemical analysis are presented in Table 1.

Table 1: Phytochemical screening of A. aspera L.

Chemical tests	Methanol	Ethanol	Acetone	Ethyl acetate	Petroleum ether	Chloroform
Flavonoids						
1) Lead Acetate Test	+	+	+	-	-	+
2) Alkaline Reagent Test	+	+	+	-	-	-
Phenol						
Ferric Chloride Test	+	+	-	-	+	-
Tannin						
1) Bramer’s Test	+	+	+	-	-	-
2) Gelatin Test	+	+	-	+	+	-
Glycosides						
1) Keller-killiani’s test	+	+	+	+	+	+
3) Liebermann’s Test	+	+	-	-	+	-
Saponins						
2) Foam Test	+	+	+	+	+	+
3) Olive oil Test	+	+	+	+	+	+
Alkaloids						
Wagner’s Test	+	+	+	+	+	+
Steroids						
1) Salkowski’s Test	+	+	+	+	+	-
Terpenoids						
1) Chloroform Test	+	+	+	+	+	+

Total Phenol Content

The figure 2 illustrates the total phenolic content (TPC) of different solvent fractions of Achyranthes aspera L., expressed as mg gallic acid equivalents (GAE) per g of extract. The phenolic content varies markedly among the solvent extracts, indicating a strong influence of solvent polarity on the extraction efficiency of phenolic compounds.

Among the fractions analyzed, the ethyl acetate extract exhibited the highest phenolic content, approximately 198 mg GAE/g, suggesting that a significant proportion of phenolic constituents in A. aspera are moderately polar in nature. The methanolic extract also showed a high TPC value (around 176 mg GAE/g), reflecting the effectiveness of polar solvents in extracting phenolic compounds.

The acetone extract demonstrated a moderate phenolic content (97 mg GAE/g), whereas the petroleum ether fraction showed the lowest TPC (33 mg GAE/g), indicating minimal extraction of phenolics by non-polar solvents. This trend confirms that phenolic compounds are predominantly polar to semi-polar molecules.

Overall, the results highlight ethyl acetate and methanol as the most efficient solvents for extracting phenolic constituents from A. aspera L. The elevated phenolic content in these extracts may contribute significantly to their observed antioxidant potential in subsequent in vitro assays.

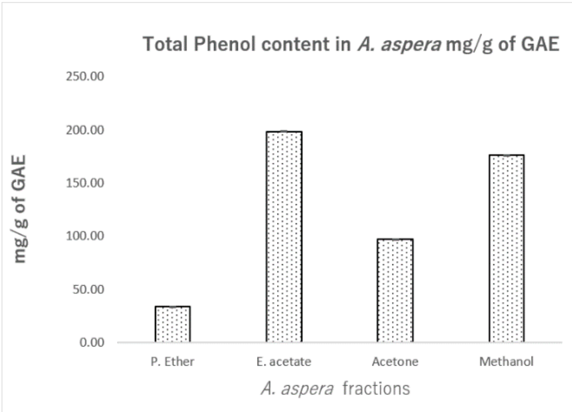

Fig 2: Total Phenol in A. aspera L. (mg/g of GAE)

Total Flavonoid Content

The graph depicts the total flavonoid content (TFC) of different solvent fractions of Achyranthes aspera L., expressed as mg quercetin equivalents (QE) per g of extract. A marked variation in flavonoid content was observed among the solvent extracts, highlighting the influence of solvent polarity on flavonoid extraction.

The methanolic extract exhibited the highest flavonoid content, approximately 48 mg QE/g, indicating that polar solvents are highly effective in extracting flavonoid compounds from A. aspera. The ethyl acetate fraction showed moderate flavonoid content (~17–18 mg QE/g), suggesting the presence of semi-polar flavonoids. The acetone extract contained a comparatively lower number of flavonoids (~11–12 mg QE/g), while the petroleum ether fraction showed the least flavonoid content (7 mg QE/g), reflecting the poor solubility of flavonoids in non-polar solvents.

Overall, the results demonstrate that flavonoids in A. aspera are predominantly polar in nature, and methanol is the most suitable solvent for their efficient extraction. The higher flavonoid content in the methanolic extract may contribute significantly to its antioxidant potential, as observed in subsequent in vitro antioxidant assays.

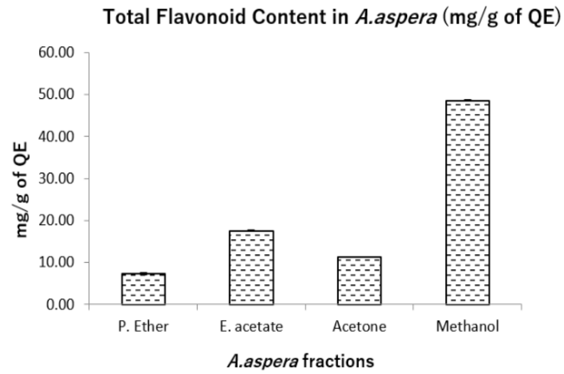

Fig 3: Total flavonoid in A. aspera L. (mg/g of QE)

In vitro antioxidant assays

Several In vitro tests were adopted to evaluate the antioxidant activity of solvent extracts at different concentrations. High antioxidant values in A. aspera L. can be related to the high concentrations of phenols and flavonoids. Antioxidants are a major primary defence system against ROS and free radicals (Brieger et al., 2012). To check this hypothesis, we studied the antioxidant properties of this plant by DPPH, ABTS and DMPD free radical scavenging assay, FRAP and phospho-molybdenum antioxidant (TAA) assay. The results obtained were compared with artificial antioxidants, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and ascorbic acid.

DPPH (2, 2-Diphenyl-1-picrylhydrazyl radical) Scavenging Assay:

The graph presents the DPPH free radical scavenging activity of the methanolic extract of Achyranthes aspera L., expressed in terms of IC_{50} values (µg), and compares it with standard antioxidants, namely ascorbic acid, butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT). The IC_{50} value represents the concentration of the sample required to scavenge 50% of DPPH radicals; therefore, a lower IC_{50} value indicates stronger antioxidant activity.

Among the tested samples, ascorbic acid exhibited the lowest IC_{50} value (~25 µg), indicating the highest radical scavenging potential. This was followed by BHT (~34 µg) and BHA (~44 µg). The methanolic extract of A. aspera showed an IC_{50} value of approximately ~47–48 µg, demonstrating appreciable antioxidant activity, although lower than the reference standards.

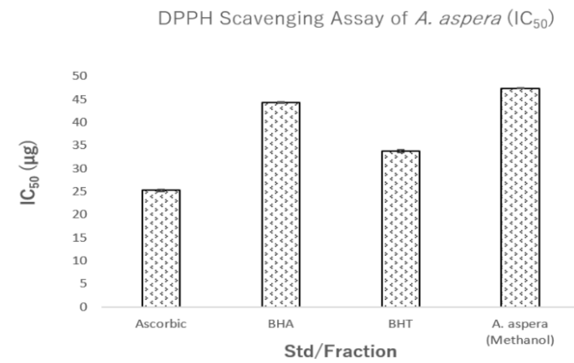

Fig 4: IC_{50} value of A. aspera methanol Fraction

The DPPH antioxidant activity of A. aspera methanolic extract shows a positive correlation with its extractive value, total phenolic content, and total flavonoid content. Although the antioxidant potential of the extract is lower than that of pure standards, its significant activity indicates the presence of potent natural antioxidants. These findings support the role of phenolics and flavonoids as major contributors to the antioxidant capacity of A. aspera and justify the selection of methanol as the preferred solvent for further phytochemical and biological investigations.

$ABTS^{\bullet+}$(2, 2-azinobis (3-ethylbenzothiazoline-6-Sulfonic Acid) Radical Scavenging Assay

The ABTS and DMPD assays confirm that the methanolic extract of Achyranthes aspera L. exhibits moderate but consistent antioxidant activity, which correlates positively with its high extractive value, phenolic content, and flavonoid concentration. Although the activity is lower than that of standard antioxidants, the results validate A. aspera as a promising natural source of antioxidants capable of scavenging multiple types of free radicals through diverse mechanisms.

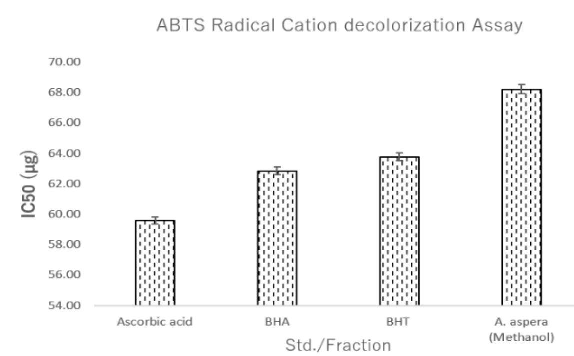

Fig 5: ABTS Radical cation decolorization Assay (IC_{50})

N, N-dimethyl-p-phenylenediamine dihydrochloride radicle ($DMPD^{\bullet+}$) scavenging assay

The methanolic extract showed the highest extractive yield, suggesting an enhanced recovery of bioactive compounds. This higher extraction efficiency supports the observed antioxidant activity across ABTS and DMPD assays. The methanolic extract possessed a high phenolic content, which plays a crucial role in ABTS and DMPD radical scavenging through electron donation and redox reactions. The moderate IC_{50} values observed in these assays correlate well with the substantial phenolic load of the extract. The highest flavonoid content was recorded in the methanolic extract. Flavonoids are known to effectively scavenge both oxygen- and nitrogen-centered radicals, thereby contributing to the antioxidant performance observed in ABTS and DMPD assays. BHT being the hydrophobic in nature did not show results in DMPD assay.

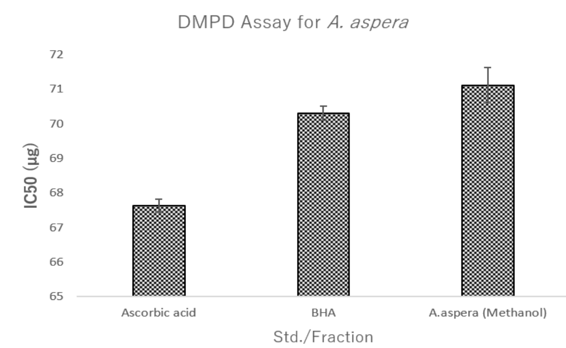

Fig 6: N, N-dimethyl-ρ-phenylenediamine (DMPD) assay

V. CONCLUSION

The present study demonstrates that Achyranthes aspera L. possesses a diverse range of bioactive phytoconstituents with significant antioxidant potential. Methanol proved to be the most effective solvent, yielding the highest extractive value and the richest phytochemical profile. Qualitative screening confirmed the presence of flavonoids, phenolics, tannins, glycosides, saponins, alkaloids, steroids, and terpenoids, predominantly in polar extracts.

Quantitative analysis revealed high total phenolic content in the ethyl acetate fraction and maximum total flavonoid content in the methanolic extract. The methanolic extract exhibited consistent antioxidant activity across DPPH, ABTS, DMPD, FRAP, and phosphomolybdenum assays, showing a positive correlation with its phenolic and flavonoid contents. Although the antioxidant activity was lower than standard antioxidants, the results validate A. aspera as a promising natural source of antioxidants and support its traditional medicinal use. Further studies are warranted to isolate and characterize the active constituents responsible for these effects.

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