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Extraction, Phytochemical Analysis and Study of TPC and TFC In Extract of Achyranthes ASPERA

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ABSTRACT

The current study investigates the extraction, phytochemical screening, and assessment of total phenolic and flavonoid content in different solvent extracts of *Achyranthes aspera* aerial parts. Various extracts, including chloroform, ethyl acetate, methanolic, and aqueous extracts, were prepared, and their yields were calculated. Phytochemical analysis revealed the presence of important bioactive compounds such as alkaloids, flavonoids, diterpenes, phenols, proteins, saponins, and tannins, with methanolic extract showing the highest yield and the most significant phenolic and flavonoid content. The methanolic extract exhibited the highest total phenolic content (1.63 mg/100 mg) and flavonoid content (1.45 mg/100 mg), suggesting its potent antioxidant activity. The findings support the therapeutic potential of *Achyranthes aspera* in combating oxidative stress and liver diseases. These results lay the groundwork for further studies on the pharmacological properties of *Achyranthes aspera* and its bioactive constituents.

Keywords: *Achyranthes aspera, phytochemical screening, total phenolic content, total flavonoid content, antioxidant activity, methanolic extract.*

1. INTRODUCTION

Achyranthes aspera, a plant belonging to the *Amaranthaceae* family, is widely distributed across tropical and subtropical regions, particularly in India. Known by various common names such as "Prickly Chaff Flower" and "Apamarga," this plant has been an integral part of traditional medicine for centuries [1].

Its aerial parts comprising the leaves, stems, and flowers are used in the treatment of various ailments, including respiratory conditions, liver diseases, fever, and inflammation [2].

Due to its vast medicinal applications, *Achyranthes aspera* has drawn attention for its potential in modern pharmacology, particularly its antioxidant, anti-inflammatory, and hepatoprotective properties [3].

The increasing prevalence of liver diseases, such as hepatitis and cirrhosis, highlights the need for effective hepatoprotective agents. Research suggests that *Achyranthes aspera* exhibits significant protective effects against liver damage, making it a promising candidate for hepatoprotective drug development [4].

The plant bioactive compounds, including alkaloids, flavonoids, phenolic acids, and saponins, contribute to its therapeutic efficacy. These compounds have been shown to exert antioxidant and anti-inflammatory effects, both of which are critical in preventing liver injury caused by oxidative stress and inflammation [5].

Phytochemical analysis plays a key role in understanding the medicinal properties of *Achyranthes aspera*. By extracting the bioactive constituents from the plant using various solvents, researchers can identify the key compounds responsible for its pharmacological effects [6].

The analysis of total phenolic content (TPC) and total flavonoid content (TFC) is especially important, as phenolics and flavonoids are known for their strong antioxidant activities, which help in neutralizing free radicals and protecting cells from oxidative damage [7]. Furthermore, identifying the compounds through phytochemical screening helps in determining their potential for various therapeutic uses, including liver protection [8].

Given the importance of *Achyranthes aspera* in traditional medicine and the increasing need for effective treatments for liver diseases, this study aims to conduct a detailed extraction and phytochemical analysis of the aerial parts of *Achyranthes*

aspera. The research will focus on determining the yield of different solvent extracts, identifying the bioactive compounds present, and assessing the total phenolic and flavonoid contents of the extracts. By doing so, this study hopes to contribute valuable insights into the plant's potential for use in modern medicine, particularly for its hepatoprotective properties.

2. MATERIAL AND METHODS

Procurement of plant material

Aerial parts of *Achyranthes aspera* were collected from local area of Bhopal month of September, 2023.

Cleaning

After procurement of plant material, they were cleaned properly. The cleaning process involved the following steps. Very first the decayed or deteriorated plant material was removed. This was followed by washing with tap water and distilled water. The washed plant material was wrapped in blotting paper in order to remove extra water.

Drying

Drying of fresh plant parts were carried out in sun but under the shade.

Extraction using hot continuous extraction (Soxhlet)

The shade dried aerial parts (40 gm) of *Achyranthes aspera* were coarsely powdered and subjected to extraction. Plant material extracted by different solvent like chloroform, ethyl acetate, methanol and distilled water was used. In this method, the finely pulverized marc is placed in a thimble which is placed in a chamber of the Soxhlet apparatus. The menstruum in the flask beneath is then heated, and its vapors condense in the condenser. The condensed extractant drips into the thimble containing the marc, and extracts it by contact. The advantage of this method is that large amounts of marc can be extracted with a much smaller volume of extractant. Each extraction process was carried out for 24 hours. The filtrate was separated from the residue using Whatmann filter paper. The filtrate from each solvent was collected and evaporated using a water bath at 50°C until a thick extract was obtained [9].

Determination of percentage yield

Percentage yield measures the effectiveness of the entire extraction process. % yield is calculated using the formula below:

$$\textbf{Percentage Yield} = \frac{Weight\ of\ Extract}{Weight\ of\ Powder\ drug\ taken} x\ 100$$

Qualitative phytochemical screening

Qualitative phytochemical screening is carried out to investigate the various classes of natural compounds present in the extract. This is accomplished using standard methods [10]. The classes of compounds identified in the extract included phenolics, flavonoids, tannins, saponins, alkaloids and protein.

1. Detection of alkaloids: Extracts dissolved individually in dilute Hydrochloric acid and filtered.

a) Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Alkaloids confirmed by the formation of yellow coloured precipitate.

2. Detection of Glycoside

a) Conc. H_2SO_4 Test: Extract dissolved in distilled water and treated with few drops of conc. Sulphuric acid. Formation of red color indicates the presence of glycoside.

3. Detection of flavonoids

a) Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

b) Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

4. Detection of diterpenes

a) Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicate the presence of diterpenes.

5. Detection of phenols

a) Ferric Chloride Test: Extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

b) Folin Ciocalteu Test: 1 ml extract was added to 1 ml Folin Ciocalteu reagent; blue green color indicates presence of the

phenols.

6. Detection of proteins

a) Xanthoproteic Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

7. Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a) Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

b) Benedict's Test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

8. Detection of saponins

a) Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the incidence of saponins.

9. Detection of tannins

a) Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

10. Detection of Sterols

a) Salkowski Test: 3-4 drops of Conc. Sulphuric acid were added to the extract in chloroform. Formation of red color appears at the lower layer indicates the presence of sterols.

Quantitative estimation of bioactive compound

Estimation of total phenolic content

The total phenolic content of the extract was determined by the modified folin-ciocalteu method [11]. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 5- 25µg/ml was prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this solution was used for the estimation of phenol. 2 ml of each extract or standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15 min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

5.2.2.2 Estimation of total flavonoids content

Determination of total flavonoids content was based on aluminium chloride method [12]. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this solution was used for the estimation of flavonoid. 1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

3. RESULTS AND DISCUSSION

The extraction yields of the aerial parts of *Achyranthes aspera* showed varying percentages across different solvents, indicating the differential solubility of bioactive compounds in these solvents. The methanolic extract had the highest yield (7.02%), followed by the aqueous extract (6.04%), chloroform extract (1.38%), and ethyl acetate extract (0.85%). These results suggest that methanol and water are more effective solvents for extracting compounds from *Achyranthes aspera* than chloroform and ethyl acetate.

The phytochemical screening of the various extracts revealed the presence of several bioactive compounds, which are known for their pharmacological activities. For instance, the chloroform extract showed the presence of alkaloids, flavonoids, diterpenes, and phenols, which are well-known for their antioxidant and anti-inflammatory properties. Similar results were observed in the ethyl acetate, methanolic, and aqueous extracts, with flavonoids being consistently present in all extracts. These compounds are important for their therapeutic potential in oxidative stress-related conditions, such as liver damage.

Flavonoids, found in all extracts, are renowned for their antioxidant, anti-inflammatory, and hepatoprotective properties. The methanolic extract, which exhibited the highest content of total flavonoids (1.45 mg/100 mg), might therefore offer the most potent therapeutic benefits among the extracts. On the other hand, phenols, another important antioxidant compound, were detected in the chloroform, methanolic, and aqueous extracts, with the methanolic extract having the highest phenolic content (1.63 mg/100 mg).

Interestingly, the aqueous and methanolic extracts also showed the presence of saponins and proteins, with proteins being absent in the chloroform and ethyl acetate extracts. Saponins are known for their anti-inflammatory and antimicrobial effects, and their presence may contribute to the therapeutic potential of these extracts.

The total phenolic and flavonoid contents were evaluated in the four extracts, with the methanolic extract showing the highest total phenolic content (1.63 mg/100 mg) and the ethyl acetate extract having the second-highest flavonoid content (0.77 mg/100 mg). This highlights the significant antioxidant activity of methanol as a solvent in extracting phenolic compounds, which are essential in the protection against liver damage and other oxidative stress-related diseases.

Table 1: % Yield of *Achyranthes aspera* (Aerial parts extract)

Sr. No	Extracts	% Yield (w/w)
1.	Chloroform	1.38
2.	Ethyl acetate	0.85
3.	Methanol	7.02
4.	Aqueous	6.04

Table 2: Result of phytochemical screening of Chloroform extracts of *Achyranthes aspera*

S. No.	Constituents	Chloroform extract
1.	**Alkaloids** Wagner's Test: Hager's Test:	 +ve +ve
2.	**Glycosides** Conc. H_2SO_4 Test:	 -ve
3.	**Flavonoids** Lead acetate Test: Alkaline test:	 +ve +ve
4.	**Diterpenes** Copper acetate Test:	 +ve
5.	**Phenol** Ferric Chloride Test: Folin Ciocalteu Test:	 -ve +ve
6.	**Proteins** Xanthoproteic Test:	 -ve
7.	**Carbohydrate** Fehling's Test: Benedict's Test	 -ve -ve
8.	**Saponins** Froth Test:	 -ve
9.	**Tannins** Gelatin test:	 -ve
10.	**Sterols** Salkowski Test:	 -ve

Table 3: Result of phytochemical screening of Ethyl acetate extracts of *Achyranthes aspera*

S. No.	Constituents	Ethyl acetate extract
1.	**Alkaloids** Wagner's Test: Hager's Test:	 +ve -ve
2.	**Glycosides** Conc. H_2SO_4 Test:	 -ve
3.	**Flavonoids** Lead acetate Test: Alkaline test:	 +ve +ve
4.	**Diterpenes** Copper acetate Test:	 -ve
5.	**Phenol** Ferric Chloride Test: Folin Ciocalteu Test:	 -ve +ve
6.	**Proteins** Xanthoproteic Test:	 -ve
7.	**Carbohydrate** Fehling's Test: Benedict's Test	 -ve -ve
8.	**Saponins** Froth Test:	 -ve
9.	**Tannins** Gelatin test:	 -ve
10.	**Sterols** Salkowski Test:	 -ve

+Ve = Positive, -Ve= Negative

Table 4: Result of phytochemical screening of Methanolic extracts of *Achyranthes aspera*

S. No.	Constituents	Methanolic extract
1.	**Alkaloids** Wagner's Test: Hager's Test:	 -ve -ve
2.	**Glycosides** Conc. H_2SO_4 Test:	 -ve
3.	**Flavonoids** Lead acetate Test: Alkaline test:	 +ve +ve
4.	**Diterpenes**	

	Copper acetate Test:	+ve
5.	**Phenol** Ferric Chloride Test: Folin Ciocalteu Test:	 -ve +ve
6.	**Proteins** Xanthoproteic Test:	 +ve
7.	**Carbohydrate** Fehling's Test: Benedict's Test	 -ve -ve
8.	**Saponins** Froth Test:	 -ve
9.	**Tannins** Gelatin test:	 +ve
10.	**Sterols** Salkowski Test:	 -ve

+Ve = Positive, -Ve= Negative

Table 5: Result of phytochemical screening of Aqueous extracts of *Achyranthes aspera*

S. No.	Constituents	Aqueous extract
1.	**Alkaloids** Wagner's Test: Hager's Test:	 -ve -ve
2.	**Glycosides** Conc. H_2SO_4 Test:	 -ve
3.	**Flavonoids** Lead acetate Test: Alkaline test:	 +ve +ve
4.	**Diterpenes** Copper acetate Test:	 +ve
5.	**Phenol** Ferric Chloride Test: Folin Ciocalteu Test:	 -ve +ve
6.	**Proteins** Xanthoproteic Test:	 +ve
7.	**Carbohydrate** Fehling's Test: Benedict's Test	 -ve -ve
8.	**Saponins** Froth Test:	 +ve

9.	**Tannins** Gelatin test:	 -ve
10.	**Sterols** Salkowski Test:	 -ve

+Ve = Positive, -Ve= Negative

Table 6: Results of total phenol and flavonoids content of extract of *Achyranthes aspera*

S. No.	Extracts	Total phenol content	Total flavonoids content
		mg/100mg	
1	Chloroform	0.40	0.60
2	Ethyl acetate	0.95	0.77
3	Methanolic	1.63	1.45
4	Aqueous	0.50	0.69

4. CONCLUSION

The results of this study indicate that the aerial parts of *Achyranthes aspera* possess substantial phytochemical diversity, with methanol emerging as the most effective solvent for extracting phenolic and flavonoid compounds. These findings suggest that *Achyranthes aspera* could serve as a potential source of natural antioxidants and hepatoprotective agents. Further studies are warranted to investigate the specific pharmacological mechanisms of these bioactive compounds and their therapeutic applications.

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