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Pharmacognostical and Phytochemical Evaluation of *Apamarga* (*Achyranthesaspera* Linn)

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Abstract: ***INTRODUCTION-*** **Apamarga** ***(*****Achyranthes aspera Linn.*****)*** ***has long been utilized in traditional medicine for managing various ailments. The entire plant, including its seeds, is known to contain alkaline substances, particularly potash. Numerous chemical constituents have been identified and isolated from different parts of the plant. Although several studies have been conducted on*** **Achyranthes aspera,** ***there remains a lack of a comprehensive review focusing on its pharmacognostical, physicochemical, phytochemical, and chromatographic evaluations.AIM AND OBJECTIVE- To conduct a thorough pharmacognostical, physicochemical, phytochemical, and chromatographic evaluation of*** **Apamarga** ***(*****Achyranthes aspera Linn.*****)*****.*** ***MATERIAL AND METHODS- This study was designed to evaluate*** **Apamarga** ***(*****Achyranthes aspera Linn.*****)*** ***through pharmacognostical, physicochemical, phytochemical, and chromatographic analyses to assess its quality, purity, and safety.OBSERVATIONS AND RESULTS- The results obtained from the pharmacognostical, physicochemical, phytochemical, and chromatographic assessments were all found to be within the acceptable quality standards.CONCLUSION- The tested sample of*** **Apamarga** ***(*****Achyranthes aspera Linn.*****)*** ***demonstrated high quality and was confirmed to be pure, safe, and authentic.***
Keywords: Pharmacognosy,Phytochemicals, **Apamarga,** ***AchyranthesasperaLinn.***

I. INTRODUCTION

Apamarga, scientifically known as *Achyranthes aspera Linn*, is a significant plant in Ayurvedic medicine. Commonly referred to as the prickly chaff flower in English, it belongs to the *Amaranthaceae* family. This plant is widely distributed across India, particularly thriving up to elevations of 900 meters. It is commonly found as a weed in diverse habitats such as roadsides, waste areas, fields, hedges, gardens, forest edges, and clearings.[1]

Apamarga is an erect, stiff annual or perennial herb with a woody base. In traditional systems of medicine, nearly all parts of the plant are utilized, with the seeds, roots, and shoots being the most therapeutically important.[2]

Table-01- *Apamarga- Scientificclassification*[3]

Kingdom	Planate
Subkingdom	Tracheobinota
Unranked	Angiosperms
SuperDivision	Spermatophyta
Order	Caryophyllales
Division	Mangoliophyta
Class	Mangoliophsida
Subclass	Caryophyllidae
Order	Caryophyllales
Family	*Amaranthaceae*
Genus	*Achyranthes*
Species	*Aspera*
Binomialname	*Achyranthusaspera*
Family	*Amaranthace*

Synonyms[4]

Shikhari, Mayuraka, Kinihi, AdhahShalya, Kharamanjari, Kubja, Vasheera, DurabhiGraha, Durgraha, Kharamanjari, Markati, MarkataPippali, Kapi Pippali, ParakPushpi, PratyakShreni.

*Ayurvedic*Properties of *Apamarga* [5-6]

Table-02- *Apamarga- Ayurvedic* Properties

Rasa	Katu, Tikta
Guna	Laghu,Ruksa,Tikshna
Virya	Usna
Vipaka	Katu
Karma	Kapha and Vata pacified. Lekhan, Visaghna, Tvak-Dosahara, Vrana-Sodhana, Dipana-Pachana, Medohar, Chedana, Vamaka, Sirovirecana, Sodhahara, Vedana sthapana. Although the fruits of Apamarga are Vipaka in nature and Madhura in rasa, they are vistambhi because they induce constipation.
Therapeuticindications	Kandu, Kushtha, Visha, Vrana, Karna-Roga, Netra-Roga, Aruchi, Chardii, Udararoga, Krmi, Hridroga, Pandu, Gandamala, Amavata, Kasa, Shwasa, Mutraghata, Visuchika, Sidhma, Nidranasa, Ashmari, Arsha, Kaphaja Timira, Praklinnavartma, Paripotaka, Pleehodara, Apachi, Sharkara, Utpataka.

Despite the availability of a few reviews on this plant, there is a lack of comprehensive studies that encompass the pharmacognostical, physicochemical, phytochemical, and chromatographic evaluation of *Achyranthes aspera Linn*.

II. AIM AND OBJECTIVE

To perform pharmacognostical, physio-chemical,phytochemical and chromatography evaluationof *Apamarga* [*Achyranthesaspera*Linn.].

III. MATERIAL AND METHODS

1) Sample Preparation- The powdered form of *Apamarga* (*Achyranthes aspera Linn.*) was processed through a vibro sifter to obtain a uniform particle size for testing. The evaluation procedures conducted included the following:

2) Pharmacognostical Study- An organoleptic assessment was performed using the naked eye and a magnifying lens to examine characteristics such as color, odor, taste, and texture.

3) Powder Microscopy- Microscopic examination of the powdered drug plays a crucial role in identifying medicinal plant materials. For this purpose, the powder was treated with various chemical reagents. While microscopy alone may not always yield conclusive results, it offers valuable complementary data when combined with other analytical methods.

4) Chemical Reagents Used for Staining:

- Safranin
- Dilute Ferric Chloride
- Methylene Blue
- Sudan Red III
- Iodine Solution
- Dilute Hydrochloric Acid (HCl)

5) Physicochemical Analysis- The physicochemical properties of Apamarga (Achyranthes aspera Linn.) were evaluated, focusing on:

6) Determination of Moisture Content / Total Soluble Solids[7]- Moisture content was measured by placing 5 grams of the powdered sample in an oven at 105°C for 5 hours. The weight of the sample was recorded at 30-minute intervals until it stabilized, indicating no further weight loss. After heating, the sample was cooled to room temperature in a desiccator prior to the final weighing.

7) Calculation Formula:

- Weight of empty Petri dish = W_1 (g)
- Weight of the drug sample = X (g)
- Weight of Petri dish with sample before drying (W_3) = W_1 + X

- Weight of Petri dish after drying = W_2 (g)
- Loss on Drying (%) = ((W_3 - W_2) × 100) / X

8) Determination of pH[8]- The pH of an aqueous solution of *Apamarga* (*Achyranthes aspera Linn.*) was determined using a digital pH meter. This test provides a quantitative measure of the acidity or alkalinity of the solution.

- A digital pH meter was utilized for the measurement.
- The meter was first calibrated using standard buffer tablets with known pH values, each dissolved in 100 ml of distilled water to prepare calibration solutions.
- The device was turned on and allowed to stabilize before use.
- The electrode was immersed in the buffer solution kept in a beaker for calibration.
- A 10% aqueous solution of the sample was then prepared, and the electrode was dipped into this solution to record the pH value.

9) Determination of Extractive Values[9]

Water-Soluble Extractive- 5 grams of the powdered *Apamarga* sample were macerated with 100 ml of distilled water in a closed flask and allowed to stand for 24 hours. The mixture was shaken continuously for 6 hours using a rotary shaker, then left undisturbed for an additional 18 hours. The solution was filtered using filter paper. The filtrate was transferred to a pre-weighed flat-bottomed dish and evaporated to dryness on a water bath. It was then dried to a constant weight in an oven at 105°C.

Calculations:

- Weight of the drug material = X g
- Weight of the empty Petri dish = W_1 g
- Weight of the Petri dish with dried extract = W_2 g
- Percentage of extractive value =W2-W1x100/X

The procedure was repeated three times, and the average value was calculated.

Alcohol-Soluble Extractive- The method used was identical to the water-soluble extractive procedure, except alcohol was used as the solvent instead of distilled water.

10) Determination of Ash Values

Total Ash- 5 grams of powdered *Apamarga* were placed in a silica crucible and evenly spread. The crucible was then placed in a muffle furnace set to 450°C and heated for approximately 6 hours or until the ash became carbon-free. After cooling in a desiccator, the crucible was weighed.

Calculations:

- Weight of empty silica crucible = A_1 g
- Weight of the sample = X g
- Weight of crucible with ash = A_2 g
- Percentage of total ash =[A2-A1/X]x100”

11) Acid-Insoluble Ash- The total ash obtained was boiled with 25 ml of 2M hydrochloric acid for 5 minutes. The insoluble residue was filtered using a Gooch crucible, thoroughly washed with hot water, ignited at a temperature not exceeding 450°C for 15 minutes, then cooled in a desiccator and weighed.

Calculations:

- Weight of the drug sample = X g
- Weight of empty crucible = G_1 g
- Weight of crucible with insoluble ash = G_2 g
- Weight of acid-insoluble ash = G_3 = G_2 - G_1
- Percentage of acid-insoluble ash =G3/X×100”

12) Determination of Water-Soluble Ash- To determine the water-soluble ash, the total ash was boiled with 25 ml of distilled water for 5 minutes. The insoluble residue was collected in a Gooch crucible, washed thoroughly with hot water, and ignited at a temperature not exceeding 450°C for 15 minutes. The weight of the insoluble matter was subtracted from the total ash weight to obtain the water-soluble ash content.

Calculations:

- Weight of the drug sample = X g

- Weight of total ash = A g
- Weight of crucible = G_1 g
- Weight of crucible with insoluble ash = G_2 g
- Weight of insoluble ash (G_3) = G_2 - G_1
- Water-soluble ash (G_4) = A - G_3

Percentage of water-soluble ash =A– [(G3)/X]x100"

13) Phytochemical Study[10]

Qualitative phytochemical analysis was conducted on both aqueous and alcoholic extracts of *Apamarga* (*Achyranthes aspera Linn.*) to identify the presence of various bioactive constituents using the following tests:

- Tests for Carbohydrates
  - Molisch's Test: 2 ml of test solution was mixed with 2 ml of Molisch's reagent and carefully layered with 1 ml of concentrated sulfuric acid. A purple ring at the interface indicates the presence of carbohydrates.
  - Benedict's Test: To 4 ml of the aqueous extract, 1 ml of Benedict's reagent was added and heated close to boiling. The appearance of green, yellow, orange, red, or brown precipitate confirms reducing sugars.
  - Fehling's Test: Equal parts of Fehling A (0.5% copper sulphate) and Fehling B (sodium potassium tartrate) were mixed. To this, 2 ml of aqueous extract was added and boiled for 5–10 minutes. A red precipitate indicates reducing sugars.
- Tests for Alkaloids
  - Dragendorff's Test: 2 ml of the test solution was treated with Dragendorff's reagent. Formation of an orange precipitate confirms the presence of alkaloids.
  - Wagner's Test: Few drops of Wagner's reagent were added to the test solution. A reddish-brown precipitate indicates alkaloids.
  - Hager's Test: A saturated solution of picric acid was added to the test extract. An orange-yellow precipitate suggests the presence of alkaloids.
- Test for Amino Acids
  - Ninhydrin Test: The test solution was heated with ninhydrin. The appearance of a deep blue or pale yellow colour indicates the presence of free amino acids.

Tests for Proteins

  - Biuret Test: A small amount of residue was mixed with 1 ml of 4% NaOH followed by a drop of 1% $CuSO_4$. Violet or pink coloration suggests the presence of proteins.
  - Xanthoproteic Test: To 2 ml of the sample, 0.5 ml of concentrated nitric acid was added. Yellow coloration indicates proteins.
  - Millon's Test: A few ml of Millon's reagent was added to the test extract. A white precipitate turning pink confirms the presence of proteins.
- Test for Saponins
  - Foam Test: The sample was shaken vigorously with water and sodium bicarbonate. Persistent honeycomb-like froth confirms saponins.
- Test for Glycosides
  - Borntrager's Test: 1 ml of benzene and 0.5 ml of dilute ammonia were added to the ethanolic extract. The formation of a reddish-pink color confirms glycosides.
- Test for Phenolic Compounds
  - Ferric Chloride Test: The extract was warmed in water, and 2 ml of ferric chloride was added. Green or blue color indicates phenolics.
- Test for Steroids
  - Salkowski Reaction: Few mg of the extract was dissolved in 2 ml chloroform and layered with 2 ml concentrated sulfuric acid. The development of a red color indicates steroids.
- Tests for Tannins
  - Ferric Chloride Test: A 5% ferric chloride solution in 90% alcohol was added to the extract. A dark green or deep blue color suggests tannins.
  - Lead Acetate Test: 10% basic lead acetate was added to the filtrate. A precipitate formation indicates the presence of tannins.

- Potassium Dichromate Test: A solution of potassium dichromate was added to the filtrate. A dark color confirms the presence of tannins.

14) Chromatographic Study[11]

Chromatography is an analytical technique used to separate a mixture into its individual components based on their molecular structure and composition. Thin Layer Chromatography (TLC) serves as a common method for identifying and isolating chemical constituents from plant extracts.

TLC Plate Specifications:Pre-coated TLC plates were used, consisting of a 0.25 mm layer of Silica Gel 60 F_{254} embedded with a fluorescent indicator. Each plate measured approximately 10 cm in length and 2 cm in width.

Activation of Plates:The silica gel plates were activated by heating them in a hot air oven at 105°C for 1.5 hours.

Mobile Phase Preparation:The solvent system used as the mobile phase was composed of Toluene : Ethyl Acetate : Formic Acid in the ratio of 7:2.5:5.

Test Solution:The test sample applied for the chromatographic study was an alcoholic extract of *Apamarga* (*Achyranthes aspera Linn.*).

Visualization Method:The developed chromatogram was visualized by exposing the plates to iodine vapors, which allowed the detection of separated spots.

Rf Value Determination:The distance of each visible spot from the origin was measured. The Retention factor (Rf) was calculated using the following formula:

Rf Value =*Distance travelled by solute from origin line ÷ Distance travelled by solvent front from origin line*

IV. RESULTS AND OBSERVATION

The observations and the results of the present study are tabulated below.

1) Pharmacognostical analysis

Photo-01- Macroscopic study of *Apamarga* [*Achyranthesaspera*Linn.]

Table-03: Organoleptic characters of dried *Apamarga* [*Achyranthesaspera*Linn.]

S. No	Parameters	Observations
1	Color	Yellowish - brown
2	Odor	Not Characteristic
3	Taste	Not Distinct
4	Texture	smooth, Leathery

Powder microscopy[12]: The presence of fibres, starch, crystals, oil glands and parenchyma were observed as shown in Figure.

Photo-02- T.S. of *Apamarga* [*AchyranthesasperaLinn.*]

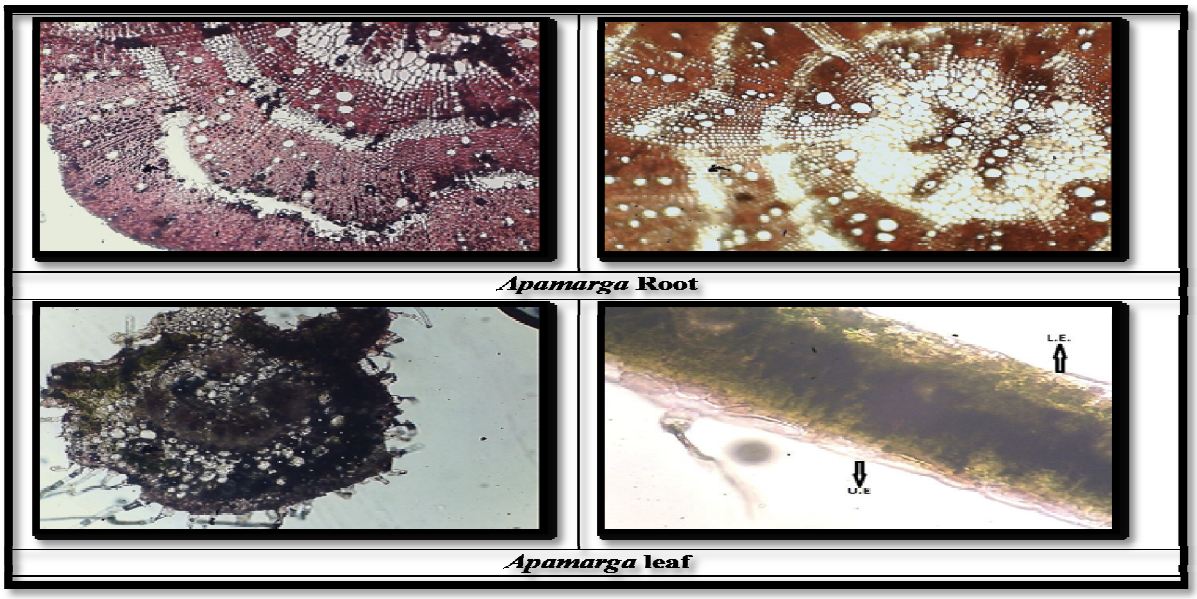
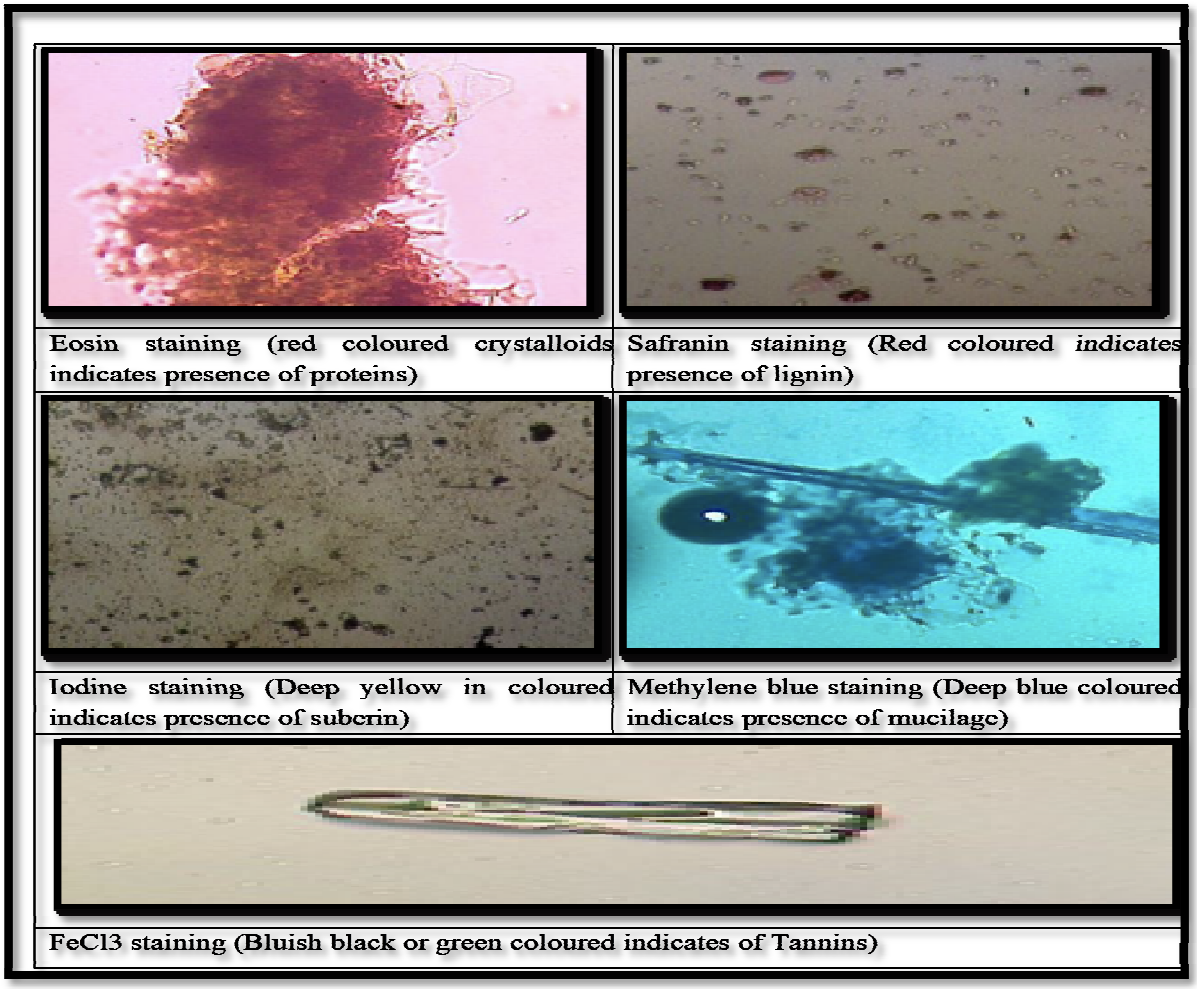


Photo-03- Powder Microscopy of *Apamarga* [*AchyranthesasperaLinn.*]



2) Physiochemical analysis-

3) Moisturecontentof sample-

Table-04: Moisturecontentof sampleof *Apamarga* [*Achyranthesaspera*Linn.]

S.NO.	Weight ofsample	Weight ofcontainer	Weightafterdryingwith container	Weightafterdryingwithout container	Value%
1.	4.9803 gm	60.8575 gm	65.5630 gm	4.7055 gm	5.5%

pH value of sample-

Figure -04- pH valueof sampleof *Apamarga* [*Achyranthesaspera*Linn.]

Table-05: pH value of sampleof *Apamarga* **[*AchyranthesasperaLinn.*]**

S.NO.	**Sample**	**pH**
1.	*Apamarga* [*Achyranthesaspera*Linn.]	**6.9**

4) Extractivevalue of sample-

Figure -05: Extractivevalueof sampleof *Apamarga* [*Achyranthesaspera*Linn.]

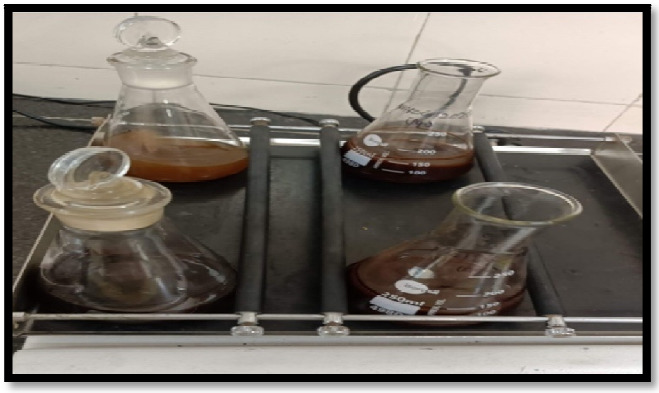


Table-06: Extractivevalueof sampleof *Apamarga* [*Achyranthesaspera*Linn.]

S.NO.	Extractivevalues	Sampleweight	Beakerweight	Beaker +extract weight	Extractweight	Extractvalue(%)
1.	Alcoholsolubleextractive Value	5.0119 gm	138.21gm	138.4184gm	0.2085gm	4.16%
2.	Water soluble extractive value	5.0120 gm	146.023 gm	146.5909gm	0.5678gm	**11.33%**

5) Ashvalue of sample-

Figure -06: Ashvalueof sampleof *Apamarga* [*Achyranthesaspera*Linn.]

Table-07: Total Ashvalueof sampleof *Apamarga* [*Achyranthesaspera*Linn.]

S.NO.	A1	X	A2	Totalash(%)
1.	39.7840 gm	4.9770 gm	40.5057 gm	14.5%

Table-08: Acid Insoluble Ashvalueof sampleof *Apamarga* [*Achyranthesaspera*Linn.]

S.NO.	X	G1	G2	G3	Totalash(%)
1.	4.9770 gm	39.7840 gm	39.9084 gm	0.1244 gm	2.5%

Table-09: Water Soluble Ashvalueof sampleof *Apamarga* [*Achyranthesaspera*Linn.]

S.NO.	X	A	G1	G2	G3	Totalash(%)
1.	5.0058 gm	0.7217 gm	31.5600 gm	31.8754 gm	0.3154gm	6.3%

6) Phytochemical study-

Figure 07: Phytochemical study of *Apamarga* [*Achyranthesaspera*Linn.]

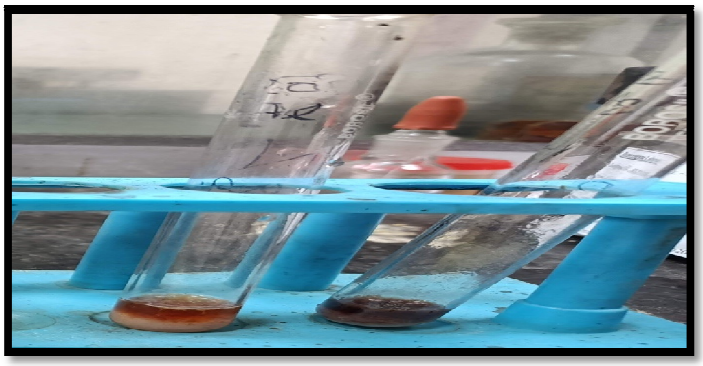


Table-10: Observations of Phytochemical parameters

Phytochemicals	Tests	Aq.ExtofApamarga	Al. Ext ofApamarga
Carbohydrates	1.1-Molish test	+	+
	1.2- Benedict test	-	-
	1.3-Fehling test	+	+
Alkaloids	2.1-Dragendorff test	-	-
	2.2-Wagner test	-	+
	2.3-Hager test	+	-

Aminoacids	3.1- Ninhydrine test	+	+
Proteins	4.1-Biuret test	+	-
	4.2-Xanthoprotic test	+	-
	4.3- Millon test	+	-
Saponin	5.1-Foam test	+	-
Glycosides	6.1- Borntrager test	+	-
PhenolicCompound	7.1- Phenolic test	+	+
Steroids	8.1- Salkowaski test	-	-
Tannins	9.1-Fecl3	+	+
	9.2- Lead acetate	+	+
	9.3-Pot. Dichromate	-	-

7) Chromatography study

Visualization was done under normal light and Iodine.

Table-11: Results of TLC of *Apamarga* [*Achyranthesaspera*Linn**.]**

Distance of solvent	R F Value	IMAGE
5.0	0.18	
	0.24	
	0.27	
	0.33	
	0.36	
	0.50	
	0.65	
	0.81	
	0.95	

V. DISCUSSION

1) Pharmacognostical study-sample is organoleptically within the limits.Table-03 and Photo-01-02-03shows the presence of fibers, starch, crystals and oil glands in the sample.

2) Physiochemical analysis- sample is stable as it has normal moisture level. The ash value which is within the standard limits is indicating the authenticity and purity of the present sample. Extractive values within the standards indicate the absence of exhausted or adulterated drugs in the sample.

3) Phytochemical study- The water extract of sample had shown positive results for the presence of carbohydrates, alkaloids, amino acids, proteins, saponins, Glycosides, phenolic compound and tannins. The alcohol extract shows presence of carbohydrates, alkaloids, amino acids, phenolic compounds and tannins.

4) Chromatography study-TLC of the Alcohol extract of sample shows bands at Rf-0.18, 0.24, 0.27, 0.33, 0.36, 0.50, 0.65, 0.81 and 0.95..

VI. CONCLUSION

On the basis of the observations, results and discussions it has been concluded that the present sample of Apamarga [Achyranthes aspera Linn.]is within all the standards of quality. All the Pharmacognostical, Physiochemical, Phytochemical and Thin Layer Chromatography study helped in identification and authentication of the sample of *Apamarga* [*Achyranthesaspera*Linn.].

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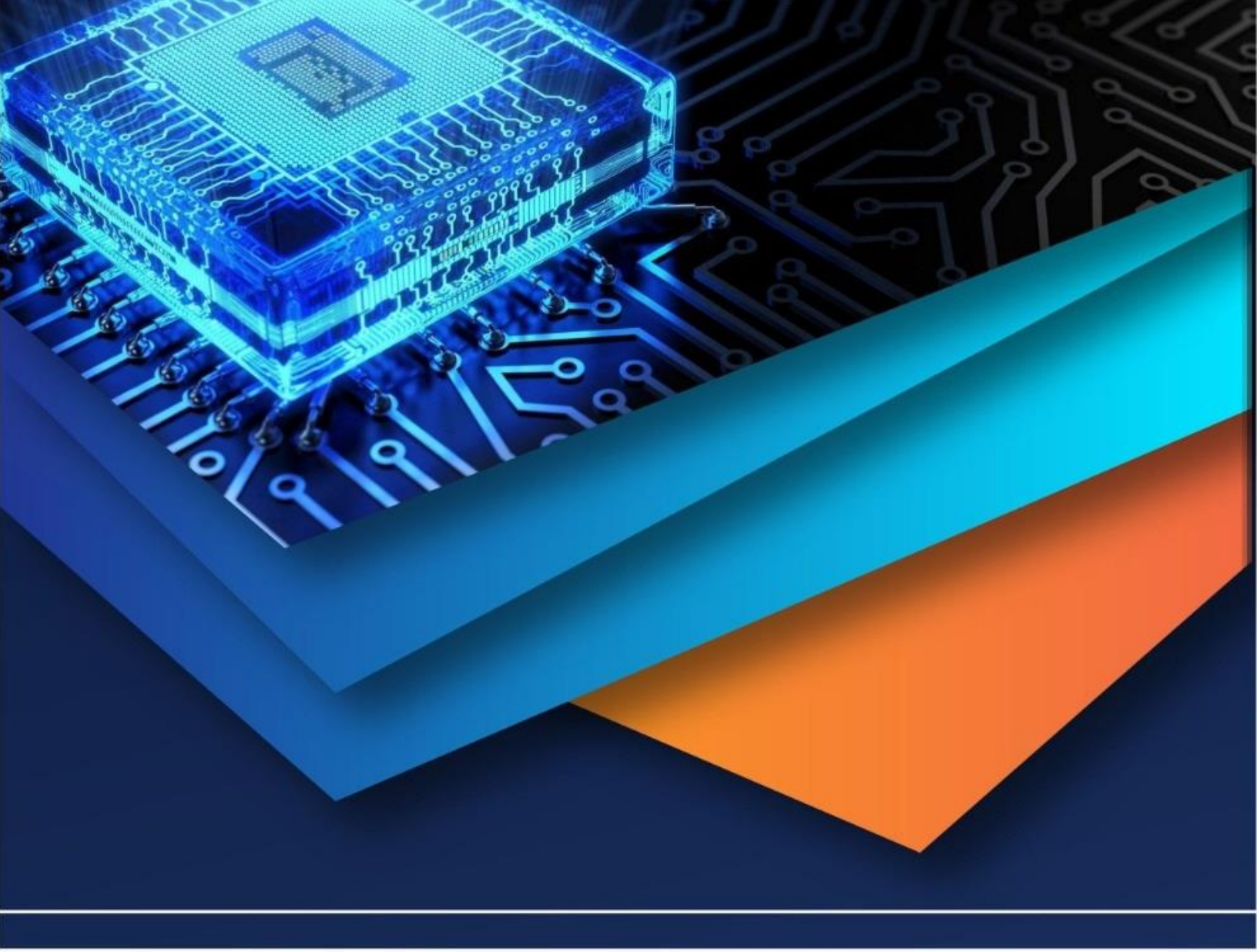

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