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COMPARATIVE PHYTOCHEMICAL SCREENING OF RHIZOMES OF HARIDRA (*CURCUMA LONGA* LINN.), AAMA-HARIDRA (*CURCUMA AMADA* ROXB.) & KRUSHNA HARIDRA (*CURCUMA CAESIA* ROXB.)

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ABSTRACT

The rhizomes of Curcuma species, including Haridra (*Curcuma longa* L.), Aama-Haridra (*Curcuma amada* R.), and Krushna Haridra (*Curcuma caesia* R.), are widely used in traditional medicine for the treatment of various ailments such as cancer, microbial infections, inflammation, skin diseases, asthma, bronchitis, leprosy, infertility, and epilepsy. These species exhibit a range of pharmacological activities, including anti-diabetic, anti-inflammatory, hepatoprotective, anticancer, antimicrobial, antioxidant, analgesic, CNS depressant, and smooth muscle relaxant effects. This study aimed to perform a preliminary phytochemical screening of the ethanolic extracts from the rhizomes of these three species to explore their bioactive compound profiles and pharmacological potential. Phytochemical analysis was conducted to detect key bioactive compounds such as alkaloids, glycosides, carbohydrates, flavonoids, saponins, tannins, phenols, steroids, resins, proteins, amino acids, and triterpenoids. Additionally, morphological analysis of the powdered rhizomes and physicochemical evaluations, including total ash value, acid-insoluble ash, alcohol-soluble extractive value, and water-soluble extractive value, were performed. The findings of this study provide valuable insights into the comparative phytochemical profiles of Haridra, Aama-Haridra, and Krushna Haridra, highlighting their pharmacological potential. These results support the need for further clinical research to establish the therapeutic efficacy and safety of these species, promoting the usage and conservation of these important medicinal plants.

KEYWORDS: *Haridra, Aama-Haridra, Krushna Haridra, Phytochemical analysis, Comparative study.*

INTRODUCTION

Haridra (*Curcuma longa* Linn.) is a perennial herbaceous plant belonging to the Zingiberaceae family, native to the Indian subcontinent and Southeast Asia. It thrives in warm climates, with temperatures ranging from 20 to 30°C.[1] This plant is highly valued for its culinary, medicinal, and aromatic properties, with its rhizomes being the source of turmeric, a vibrant spice recognized for its distinctive bitter, pungent taste and pepper-like aroma. The rhizomes of Haridra contain 3-6% polyphenolic compounds collectively referred to as curcuminoids, which include curcumin, demethoxycurcumin, and bisdemethoxycurcumin. These compounds are largely responsible for the plant's diverse pharmacological effects[2,3], such as antimicrobial, antidiabetic, hypolipidemic, anti-inflammatory, hepatoprotective, anticancer, antioxidant, analgesic, and free radical scavenging properties.[1,2]

Aama Haridra (*Curcuma amada* Roxb.)**,** another member of the Zingiberaceae family, is widely distributed in regions such as India, Indonesia, Malaysia, northern Australia, and Thailand. The rhizomes of this plant are sympodially branched and feature scaly rings at the nodes. Known as "Mango-Ginger" for its raw mango-like flavor, Aama Haridra contains essential oils with key components such as ar-curcumene (28.1%), β-curcumene (11.2%), camphor (11.2%), curzerenone (7.1%), and 1,8-cineole (6.0%). Traditionally, it is used as an appetizer, anti-allergic, anti-inflammatory, antipyretic, aphrodisiac, diuretic, and laxative, and is commonly employed in the treatment of conditions like itching, skin diseases, and asthma. Additionally, Aama Haridra is incorporated into various culinary preparations, such as mango pickles and chutneys, due to its distinctive flavor.[5]

Krushna Haridra (Curcuma caesia Roxb.), also from the Zingiberaceae family, grows wild in northeastern and

central India, as well as in parts of Southeast Asia, including Malaysia, Thailand, and Indonesia. Its rhizomes have been used in traditional medicine for treating a wide range of ailments, including leprosy, bronchitis, asthma, cancer, epilepsy, fever, wounds, impotence, infertility, and pain. Often referred to as black turmeric or black zedoary, Curcuma caesia is characterized by its bluish-black rhizomes. Despite being considered endangered in its native range in India, the species remains underexplored in terms of cultivation and commercialization. The rhizomes of Krushna Haridra contain bioactive compounds such as ar-curcumene, ar-turmerone, camphor, and bornyl acetate, which contribute to its therapeutic properties, including use as a smooth muscle relaxant, and for treating conditions such as leprosy, cancer, leuco-derma, asthma, and menstrual disorders.[6,7]

MATERIALS AND METHODS

Collection and Authentication of Plants

The fresh rhizomes of Haridra (*Curcuma longa L.*), Aama Haridra (*Curcuma amada R.*), and Krushna Haridra (*Curcuma caesia R.*) were collected from Rishikul Ayurvedic College, Haridwar (Uttarakhand) India, for a comparative study and quality standardization. Upon collection, the rhizomes were thoroughly washed with tap water, sliced, and then dried in the shade. After drying, the samples were ground into a moderately coarse powder. To ensure uniformity, the coarse powder was sieved through mesh No. 16 and stored in a cool, dry location for subsequent analysis.

Reagent/Chemical and Equipments

- **Reagent/chemical used**

Distilled water, Glycerol solution, Safranine, hydrochloric acid, Ethanol, Methanol, Magnesium powder, FeCl3 solution, Fehling's solution A and B, Chloroform, Acetic anhydride, Sulphuric acid, Mayer's reagent, Hager's reagent, Acetone.

- **List of equipment's and apparatus**

Measuring tape, Magnifying glass, Grinder, Gloves, Tissue paper, Droper, Sharp razor blades, Brush, Microscope, Slides, Cover slip, No. 250 sieve, No. 16 sieve, Digital balance, Petry dish, Hot air oven, Desiccator, Silica crucible, Muffle furnace, Ash less filter paper, Hot-plate, Silver paper, Stand, Vial, Centrifuge machine, Specula, Tripod stand, Funnel, Flask, Shaker, Flat- bottomed shallow dish, Water bath, Test tube, Measuring cylinder.

Extraction Procedure

The dried plant material was finely ground and prepared for extraction. Successive extractions were performed using solvents with increasing polarity, including n-Hexane, ethanol, methanol and water. Each extraction process was carried out for approximately 72 hours, or until the solvent became colorless.[8] The solvents were subsequently evaporated under reduced pressure using a rotary vacuum evaporator, yielding the crude extract. The final extract was stored in a refrigerator for preservation.[9]

PHYSIO-CHEMICAL PARAMETERS

Moisture Content (Loss on Drying)

10 g of the powdered drug was accurately weighed and placed in a tarred Petri dish. The sample was dried at 105°C in a hot air oven for one hour and then re-weighed. The loss on drying was calculated by comparing the initial and final weights, representing the moisture content of the sample.

Total Ash Value

5 g of the powdered drug was accurately weighed and incinerated in a silica crucible at a temperature not exceeding 450°C in a muffle furnace until it was free from carbon. The residue was then cooled and weighed. The total ash value was expressed as a percentage of the weight of the air-dried drug.

Acid Insoluble Ash Value

1gm of ash was accurately weighed and boiled with 25 mL of hydrochloric acid for 5 minutes in a crucible, covered with a watch glass, over a water bath. After boiling, the mixture was allowed to cool. The watch glass was rinsed with 5 mL of hydrochloric acid, and the rinsings were added to the crucible. The mixture was filtered through pre-weighed Whatman filter paper. The residue on the filter paper was dried, weighed, and recorded. The acid-insoluble ash value was calculated as the percentage of the weight of the residue after subtracting the weight of the filter paper.

Water Soluble Ash Value

1gm of accurately weighed ash was boiled with 25 mL of distilled water for 5 minutes in a crucible, covered with a watch glass, on a water bath. After boiling, the solution was allowed to cool. The watch glass was rinsed with 5 mL of distilled water, and the rinsings were added to the crucible. The mixture was filtered, and the residue remaining on the filter paper was dried and weighed. The percentage of the residue was subtracted from 100% to calculate the water-soluble ash value of the sample.

Alcohol Soluble Extractive Value

2 gm of coarsely powdered, air-dried drug were macerated with 100 ml of alcohol in a closed flask for 24 hours. During the first 6 hours, the flask was shaken frequently, and then it was allowed to stand for the remaining 18 hours. The macerated mixture was then filtered rapidly, taking precautions to prevent the loss of the solvent. Ten milliliters of the filtrate were transferred to a tarred flat-bottomed shallow dish, evaporated to dryness, and dried at 105°C to a constant weight. The percentage of alcohol-soluble extractive was calculated with reference to the weight of the air-dried drug.

Water Soluble Extractive Value

2 gm of coarsely powdered, air-dried drug were macerated with 100 ml of chloroform water in a closed

flask for 24 hours. The flask was shaken frequently during the first 6 hours and allowed to stand for the remaining 18 hours. The mixture was then filtered rapidly, ensuring no solvent loss. Ten milliliters of the filtrate were transferred to a tarred flat-bottomed shallow dish, evaporated to dryness, and dried at 105°C to a constant weight. The percentage of water-soluble extractive was calculated with reference to the weight of the air-dried drug.

PHYTOCHEMICAL ANALYSIS OF CRUDE EXTRACT[1,2]

The crude extract obtained by solvent extraction was subjected to various qualitative tests to detect the presence of common chemical constituents as
Alkaloids, Glycosides, Carbohydrates, Steroids, Saponins, Tannins, Flavonoids, Proteins etc.

Tests for Alkaloids

Mayer's test

1 ml of the extract and 1 ml of Mayer's reagent (potassium mercuric iodide solution) were mixed. The formation of a whitish-yellow or cream-colored precipitate indicated the presence of alkaloids.

Tests for Glycosides

Killer-killani test

2ml of the extract were dissolved in glacial acetic acid, followed by the addition of one drop of 5% ferric chloride ($FeCl_3$) solution and concentrated sulfuric acid (H_2SO_4). A reddish-brown color at the junction of the two liquid layers, with a bluish-green color in the upper layer, indicated the presence of glycosides.

Baljet's test

To 1 ml of the extract, 1 ml of sodium picrate solution was added. The appearance of a yellow to orange color indicated the presence of glycosides.

Tests for Carbohydrate

Molisch's test

A small amount of the extract was dissolved in ethanol, and a few drops of 20% w/v solution of α-naphthol in ethanol (90%) were added. 1ml of concentrated sulfuric acid was carefully added along the side of the test tube. The formation of a reddish-violet ring at the junction of the two layers indicated the presence of carbohydrates.

Fehling's test

The extract was heated with dilute hydrochloric acid, neutralized with sodium hydroxide (NaOH), and then treated with Fehling's solution A & B. The formation of a brick-red precipitate indicated the presence of carbohydrates.

Tests for Flavonoids

Shinoda test

To the test solution, a few magnesium turnings and concentrated hydrochloric acid were added dropwise. The appearance of pink, scarlet, crimson red, or occasionally green to blue color confirmed the presence of flavonoids.

Alkaline reagent test

A few drops of sodium hydroxide solution were added to the test solution. The formation of an intense yellow color that turned colorless upon the addition of dilute acid indicated the presence of flavonoids.

Test for Tanins

To a small quantity of extract (about 2 mg), a few drops of 5% w/v $FeCl_3$ solution were added dropwise, and any change in color was noted. A blue or green color indicates the presence of tannins.

Test for Phenolics

Ferric chloride test

The extract (50 gm) was dissolved in 5 ml of distilled water, and a few drops of 5% ferric chloride solution were added. The formation of a dark green color indicated the presence of phenolic compounds.

Gelatine test

The extract (50 mg) was dissolved in 5 mL of distilled water, and 2 mL of 1% gelatin solution containing 10% sodium chloride was added. The formation of a white precipitate confirmed the presence of phenolic compounds.

Tests for Saponins

Foam test

A small portion of the extract was taken in 2 ml of distilled water, and a pinch of sodium carbonate was added. The mixture was shaken thoroughly. The formation of persistent foam confirmed the presence of saponins. Aqueous and alcoholic extract were tested directly.

Tests for Steroids[11]

Salkowski test

The extract was dissolved in chloroform, and an equal volume of concentrated sulfuric acid (H_2SO_4) was added. The appearance of a bluish-red to cherry color in the chloroform layer and green fluorescence in the acid layer indicated the presence of steroids.

Test for Resins

2ml of the extract were dissolved in acetone, and the solution was poured into distilled water. The formation of turbidity indicated the presence of resins.

Test for Protein and Amino Acid

Ninhydrin test

To 2 mL of the extract, two drops of ninhydrin solution (10 mg of ninhydrin in 200 mL of acetone) were added. The formation of a purple color indicated the presence of amino acids.

Millon's test
To 2 ml of the extract, a few drops of Millon's reagent were added. A white precipitate formed, which turned red upon heating, indicating the presence of proteins and amino acids.

Test for Triterpenoids
Libarmann Burchard Test
For this test 2 mg extract was dissolved in 1 ml of chloroform and mixed thoroughly. Acetic anhydride was added dropwise, followed by 1 mL of concentrated sulfuric acid added carefully along the sides of the test tube. The formation of a green color ring indicated the presence of triterpenoids.

RESULTS

The organoleptic evaluation of crude samples of *Curcuma longa*, *Curcuma amada*, and *Curcuma caesia* revealed variations in their physical characteristics, including color and taste. The rhizomes exhibited a range of colors: brown for *Curcuma longa*, slightly brown for *Curcuma amada*, and slightly greyish for *Curcuma caesia*. The inner surfaces also differed, showing yellow in *Curcuma longa*, creamy white in *Curcuma amada*, and greyish in *Curcuma caesia*. The tastes of these rhizomes were distinctly characteristic: *Curcuma longa* was slightly bitter, *Curcuma amada* had a raw mango-like sweet flavor, and *Curcuma caesia* was acrid.

Phytochemical screening of the ethanolic extracts from these three species indicated the presence of bioactive secondary metabolites. *Curcuma longa* (Haridra) showed the presence of alkaloids, glycosides, carbohydrates, steroids, phenolic compounds, tannins, saponins. Similarly, the ethanolic extract of *Curcuma amada* (Aama Haridra) revealed glycosides, carbohydrates, steroids, phenolic compounds, tannins, flavonoids, and terpenoids. The ethanolic extract of *Curcuma caesia* (Krushna Haridra) was found to contain alkaloids, glycosides, carbohydrates, steroids, phenolic compounds, tannins, flavonoids, steroids, and terpenoids.

The major groups of metabolites identified include carbohydrates, tannins, flavonoids, glycosides, alkaloids, and other bioactive compounds. Detailed results of the organoleptic evaluation and phytochemical screening are provided in Tables 3, 4, and 5.

Table No. 1: Organoleptic characters of crude samples.

S.N.	Parameter	DESCRIPTION OF RHIZOMES		
		C. longa L.	***C. amada R.***	***C. caesia R.***
1.	Color	Brown	Slightly brown	Slightly Brown
2.	Odor	Characteristic	Aromatic or Characteristic	Characteristic
3.	Taste	Slightly Bitter	Raw mango like smell and sweet in taste	Acrid
4.	Texture	Yellowish and Crustaceous	Creamy white and Crustaceous	Slightly Greyish and Crustaceous
5.	Touch	Rough	Rough	Rough

Table No. 2: Organoleptic characters of powder samples.

S.N.	Parameter	DESCRIPTION OF POWDER OF THE SAMPLE		
		C. longa L.	***C. amada R.***	***C. caesia R.***
1.	Color	Yellow	Creamy white	Slightly Greyish
2.	Odour	Characteristic	Aromatic or Characteristic	Characteristic
3.	Taste	Slightly Bitter	Raw mango like smell and sweet in taste	Acrid
4.	Texture	Yellowish and Coarse	Creamy white and Coarse	Slightly Greyish and Coarse
5.	Touch	Rough	Rough	Rough

Haridra	Aama-Haridra	Krushna Haridra
		

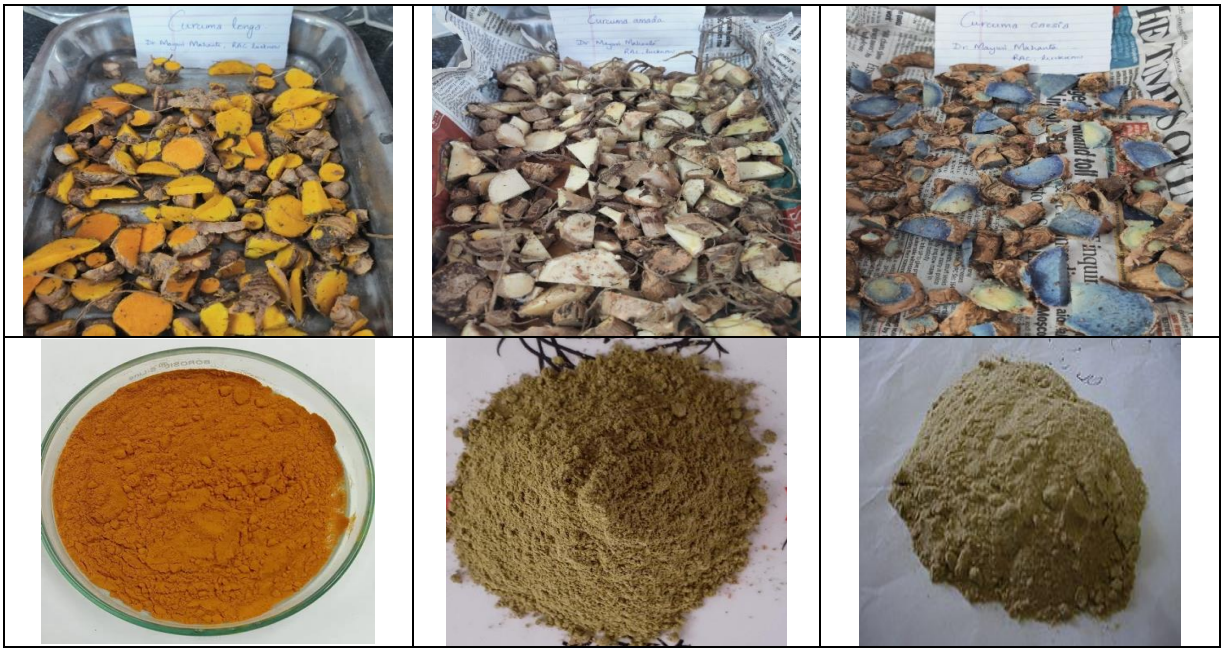

Table No. 3: Phytochemical screening of ethanolic extract of Haridra Rhizomes.

S.No	Identification Test	Test Name	Present	Absent
1.	Alkaloids	Mayer's test	+	
2.	Glycosides	Killer-killani test	+	
		Baljet test	+	
3.	Carbohydrates	Molisch's test	+	
		Fehling test	+	
4.	Flavonoids	Shinoda's test	+	
		Alkaline reagent test		-
5.	Tannins		+	
6.	Phenols	Ferric chloride test	+	
		Gelatine test	+	
7.	Saponins	Foam test	+	
8.	Steroids	Salkowski test	+	
9.	Resins	Turbidity test	+	
10.	Protein and Amino acid	Ninhydrin test		-
		Millon's test		-
11.	Triterpenoids	Libermamm-Burchard test		-

Table No. 4: Phytochemical screening of ethanolic extract of Aama-Haridra Rhizomes.

S.No	Identification Test	TestName	Present	Absent
1.	Alkaloids	Mayer's test		-
2.	Glycosides	Killer-killani test	+	
		Baljet test	+	
3.	Carbohydrates	Molisch's test	+	
		Fehling test	+	
4.	Flavonoids	Shinoda's test	+	
		Alkaline reagent test	+	
5.	Tannins		+	
6.	Phenols	Ferric chloride test	+	
		Gelatine test	+	
7.	Saponins	Foam test		-
8.	Steroids	Salkowski test	+	
9.	Resins	Turbidity test	+	
10.	Protein and Amino acid	Ninhydrin test		-
		Millon's test		-
11.	Triterpenoids	Libermamm-Burchard test	+	

Table No. 5: Phytochemical screening of ethanolic extract of Krushna Haridra Rhizomes.

S.No	Identification Test	TestName	Present	Absent
1.	Alkaloids	Mayer's test	+	
2.	Glycosides	Killer-killani test	+	
		Baljet test	+	
3.	Carbohydrates	Molisch's test	+	
		Fehling test	+	
4.	Flavonoids	Shinoda's test	+	
		Alkaline reagent test	+	
5.	Tannins		+	
6.	Phenols	Ferric chloride test	+	
		Gelatine test	+	
7.	Saponins	Foam test		-
8.	Steroids	Salkowski test	+	
9.	Resins	Turbidity test	+	
10.	Protein and Amino acid	Ninhydrin test		-
		Millon's test		-
11.	Triterpenoids	Libermamm-Burchard test	+	

Table No. 6: Physico-chemical Parameters.

S.No.	Sample	Moisture Content In %	Total Ash Value In %	Acid Insoluble Ash Value In %	Alcohol Soluble Ext. Value In %	Water Soluble Ext. Value In %
1.	*C. longa* L.	7.96 %	8 %	0.85 %	8.08 %	16.10 %
2.	*C. amada* R.	13.50 %	15.4%	0.43 %	8.94 %	3.3 %
3.	*C. caesia* R.	19.41 %	9.1 %	1.1 %	13.5 %	16.5 %

CONCLUSION

The present study highlights the comparative phytochemical screening of the rhizomes of *Curcuma longa* (Haridra), *Curcuma amada* (Aama Haridra), and *Curcuma caesia* (Krushna Haridra), revealing the presence of pharmacologically active metabolites in all three species. The ethanolic extract of Haridra was found to contain alkaloids, glycosides, carbohydrates, steroids, phenolic compounds, tannins, and saponins. Similarly, Aama Haridra and Krushna Haridra also exhibited a rich phytochemical profile, including glycosides, carbohydrates, steroids, phenolic compounds, tannins, flavonoids, and terpenoids, with Krushna Haridra additionally containing alkaloids. These findings suggest that all three species share a comparable phytochemical profile, which may contribute to their similar therapeutic effects.

The determination of ash values provided insight into the purity of the samples. Aama Haridra exhibited a higher total ash content, indicating a greater presence of impurities such as carbonates, oxalates, and silicates, while Krushna Haridra had higher acid-insoluble ash content, suggesting a greater amount of siliceous matter. Extractive value analysis revealed that Krushna Haridra had the highest alcohol-soluble extractive value, indicating a higher concentration of alcohol-soluble active compounds, while Haridra showed the highest water-soluble extractive value, suggesting a higher content of water-soluble constituents.

Given the phytochemical similarities among Haridra, Aama Haridra, and Krushna Haridra, it is evident that the latter two species have significant potential for medicinal use. Promoting the cultivation and utilization of these species, particularly endangered ones like Krushna Haridra and Aama Haridra, is crucial. However, rigorous clinical studies are essential to establish their therapeutic efficacy and safety for treating specific diseases. Such research will help validate their medicinal applications and expand their role in both traditional and modern healthcare systems.

In conclusion, the sustainable cultivation of medicinal plants like *Curcuma longa*, *Curcuma amada*, and *Curcuma caesia* is imperative for ensuring the availability of authentic, high-quality raw materials. This approach will help prevent adulteration and substitution, ensuring the integrity of herbal medicines and supporting the preservation of endangered species for future generations.

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