CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187

https://doi.org/10.5281/zenodo.15576363

https://www.iajps.com/volumes/volume12-may-2025/41-issue-05-may-25/

Available online at: http://www.iajps.com *Research Article*

IN VITRO ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF AQUEOUS EXTRACT OF *HOLARRHENA ANTIDYSENTERICA*

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Abstract:

The present study investigates the in vitro antioxidant and antimicrobial properties of the aqueous extract of Holarrhena antidysenterica, a medicinal plant widely recognized in traditional systems for treating gastrointestinal and infectious diseases. The extract was subjected to preliminary phytochemical screening, which confirmed the presence of glycosides, saponins, phenols, tannins, proteins, sterols, and carbohydrates. The antioxidant potential was evaluated using the DPPH radical scavenging method, and the extract exhibited dose-dependent activity with a maximum inhibition of 42.26% at 100 µg/ml and an IC_{50} value of 88.84 µg/ml, compared to ascorbic acid (IC_{50}: 20.67 µg/ml). Antimicrobial activity was assessed against Staphylococcus aureus and Klebsiella pneumoniae using the agar well diffusion method. The extract showed moderate antibacterial activity, with a maximum zone of inhibition of 11.3 mm and 10.3 mm, respectively, at the highest tested concentration (100 mg/ml), while ciprofloxacin exhibited significantly larger zones. These findings support the traditional therapeutic use of Holarrhena antidysenterica and indicate its potential as a source of natural antioxidant and antimicrobial agents.

Keywords: *Holarrhena antidysenterica, antioxidant activity, DPPH assay, antimicrobial activity, aqueous extract, phytochemical screening, Staphylococcus aureus, Klebsiella pneumoniae*

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Please cite this article in press ***Palak Sahu*** *et al.,* ***In Vitro Antioxidant And Antimicrobial Activities Of Aqueous Extract Of Holarrhena Antidysenterica.,*** *Indo Am. J. P. Sci, 2025; 12(05).*

INTRODUCTION:

Medicinal plants are a vital source of therapeutic agents and have been used extensively in traditional healthcare systems for centuries. Among them, *Holarrhena antidysenterica* (family: Apocynaceae), commonly known as Kutaja or Indrajav, holds a prominent place in Ayurvedic and Unani medicine for its efficacy in treating gastrointestinal disorders, particularly dysentery and chronic diarrhea (Kirtikar & Basu, 2001; Nadkarni, 2002). Various parts of the plant, especially the bark and seeds, are rich in bioactive compounds such as steroidal alkaloids (e.g., conessine), flavonoids, tannins, and phenolics (Rastogi & Mehrotra, 1999).

Recent studies have validated many traditional claims by demonstrating the pharmacological activities of *H. antidysenterica*, including antioxidant, antidiabetic, anti-inflammatory, and antimicrobial properties (Yadav et al., 2010; Sharma et al., 2014). Antioxidants from natural sources have drawn significant attention due to their role in neutralizing free radicals and preventing oxidative stress-related diseases such as cancer, atherosclerosis, and neurodegeneration (Halliwell & Gutteridge, 2007; Sies, 1997). The presence of polyphenols and flavonoids in *H. antidysenterica* contributes to its strong radical scavenging potential, making it a suitable candidate for natural antioxidant therapy (Pietta, 2000).

In parallel, the global rise in antimicrobial resistance (AMR) has necessitated the search for novel antimicrobial agents, particularly from plant sources. Several studies have reported the antimicrobial effects of *H. antidysenterica* against a broad spectrum of pathogenic bacteria, including *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Dwivedi & Gupta, 2012; Alam et al., 2011). These effects are primarily due to the presence of alkaloids like conessine, known to possess potent antibacterial activity (Mehta et al., 1993).

Given this background, the present study was designed to investigate the in vitro antioxidant activity of the aqueous extract of *Holarrhena antidysenterica* using the DPPH free radical scavenging method and to evaluate its antimicrobial activity against selected microbial strains. The findings are expected to contribute to the growing body of evidence supporting the ethnopharmacological use of this plant and to explore its potential as a natural source for developing antioxidant and antimicrobial therapies.

MATERIAL AND METHODS:

Material

The materials used in this study included various analytical grade chemicals and reagents procured from reputable suppliers. Key reagents such as potassium mercuric iodide, iodine, potassium iodide, and picric acid were obtained from Thomas Baker and Loba Chemie Pvt. Ltd., Mumbai. Other essential chemicals including sodium hydroxide, sodium nitroprusside, ferric chloride, gelatin, and lead acetate were supplied by S.D. Fine Chem. Ltd., Mumbai. Organic solvents like methanol, ethanol, and chloroform were sourced from Qualigens Fine Chemicals, Mumbai. Additional reagents such as Folin-Ciocalteu reagent and Fehling's solution were purchased from Loba Chemie Pvt. Ltd. and Central Drug House Ltd., New Delhi, respectively.

Methods

Selection and collection of plant material

The plant has been selected on its availability and folk use of the plant. Every parts of the plant like bark, leaves, flowers, roots, fruits and seeds may contain active secondary metabolites. Bark of *Holarrhena antidysenterica* were collected from ruler area of Bhopal (M.P.) in the month of January, 2025.

Extraction procedure by maceration process

Following procedure was adopted for the preparation of extract from the shade dried and powdered herbs (Mukherjee, 2007). Dried powdered bark (20 gram) of *Holarrhena antidysenterica* has been extracted with aqueous using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40°C.

Determination of percentage yield

After extraction, yield of the plant extracts obtained were calculated in grams and then converted it into percentage. Following formula was adopted for determination of percentage yield of selected plant materials. The percentage yield of each extract was calculated by using following formula:

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

Phytochemical screening

Phytochemical constituents are of two type primary bioactive constituents (chlorophyll, proteins, amino acids, sugar etc.) and secondary bioactive constituents include (alkaloids, terpenoids, phenols, flavonoids etc.). Phytochemical examinations were

carried out for all the extracts as per the standard methods (Kokate, 1994).

Quantitative estimation of bioactive compounds

Total phenolic content estimation

The total phenolic content of the extract was determined by the modified folin-ciocalteu method. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10- 50µg/ml was prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of extract and each 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 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer (Mishra *et al.*, 2017).

***In-vitro* antioxidant activity using DPPH method**

Total free radical scavenging capacity of extract from *Holarrhena antidysenterica* was estimated according to the previously reported method with slight modification (Parkhe and Jain, 2018). Solution of DPPH (6 mg in 100ml methanol) was prepared and stored in dark place. Different concentration of standard and test (10-100 µg/ml) was prepared. 1.5 ml of DPPH and 1.5 ml of each standard and test was taken in separate test tube; absorbance of this solution was taken immediately at 517nm. 1.5 ml of DPPH and 1.5 ml of the methanol was taken as control absorbance at 517nm.

The percentage inhibition of free radical DPPH was calculated from the following equation: % inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] × 100%.

In vitro* antimicrobial activity of aqueous extract of *Holarrhena antidysenterica

At first, all instruments which were used in laboratory were made sterile, all glassware's like Erlenmeyer flask, graduated cylinders, stirring rods, beakers, test tubes, petri dishes, inoculating loops, that were used in the assay were placed in an autoclave at 121°C under 15 psi pressure for 25 min by using Autoclave and followed aseptic technique method. Nutrient agar media (NAM) was prepared for growing of bacteria inside the laboratory. The standard size (100mm× 15mm) petri dishes as required for whole experiment. For preparation of NAM, 13 gram powder was mixed with 1000 ml of distilled water and stirred to obtain homogenized mixture. After which, NAM mixture were placed in Autoclave under 15 psi pressure, at 121°C for 25 min for sterilization of media. After that poured the culture media into petri dishes at ratio of 20 ml/dish and was left half covered on the table to let the agar cool down and solidify at room temperature.

Agar well diffusion method

Agar well-diffusion method was followed to determine the antimicrobial activity (Bauer *et al.*, 1966). Nutrient agar (NA) plates were swabbed (sterile cotton swabs) with fresh broth culture of bacteria. Wells (6mm diameter) were made in each of these plates using sterile cork borer. 100mg/ml, 50mg/ml and 25mg/ ml solution was prepared in different extracts. About 100 µl of different concentrations of extract were added sterile micropipette into the wells and allowed to diffuse at room temperature for 2hrs. Control experiments comprising inoculums distilled water were set up. The plates were incubated at 37°C for 24 h for bacterial pathogens. The diameter of the inhibition zone (mm) was measured and the activity index was also calculated. Triplicates were maintained and the experiment was repeated thrice, for each replicates the readings were taken in three different fixed directions and the average values were recorded.

RESULTS AND DISCUSSION:

The present study aimed to evaluate the in vitro antioxidant and antimicrobial activities of the aqueous extract of *Holarrhena antidysenterica*, along with its phytochemical profile. The percentage yield of the aqueous extract was found to be 3.20% w/w, which is within a reasonable range for water-based extractions and reflects the efficiency of the extraction method used.

Phytochemical screening of the aqueous extract revealed the presence of various secondary metabolites such as alkaloids (only Hager's test positive), glycosides, saponins, phenols, proteins, tannins, sterols, and some carbohydrates. These compounds are known for their biological activities, especially antioxidant and antimicrobial properties. Notably, flavonoids were absent in the aqueous extract, which may partially explain the moderate antioxidant activity observed.

The antioxidant activity assessed by the DPPH radical scavenging method demonstrated that the aqueous extract had dose-dependent activity, with a maximum inhibition of 42.26% at 100 µg/ml and an IC_{50} value of 88.84 µg/ml. In comparison, the standard antioxidant ascorbic acid exhibited significantly stronger activity with an IC_{50} of 20.67 µg/ml. Although the extract's antioxidant potential

was moderate, the presence of phenolic compounds, as confirmed by both the ferric chloride and Folin-Ciocalteu tests, likely contributed to the radical scavenging effect.

The antimicrobial activity of the extract was evaluated against *Staphylococcus aureus* and *Klebsiella pneumoniae*. The aqueous extract exhibited concentration-dependent inhibitory effects against both bacterial strains. At 100 mg/ml, the extract showed zones of inhibition of 11.3 mm for *S. aureus* and 10.3 mm for *K. pneumoniae*. Although these values were lower than those observed for ciprofloxacin (standard drug), the results indicate that *Holarrhena antidysenterica* possesses mild antibacterial activity, which could be attributed to its alkaloid and tannin content. The results also highlight that *S. aureus*, a Gram-positive bacterium, was slightly more susceptible to the extract than *K. pneumoniae*, a Gram-negative bacterium. This may be due to the structural differences in the bacterial cell walls, with Gram-negative bacteria typically being more resistant to plant-based extracts.

Table 1: % Yield of *Holarrhena antidysenterica*

S. No.	Extract	% Yield (w/w)
1.	Aqueous	3.20%

Table 2: Result of phytochemical screening of aqueous extract of *Holarrhena antidysenterica*

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** Alkaline Reagent Test: Lead acetate Test:	 -Ve -Ve
4.	**Saponins** Froth 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.	**Diterpenes** Copper acetate Test:	 -Ve
9.	**Tanins** Gelatin Test	 +Ve
10.	**Sterols** Salkowski Test	 +Ve

[+Ve= Positive; -Ve= Negative]

Table 3: % Inhibition of ascorbic acid and extract of *Holarrhena antidysenterica* using DPPH method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Aqueous extract
1	10	45.77	2.94
2	20	48.85	4.47
3	40	59.98	17.77
4	60	63.32	28.10
5	80	73.32	37.72
6	100	74.65	42.26
IC 50 value (µg/ml)		**20.67**	**88.84**

Table 4: Antimicrobial activity of *standard drug* against selected microbes

S. No.	Name of drug	Microbes	Zone of Inhibition (mm)		
			10 µg/ml	20 µg/ml	30 µg/ml
1	Ciprofloxacin	*Staphylococcus aureus*	15±0.47	18±0.5	22±0.57
		Klebsiella pneumoniae	17±0	20±0.86	25±00.94

Table 5: Antimicrobial activity of aqueous extract of *Holarrhena antidysenterica*

S. No.	Name of microbes	Zone of inhibition (mm)		
		25mg/ml	50 mg/ml	100mg/ml
1.	*Staphylococcus aureus*	6±0	9.6±0.74	11.3±0.94
2.	*Klebsiella pneumoniae*	6±0	8±0	10.3±0.5

CONCLUSION:

In conclusion, the aqueous extract of *Holarrhena antidysenterica* demonstrated moderate antioxidant and antimicrobial activities, supporting its traditional use in the treatment of infections and oxidative stress-related conditions. Further research involving purification of active constituents and in vivo evaluation is warranted to better understand its therapeutic potential.

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