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Original Research Paper

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EVALUATION OF ANTIOXIDANT ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF *HARDWIKIA BINATA* (ROXB.)

Varsha D. Hutke* Associate Professor, P.G. Department Of Botany, Govt. Vidarbha Institute Of Science And Humanities, Amravati *Corresponding Author

Vaishali Rathod P.G. Department Of Botany, Govt. Vidarbha Institute Of Science And Humanities, Amravati.

ABSTRACT The genus Hardwickia of the family Caesalpiniaceae is represented by a single species Hardwickia binata Roxb. The present study deals with screening of secondary metabolites and antioxidant activity of different extracts of leaf and bark of Hardwickia binata. Phytochemical screening revealed the presence of various secondary metabolites viz. alkaloids, saponins, phenolics compound, tannins, phenols and steroids. Both leaf and bark extracts scavenged free radicals significantly. Whereas aqueous extract of bark and ethanol extract of leaf exhibited good antioxidant property.

KEYWORDS : Hardwickia Binata, Phytochemistry, Antioxidant Activity, DPPH

INTRODUCTION

Plants in all facets of life have served a valuable starting material for drug development. It has proven to be most useful in the treatment of diseases and they provide an important source of all the world's pharmaceuticals. Phytochemicals are used as templates for lead optimization programs, which are intended to make safe and effective drugs. Many plants are found to contain chemical compounds, which are used as natural medicines to treat common bacterial infections. These medicinal plants have been regularly used in Traditional System of Indian Medicine because of minimal side effect and cost effectiveness which provide scientific support to the therapeutic use of the plants in tribal medicine (Prasad *et al*., 2012). Most important of these bioactive constituents of plants are steroids, terpenoids, carotenoids, flavonoids, alkaloids, tannins, saponins and glycosides.

Interest in the role of antioxidants in human health has prompted research in the fields of food science and medicinal herbs to assess the role of herbs as antioxidants. (Prasad *et al*., 2012). The use of spices and herbs as antioxidants and antimicrobial agents in foods is becoming of increasing importance. (Gandhiappan and Rengasamy 2012). The genus *Hardwickia* of the family Caesalpiniaceae is represented by a single species *Hardwickia binata* Roxb. The bark decoction is used in the treatment of diarrhea, piles, skin diseases, dysentery and worms. The present study was carried out to analyze the preliminary phytochemical screening and antioxidant activity of leaves and bark of *Hardwickia binata*.

MATERIALS AND METHODS

Plant Material

Figure-1. *Hardwickia binata* Roxb

Leave and bark of *Hardwickia binata* were collected from the area of Amravati. The plant was identified with the help of floras of Cook, 1967 and Dhore, 1986 and voucher specimen was deposited to Botany Department, Govt. Vidarbha institute of science and humanities, Amravati. The collected materials were washed under running tap water to remove the surface pollutants and air dried under shade. After drying, the plant materials were ground well using mechanical blender into fine powder and stored in airtight containers with proper labeling.

Preparation Of Extracts

Crude plant extracts were prepared by Soxhlet extraction method (Tiwari, *et al*, 2011). About 50 gm of powdered plant material was uniformly packed into a thimble and extracted with 500 ml of different solvents separately. Solvents used were ethanol, methanol, acetone and distilled water. The process of extraction continued for 24 hours or till the solvent in siphon tube of an extractor became colorless. After that the extracts were taken in the beakers and kept on hot plate and heated at 30-40°C till all the solvents got evaporated. Dried extracts were kept in refrigerator until used.

Preliminary Phytochemical Screening

Chemical tests were carried out for above four extracts using standard procedures to identify the phytochemicals (Harborns 1973)

Qualitative Phytochemical Test

The solvent free extract obtain as above was then subjected to qualitative preliminary phytochemical screening for identification of various plants constituents following the standard methods.

Test For Alkaloids

Solvent free extracts, 50 mg was stirred with few ml of dilute HCL and filtered. The filtrate was tested with various alkaloidal reagents as follows:

Mayer's Test

Few ml of filtrate and a drop or two of Mayers reagent were added by the side of the test tube. A white or creamy ppt indicates the presence of alkaloids.

Wagner's Test

To a few ml of filtrate, few drops of Wagner's reagent were added by the side of the test tube. A reddish- brown ppt confirms the presence of alkaloids.

Hager's Test

To a few ml of filtrate,1 or 2 ml of Hager's reagent (saturated aqueous solution of picric acid) were added. A prominent

yellow ppt indicates the presence of alkaloids.

Test For Phenolic Compound
Lead acetate test
The extract (50mg) was dissolved in distilled water and to this; 3ml of 10% lead acetate solution was added. A bulky white ppt indicates the presence of phenolic compounds.

Test For Tannins
About (0.5g) of the plant extract was added in 10 ml of water in test tube and filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue- black coloration.

Test For Proteins
To 2 ml of protein solution 1ml of 40% NaOH solution and 1 to 2 drops of 1% CuSO4 solution was added. A violet color indicated the presence of peptide linkage of the molecule.

Test for amino acids
To 2 ml of sample was added to 2 ml of Ninhydrin reagent and kept in water bath for 20 minutes. Appearance of purple color indicated the presence of amino acid in the sample.

Test For Reducing Sugars
To 2 ml of extract 2 drops of Molisch's reagent was added and shaken well. 2ml of conc. H_2SO_4 was added on the sides of the test tube. A reddish violet ring appeared at the junction of two layers immediately indicated the presence of carbohydrates.

Test For Glycoside
Each extract was hydrolyzed with HCL and neutralized with NaOH solution. A few drops of Fehling's solution A and B were added to each mixture. Formation of red ppt indicates the presence of glycosides.

Test For Flavonoids
(a) 0.2 g of each extract was dissolved in diluted NaOH and few drops of HCL were added. A yellow solution that turn colorless indicates the presence of flavonoids
(b) To 2 ml of test solution, 0.5ml alcohol was mixed. Then a bit of magnesium and 1 or 2 drops of con. HCL were added and heated. The mixture was analyzed for reaction.

Test For Phenols
To 2 ml of test solution, alcohol and then few drops of neutral ferric chloride solution was added. A dark green clour indicated the presence of phenolic compound.

Test For Coumarins
3 ml of 10% NaOH was added to 2 ml of aqueous extract, formation of yellow color indicates the presence of coumarins.

Test For Resins
To the 0.2 g of each extract, 10 ml of glacial acetic acid was added then heated and cooled. A drop of conc. H2SO4 was added. Purplish red color shows the presence of resins.

Test For Steroids/ Terpenoids
1 ml of the extract was dissolved in 10 ml of chloroform and equal volume of con. H2SO4 was added by the side of the test tube. The upper layer turns red and H2SO4 layer showed yellow with green fluorescence indicated the presence of steroids.

Antioxidant Activity With DPPH Assay
The antioxidant activity of the plant extract, was estimated utilizing 2, 2-diphenyl-1-picrylhydrazyl (DPPH) (Blois,1958). Five concentrations (25, 50, 75, 100ug/ml) of each sample were prepared. 0.1 Mm solution of DPPH in methanol was prepared and 180 μl of this solution was added to 20μl of different plant extracts in 96 well plates and incubated for 30 min at room temperature in the dark. Ascorbic acid was used as a positive control. The DPPH radical-scavenging activity was determined by measuring the absorbance at 490nm and calculated using the equation (Badami and Gupta 2005).

$$I\% = (Ac - As) / Ac \times 100 \ldots\ldots (1)$$

Where, Ac – absorbance of the control
As – absorbance of the sample

RESULT AND DISCUSSION

The phytochemical screening of the leaves and bark of *Hardwickia binata*. were performed and presented in table–1. The qualitative phytochemical analysis of *Hardwickia binata* leaves and bark contains alkaloids, saponins, Phenolic compound, tannins, phenol and steroids. Analogous to the result of a present investigation Gunaselvi *et al* (2010), Sharanabasappa *et al* (2007) and Deshmukh and Ghanawat (2020) also reported that the phytochemical study revealed the presence of phenols, saponins, glycosides and tannins.

The antioxidant activity of leaves and bark of *Hardwickia binata* were carried out by measuring reducing ability free radical scavenging activity with various extracts (ethanol, methanol, acetone and water) by using DPPH assay (Table-2). Ascorbic acid was used as standard control and results are depicted in Fig. 3. The free radical scavenging of bark extract (Fig.2) in water was exhibited significant antioxidant activity as compared to all other extracts with IC50 11.59 μg/ml (Fig.4). In previous work methanol extract of leaves of *H. binata* exhibited good results (Hamid *et al*, 2018). In case of leaf ethanol (12.45 μg/ml) showed higher antioxidant activity. Results of present study about the ethanol extract are in support of the findings of Hutke and Naswale, (2020).

TABLE 1:- Phytochemical Analysis Of Various Extracts From Leaves And Bark Of *Hardwickia Binata*

Phytochemical components	Leaf				Bark			
	Ethanol	Methanol	Acetone	Water	Ethanol	Methanol	Acetone	Water
Alkaloid	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+	+
Phenolic compounds	+	+	+	+	+	+	+	+
Tannin	+	+	+	+	+	+	+	+
Protein	+	+	-	+	+	+	_	+
Amino acid	-	+	-	-	-	+	-	-
Glycosides	+	+	+	+	-	-	+	-
Flavonoids	_	-	-	_	-	-	-	-
Phenols	+	+	-	+	+	+	-	+
Coumarins	+	+	+	+	-	-	-	-
Resins	-	+	+	-	+	+	-	-
Steroids/ Terpenoids	+	+	+	+	+	+	+	+
Anthraquanine	-	-	-	-	+	-	-	-

Keys: (+) = indicates present, (-) = indicates absent

TABLE2:- Evaluation Of DPPH Free Radical Scavenging Activity Of *Hardwickia Binata* (leaf And Bark)

Extracts	Concentration (μg/ml)	Leaf		Bark	
		%Inhibition (Mean± SD) n=2	IC50 (μg/ml)	%Inhibition (Mean±SD) n=2	IC50 (μg/ml)
Ethanol	25	43.71±1.34	12.45	45.13±1.72	32.23
	50	50.63±3.11		58.19±2.14	
	75	53.61±2.27		59.16±1.38	
	100	61.38±3.51		64.26±2.14	

Methanol	25	51.31±1.11	19.34	49.10±1.75	33.84
	50	53.18±1.31		50.77±1.56	
	75	54.34±1.17		52.53±5.69	
	100	56.79±1.27		64.85±3.95	
Acetone	25	53.51±0.52	25.82	50.87± 2.17	27.87
	50	49.53±6.70		62.21±2.79	
	75	47.71±2.16		66.36±2.54	
	100	46.00±1.25		75.69±1.91	
Aqueous	25	43.29±0.13	31.84	40.12±7.10	11.59
	50	59.00±0.76		49.32±1.49	
	75	63.82±0.39		52.43±2.84	
	100	65.38±0.32		58.16±1.39	
Ascorbic Acid	25	52.76±2.55	5.78	52.76±2.55	5.78
	50	63.76±2.83		63.76±2.83	
	75	77.21±1.92		77.21±1.92	
	100	81.66±2.82s		81.66±2.82s	

FIGURE-2. ANTIOXIDANT ACTIVITY OF *HARDWICKIA BINATA* (LEAF AND BARK) EXTRACTS

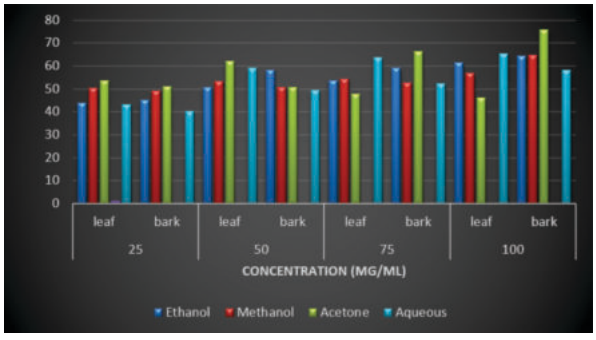


FIGURE-3. ANTIOXIDANT ACTIVITY OF ASCORBIC ACID

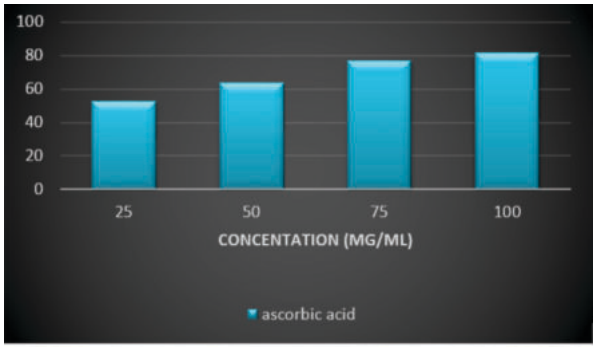
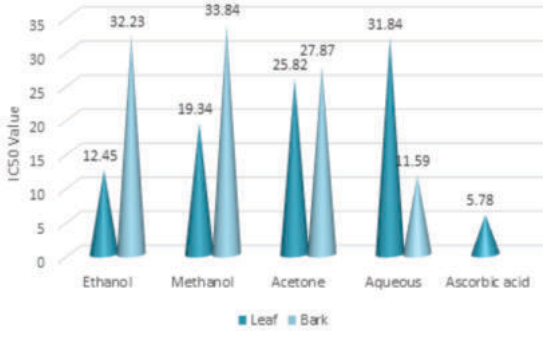


FIGURE-4: IC50 VALUES OF *HARDWICKIA BINATA* (LEAF AND BARK) EXTRACTS



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