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Research Article

PHYTOCHEMICAL SCREENING AND EVALUATION OF ANTIOXIDANT ACTIVITY OF *HETEROPHRAGMA ADENOPHYLLUM Linn.*

Varsha D. Hutke* and Vaishali Kale

Department of Botany, Govt. Vidarbha Institute of Science and Humanities, Amravati

A R T I C L E I N F O

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A B S T R A C T

Present study was conducted to screen secondary metabolites and antioxidant activity of different extracts of ***Heterophragma adenophyllum*** *Linn.* Four different solvents were used to extract the bioactive compounds from leaves and bark. Analysis of phytochemical screening reveals the presence of alkaloid, Phenolic compounds, tannins, saponins, glycosides, flavonoids, steroid, and terpenoids. The extracts were screened for its potential of antioxidant activity using DPPH radical scavenging activity. The results indicated that the maximum activity was found in ethanol extract of bark.

INTRODUCTION

Men have always used natural resources of healing substances to cure human diseases. Efforts to cure the diseases by means of traditional phytotherapy have been made in all parts of the world (Bodekar *et al*., 2005). Medicinal plants are well known to the local population and are used for the treatment of their ailments since time immemorial. Plant derived medicines are widely used because they are relatively safer than the synthetic alternatives, are easily available and cheaper (Iwu et al., 1999). It is generally assumed that frequent consumption of plant-derived phytochemicals from vegetables, fruit, tea, and herbs may contribute to shift the balance toward an adequate antioxidant status (Halliwell, 1996). Thus interest in natural antioxidant, especially of plant origin, has greatly increased in recent years (Jayaprakash and Rao, 2000). *Heterophragma adenophyllum* is a large tree belonging to the family bignoniaceae, have huge importance in the medicinal field. It is used in constipation, muscular tension and also prescribed as drink in viper bite. The present study was carried out to analyze the preliminary phytochemical screening and antioxidant activity of leaves and stem of *Heterophragma adenophyllum*.

MATERIALS AND METHODS

Plant material

Leave and bark of *Heterophragma adenophyllum* were collected from the area of Amravati.

Corresponding author:* **Varsha D. Hutke
Department of Botany, Govt. Vidarbha Institute of Science and Humanities, 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.

Figure 1 *Heterophragma adenophyllum Linn.*

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 30-40ºC till all the solvent 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.l% 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% $CuSO_4$ 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

1. 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
2. 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. H_2SO_4 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. H_2SO_4 was added by the side of the test tube. The upper layer turns red and H_2SO_4 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).

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

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

RESULT AND DISCUSSION

The phytochemical characters of the leaves and bark of *Heterophragma adenophyllum* were investigated and presented in table–1. The qualitative phytochemical analysis of *Heterophragma adenophyllum* leaves and bark contains alkaloids, saponins, Phenolic compound, tannins, flavanoids, phenol and steroids. Results are in support of previous workers (Satani *et al*, 2017 and Akhtar *et al*, 2012).

The antioxidant activity of leaves and bark of *Heterophragma adenophyllum* were evaluated by measuring reducing ability free radical scavenging activity with various extracts (ethanol, methanol, acetone and water) by using DPPH assay, ascorbic acid was used as standard control and results are depicted in Table-2.

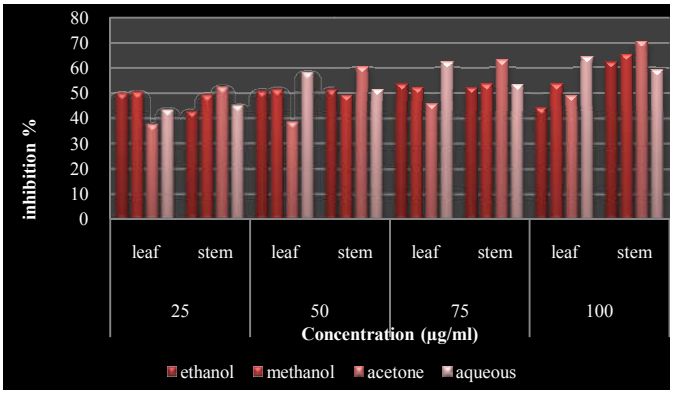

Figure 2 Antioxidant activity of *Heterophragma adenophyllum* (Leaf and Stem) extracts

Table 1 Phytochemical analysis of various extracts from leaves and bark of *Heterophragma adenophyllum*

Phytochemical components	Leaf				Stem			
	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

Table 2 Evaluation of DPPH free radical scavenging activity of of *Heterophragma adenophyllum* (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	49.71 ±1.44		44.14±1.72	
	50	50.63±3.23		51.29±2.14	
	75	53.51±2.17	36.16	52.19±1.38	11.83
	100	61.48±3.71		62.36±2.14	
Methanol	25	50.21±2.11		49.10±1.73	
	50	51.28±1.71		50.77±3.23	
	75	52.18±1.09	16.9	53.53±2.49	13.63
	100	53.56±1.03		65.35±5.17	
Acetone	25	37.51±0.52		52.27± 2.17	
	50	38.53±3.70		60.41±2.79	
	75	45.71±2.16	21.12	63.16±2.54	24.87
	100	49.00±1.25		71.59±1.91	
Aqueous	25	43.29±0.13		45.02±6.10	
	50	57.97±0.76		51.32±2.01	
	75	62.31±0.57	21.13	53.48±2.92	32.53
	100	64.38±0.42		59.16±1.88	
Ascorbic acid	25	55.76±2.87		55.76±2.87	
	50	61.76±2.34		61.76±2.34	
	75	79.21±1.66	5.05	79.21±1.66	5.05
	100	74.66±2.72		74.66±2.72	

The free radical scavenging of bark extract (Fig.2 & 4) in ethanol was found to be higher as compared to all other extracts with IC50 11.83 µg/ml. Methanol extract also showed good results with IC50 13.63 µg/ml. In case of leaf methanol (16.9 µg/ml) exhibited significant antioxidant activity. Similar to the present work Surana *et al*. (2016) also reported the antioxidant properties in leaves and bark of *Heterophragma adenophyllum*. Their study also assured free radical scavenging potential of bark at a good level. The bark found to have potential free radical scavenging activity against DPPH (Wood *et al*, 2008). Results of present study are in support of this findings.

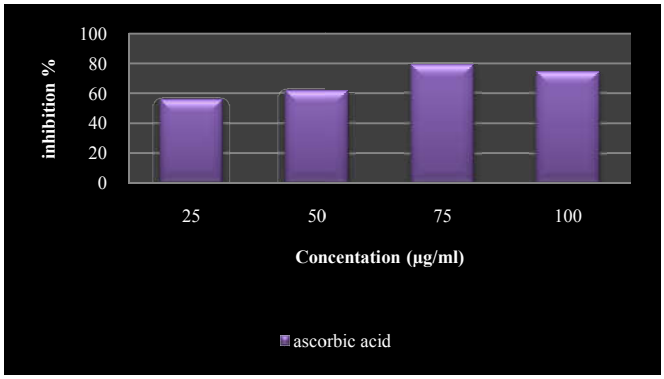

Figure 3 Antioxidant activity of Ascorbic acid

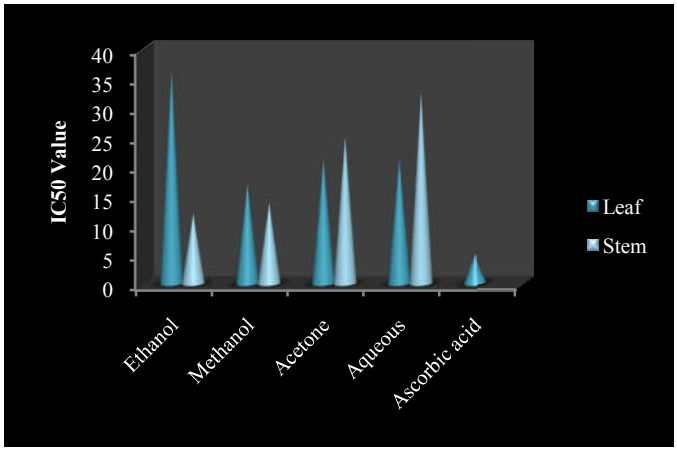

Figure 4 IC50 values of *Heterophragma adenophyllum* (Leaf and Stem) extracts

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