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Preliminary phytochemical analysis of leaves of *Bidens pilosa* L.

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Abstract

Bidens pilosa is a representative perennial herb, globally distributed across temperate and tropical regions. *B. pilosa* has been traditionally used in foods and medicines without obvious adverse effects. Despite significant progress in phytochemical and biological analyses of *B. pilosa* over the past few years, comprehensive and critical reviews of this plant are anachronistic or relatively limited in scope. It has known for its number of pharmacological activities like Hepatoprotective activity, Anti-inflammatory, Wound healing, Antidiabetic activity, Hypotensive effect, Immunomodulating property, bronchial catarrh, dysentery, diarrhoea.

The present study was aimed to investigate phytochemical present in the leaves extract of *Bidens pilosa*. Initially dried powder of *Bidens pilosa* was extracted successively in Chloroform, Acetone-Water and Chloroform-Water and tested for the presence of different phytochemicals.

Keywords: *Bidens pilosa* Linn, extracts and phytochemistry

Introduction

Man and animals depends on the plants for their very existence. Our environment is characterized by richly diversified plant life. Plant diversity is composed of more than 5,00,000 botanical species. Plants constitute a vital component of the biodiversity as they play a key role in maintaining earth's environmental equilibrium and ecosystem stability. Herbal medicine is known to be the oldest form of healing. It originated from ancient Greek as far back as 1600BC (Baker, 1970) ^[1]. With Herbal Renaissance happening all over the globe, medicinal herbs are staging a phenomenal comeback. Ethnobotanical information from India estimates that more than 6000 higher plant species forming about 40% of the higher plant diversity are used in its codified and folk healthcare traditions (Ved and Goraya, 2007) ^[2]. In India, Ayurvedic System of medicine has existed for over four thousand years. From ancient literature it is evidence that the various parts of the plants were used in Siddhha, Ayurveda and Unani medicines for the treatment of diseases of human being.

In the present study we investigated 16 phytochemicals qualitatively from various extracts.

Experimental

Plant Material

The leaves of *Bidens pilosa* Linn. were collected from, Sidhi district, Madhya Pradesh during Feb 2020. It was authenticated by Retd. Prof. A.A. Khan, Department of Botany, Govt. Girls P.G. College, Rewa, Madhya Pradesh.

Preparation of Extract

Chloroform Extract

The collected leaves of *Bidens pilosa* Linn were washed and dried under shade. The coarse powder of the leaves (400 gm) was soaked in 500 ml of Chloroform and extracted in the cold for 2 days with occasional shaking. The solvent from the total extract was filtered and filtrate was dried under shade, it was used for phytochemical screening.

Chloroform-Water Extract

The residue obtained from Chloroform extract was mixed with 600 ml of distilled water and extracted in the cold for 2 days with occasional shaking. The solvent from the total extract was filtered and filtrate was concentrated on water bath for 3 hrs, it was used for phytochemical screening.

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Acetone-Water Extract

500 gm of coarse powder of leaves of *Bidens pilosa* Linn were mixed with 300 ml of distilled water and 300 ml of Acetone in cold for 2 days. The solvent from the total extract was filtered and filtrate was concentrated on water bath for 3 hrs, remaining filtrate was used for phytochemical analysis.

Chemicals and Drugs

All the chemicals and solvents were of Analytical grade from SD Fine Chemicals Pvt. Limited, Bombay.

Phytochemical Analysis

The individual extract was subjected to the qualitative phytochemical screening for the presence of some chemical constituents. Phytochemical test were carried out adopting standards procedure (Trease *et al.* 1983, Kokate *et al.* 1997, Hegde *et al.* 2010) [3-5]. Test were performed for Steroids, Tannin, Saponin, Anthocyanin, Coumarins, Emodins, Alkaloids, Proteins, Amino acids, Diterpenes, Phytosterol, Phenol, Phlobatannins, Leucoanthocyanin, Cardial glycosides and Flavonoids.

Steroid

1ml extract was dissolved in 10 ml of chloroform & equal volume of concentrated H₂SO₄ acid was added from the side of test tube. The upper layer turns red and H₂SO₄ layer showed yellow with green fluorescence. This indicates the presence of steroid.

Tannin

- 2ml extract was added to 1% lead acetate a yellowish precipitate indicates the presence of tannins.
- 4ml extract was treated with 4 ml FeCl₃ formation of green colour indicates that presence of condensed tannin.

Saponin

5 ml extract was mixed with 20 ml of distilled water then agitated in graduated cylinder for 15 min formation of foam indicates Saponin.

Anthocyanin

2 ml of aqueous extract is added to 2 ml of 2N HCl & NH₃, the appearance of pink red turns blue violet indicates presence of Anthocyanin.

Coumarin

3 ml of 10% NaOH was added to 2 ml of aqueous extract formation of yellow colour indicates coumarins.

Emodins

2 ml of NH₄OH and 3 ml of benzene was added to extract appearance of red colour indicates presence of emodins.

Alkaloids

A quantity (3 ml) of concentrated extract was taken into a test tube and 1 ml HCl was added the mixture was heated gently for 20 min cooled and filter, the filtrate was used for following test.

a) Wagner test: Filtrate was treated with Wagner's reagent; formation of brown reddish precipitate indicates presence of alkaloids.

b) Hager's test: Filtrate was treated with Hager's reagent, presence of alkaloids confirmed by the yellow colored precipitate.

Proteins

Xanthoproteic test: Extract was treated with few drops of concentrated HNO₃ formation of yellow indicates the presence of proteins.

Amino acids

Ninhydrin test: To the 2 ml extract 2 ml on ninhydrin reagent was added & boil for few minutes, formation of blue colour indicates the presence of amino acid.

Diterpenes

Copper acetate test: Extract were dissolved in water and treated with 10 drops of copper acetate solution, formation of emerald green colour indicates presence of diterpenes.

Phytosterol

Salkowski's test: Extract was treated with chloroform and filtered. The filtrate was treated with few drops of concentrated H₂SO₄ and shakes, allow standing, appearance of golden red indicates the positive test.

Phenol

Ferric Chloride test: Test extract were treated with 4 drops of Alcoholic FeCl₃ solution. Formation of bluish black colour indicate the presence of Phenol.

Phlobatannins

Deposition of red ppt when aqueous extract of each plant sample is boiled with 1% Aqueous HCl was taken as evidence for presence of Phlobatannins.

Leucoanthocyanin

5 ml of isoamyl alcohol added to 5 ml of aqueous extract, upper layer appear red in colour indicates the presence of Leucoanthocyanin.

Cardial Glycosides

Keller-Killani Test: Plant extract treated with 2 ml glacial acetic acid containing a drop of FeCl₃. A brown colour ring indicates the presence of positive test.

Flavonoid

- Alkaline reagent test:** Extract was treated with 10% NaOH solution, formation of intense yellow colour indicates presence of Flavonoid.
- NH₄OH test:** 3 ml of extract were 10% NH₄OH solution development of yellow fluorescence indicates positive test.
- Mg turning test:** Extract were treated with Mg turning and add conc. HCl to this solution add 5ml of 95% ethanol, formation of crimson red colour indicates Flavonoid.
- Zn test:** 2 ml extract were treated with Zn dust and conc. HCl development of red colour indicates presence of Flavonoid.

Result and Discussion

Present study deals with qualitative analysis of leaves extract of *Bidens pilosa* Linn. Table no. 1 shows the results of phytochemical analysis of leaves of *Bidens pilosa* Linn. Chloroform extract of leaves of *Bidens pilosa* Linn shows the presence of Steroid, Saponin, Coumarins, Alkaloids, Amino acids, Diterpenes, Phenol and Flavonoids whereas Tannin, Anthocyanin, Emodins, Proteins, Phytosterol, Phlobatannin, Leucoanthocyanin and Cardial Glycosides were absent.

Acetone-Water extract of leaves of *Bidens pilosa* Linn shows the presence of Steroid, Tannin, Saponin, Anthocyanin, Coumarins, Alkaloids, Diterpenes, Phenol and Flavonoids whereas Emodins, Proteins, Amino acids, Phytosterol, Phlobatannin, Leucoanthocyanin and Cardial Glycosides were absent.

Chloroform -Water extract of leaves of *Bidens pilosa* Linn shows the presence of Steroid, Tannin, Saponin, Anthocyanin, Coumarins, Alkaloids, Amino acids, Diterpenes, Phenol and Phlobatannin whereas Emodins, Proteins, Phytosterol, Leucoanthocyanin, Cardial Glycosides and Flavonoids were absent.

Kewuchi Jude *et al.* (2009) ^[6] was reported Six phytochemical from the leaves of *Bidens pilosa* Linn. Ayyappa Das *et al.* (2009) ^[7] was calculated Eight secondary metabolites from the Aqueous and Methanolic leaf extract of *Bidens pilosa* Linn. Dhanabalan *et al.* (2008) ^[8] also shows the presence of Eight phytochemicals as Alkaloids, Tannin, Saponin, Steroid, Phlobatannin, Terpenoids, Flavonoids and Cardiac glycosides form the Methanolic extract of leaves of *T. procumbens* Linn.

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Table 1: Phytochemical analysis of leaves of *Bidens pilosa* L.

S. No.	Phytochemicals	A.W.E	C.E.	C.W.E.
1.	Steroids	+	+	+
2.	Tannin			
	Lead acetate	+	-	+
3.	Ferric chloride	+	-	+
	Saponin	+	+	+
4.	Anthocyanin	+	-	+
5.	Coumarins	+	+	+
6.	Emodins	-	-	-
7.	Alkaloids			
	Wagner Test	+	+	+
8.	Hager Test	+	+	+
	Proteins Xanthoproteic Test	+	+	+
9.	Amino acids Ninhydrin Test	-	+	+
10.	Diterpenes	+	+	+
11.	Phytosterol Salkowski Test	-	-	-
12.	Phenols	+	+	+
13.	Phlobatannin	-	-	+
14.	Leucoanthocyanin	-	-	-
15.	Cardial Glycosides Kellar-Killiani Test	-	-	-
16.	Flavonoids	+	+	-
	Alkaline reagent test NH ₄ OH	+	+	-
	Mg turning test	+	+	-
	Zn Test	-	+	+

+ = Present; - = Absent; A.W.E- Acetone Water Extract; C.E.- Chloroform Extract, C.W.E.-Chloroform Water Extract

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