Available online at www.ijpab.com

ISSN: 2320 – 7051
Int. J. Pure App. Biosci. **3 (2):** 152-155 (2015)

INTERNATIONAL JOURNAL OF PURE & APPLIED BIOSCIENCE

Research Article

OPEN ACCESS

Total phenolic and primary metabolites in *Verbesina encelioides*

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ABSTRACT

The phytochemical screening carried out on Verbesina encelioides *showed the existence of beneficial phytonutrients present in it. Verbesina* encelioides *is a medicinal plant that used to treat stomach problems and is an excellent blood purifier, the results showed that Verbesina* encelioides *is beneficial herbal plant that showed the presence of phenolic compounds, flavonoid and some specific metabolites. The results of the phytochemical screening of medicinal plants was discussed in relations to their usefulness to mankind.*

Keywords: *Verbesina* encelioides, *golden crown beard, terpenoids , flavonoids, Asteraceae*

INTRODUCTION

The Study of presence of phytochemical is the preliminary step for the standardization of Therapeutic medicines, Examination of phytochemical values gives valuable information about crude drug related to its morphology and secondary and primary metabolites. These study counts its own importance for various pharma books[1]. Most of the therapeutic plants in India needs standardization that has not been identified yet. So, these studies gives a proper scientist information about the proper standardization of drug,(3), Verbesina is a less dense populated group native to South western United States and Mexican platueau,,This species originate in the arid grass and scrublands .Most of the species of this group have economical as well as ornamental values.

Verbesina encelioides is known as golden crown beard, comes in daisy family (*Asteraceae*) [2] and counts a genus of flowering plants, has occurred into different regions of the globe. This plant is mostly used to treat stomach problems. The roots consumed for retention of water in body, bladder inflammation and used as blood purifier[4]. The main pharma compound from various parts of the plant is terpenoids, flavonoids, aromatic compounds and phenolic complexes etc[5]. Plant leaf is cast-off to cure pain in legs so as to cure rheumatism, and juice of the plant has its own importance as purgative compound.

The height of the plant is 1 to 5 feet[6,7]. The flower look like Sun flower[8]. Flower heads can both that is unsociable, or in clusters of up to 3 heads[8,9]. Seeds are grayish-brown in colour, flat in shape, and feathered on the margins[7]. Size of the seed is stuck between 5.4mm to 6.7mm by 3.1mm to 3.64 mm[10]. Leafs, seeds and stalk of *V. encelioides*, are enclosed with fine hairs[7]. *Verbesina encelioides* is distinguished from the orchard sunflower by the opposite leaves on the lower part of the plant, and it has considerably a small flower heads[8].

The present study was carried out to calculate the presence of some primary metabolites and phenolic compounds.

MATERIAL AND METHOD

For Carbohydrates Extraction and Quantification (Total Soluble Sugars)

The dried plant materials stem, root, leaf, and callus (50 mg each) were homogenized separately in a mortar and pestle with 20 ml of 80% ethanol and left overnight. Each of the sample was centrifuged at 1200 rpm for 15 min, the supernatants were collected separately and concentrated on hot water. Distilled H_2O was poured to mark volume up to 50 ml and processed further for quantitative analysis.

For Starch Extraction and Quantification

The residual mass obtained after extraction of total soluble sugars of each of the test samples was suspended in 5.0 ml of 52% per chloric acid. Later, 6.5 ml of water was added to each sample and the mixture was shaken vigorously for 5 min.

Quantitative Estimation

1 ml aliquot of each sample was used for the estimation of carbohydrates using the phenol-sulphuric acid. A standard regression curve of standard sugar (glucose) was prepared. A stock solution of glucose 100 µg / ml was prepared in distilled water. From this solution, 0.1 to 0.8 ml was pipette out into eight separate test tubes and volume was made up to 1 ml with. Distilled H_2O. Tubes were placed in cold or on ice; 1 ml from 5% phenol was added in each tube and shaken gently. 5 ml of conc. sulphuric acid was rapidly poured so that the steam hits the liquid and tubes were gently shaken during the addition of the acid. Finally the mixture was allowed to stand on a water bath at 26-30^oC for 20 min. The characteristic yellow orange colour was developed. The O.D was taken on 490*nm* using spectrometer after setting for 100% transmission against a blank (distilled water). Standard regression curve was computed between the known concentrations of glucose and their respective O.D, that trailed Law of Beer's.

All samples were analysed in the same way as described above and contents of the total soluble sugars and starch were calculated by computing optical density of each of the samples with standard curve.

Proteins Extraction and Quantification

The test samples (50 mg each) were separately homogenized in 10 ml of cold 10% trichloroacetic acid (TCA) for 30 min and placed for a day on 4^oC. These mixtures were centrifuged separately and supernatants were discarded. Each of the residues was again suspended in 10 ml of 5% TCA and heated at 80^oC on a water bath for 30 min. The samples were cooled, centrifuged and the upper layers were thrown away. Then the left over material was cleaned with Distil H_2O, dissolved in 10 ml of 1N NaOH, and left overnight at room temperature.

Quantitative Estimation

Each of the above samples (1ml) was taken and the total protein content was estimated using the spectrophotometer. A graph of protein (bovine serum albumin, BSA) was made. bovine serum albumin (Sigma Chem. Co., St. Louis, USA) was prepared in 1N NaOH (1mg/ml). Eight concentrations (ranging from 0.1 to 0.8 mg/ml) were separately measured in test tubes and the volume of each was made up to 1ml by adding distil H_2O. 5mili litre of alkaline solution was added in every sample (Prepared by mixing 50 ml of 2% Na_2CO_3 in 0.1 N NaOH and 1 ml of 0.5% $CuSO_4.5H_2O$ in 1% Sodium potassium tartarate) was added and kept at RT for 10 min. In each sample 0.5 ml of Folin-Ciocalteau reagent (commercially available reagent was diluted with equal volume of distilled water just before use) was added rapidly with immediate mixing and OD of each sample was measured after 30 min at 750 nm using spectrophotometer against the blank. Five replicates of each concentration were taken and the average value was plotted against their respective concentrations to compute a graph.

All samples were processed in the same manner and the concentration of the total protein content in each sample was calculated by referring the optical density of each sample with standard curve. Five replicate samples were taken in each case and mean value was calculated

Phenol Extraction and Quantification

The test samples dried and powdered and 200 mg was macerated with 80% ethanol (10 ml) for 2 hr and left for overnight at RT. After centrifugation the upper layer was collected separately and volume of each was raised to 40ml with 80% ethanol. For the estimation of total phenol a standard curve of galic acid was prepared.

A stock solution (40%) of galic acid was prepared in 80% ethanol, from which 0.1 to 0.9 ml was transferred into test tubes separately and the volume in each case was raised to 1 ml in 80% ethanol. Then, 1 mili litre of folin- Ciocalteau chemical (dilute the reagent 1:2 with distilled water) and 2 ml of 20% of Na_2CO_3 solution was added. The mixture was shaken vigorously. Each of the tubes was boiled on water bath and cooled at room temperature and diluted to 25 ml with distilled water. The readings were taken using spectrometer against blank at 750nm. The experiment performed in triplicate and average OD was plotted against respective concentration to compute a regression curve. Each of the test samples was treated and processed, and readings were calculated and amount of phenol was calculated from the mean value by referring the OD of the test sample with regression curve of the standard.

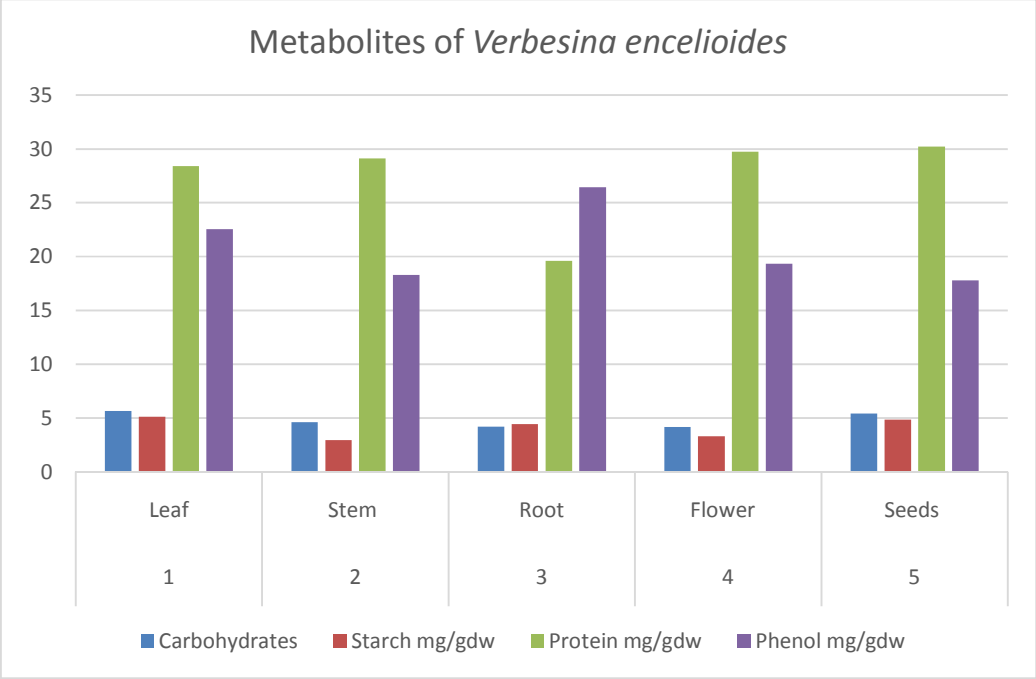
RESULT AND DISSCUSION

Verbesina encelioides shows the presence of carbohydrate, starch, protein and phenols. The below mentioned table displays the presence of all metabolites .The maximum amount of Carbohydrate in leaf (5.65mg) minimum amount in flower (4.17mg)

Table 1.1. Primary metabolites and total phenol of *Verbesina encelioides*

S.No.	Plant parts	Carbohydrate mg/gdw	Starch mg/gdw	Protein mg/gdw	Phenol mg/gdw
1	**Leaf**	**5.65**	**5.12**	**28.41**	**22.55**
2	**Stem**	**4.61**	**2.94**	**29.13**	**18.31**
3	**Root**	**4.20**	**4.43**	**19.60**	**26.43**
4	**Flower**	**4.17**	**3.3**	**29.73**	**19.33**
5	**Seeds**	**5.40**	**4.87**	**30.23**	**17.78**

Fig. 1.1 primary metabolites and total phenol of *Verbesina encelioides*



DISSCUSION

The results demonstrate that test herb's leaf, stem, flower, seeds has presence of protein to the maximum whereas in root, phenolic compound present in it to the maximum amount, and starch is in least amount in all the plant parts.

Acknowledgement

Authors acknowledge with thanks the financial support from Department of Science andTechnology, Government of Rajasthan, in the form of Centre with Potentials for Excellence in Biotechnology, sanction no. F7(17) (9) Wipro/Gaprio/2006/7358-46(31/10/2008).

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