

# Isolation and Purification of Bioactive Compounds from *Verbesina encelioides* : An Underexploited Medicinal Plant

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## Abstract

*Verbesina encelioides* is an important medicinal plant of family Asteraceae. This plant is commonly known as golden crown beard and it shows aggressive and dominant growth abilities. The present study was carried out to assess the phytochemical constituents of the whole plant part of *Verbesina encelioides* and the identification of bioactive compounds was done using thin layer chromatography.

**Key words :** *Verbesina encelioides*, Phytochemical constituents, Plant extract, Therapeutic properties, TLC

## Introduction

Nature has provided a complete storehouse of remedies to cure all ailments of humankind. The history of herbal medicines is as old as human civilization. Today, there is a vast store of knowledge concerning therapeutic properties of different plants. Plants still remain one of the major sources of drugs in modern as well as in traditional system of medicine. Phytochemicals are non-nutritive plant chemicals that have protective or diseases preventive properties (Nisharaj and Radhamany, 2012; Moses *et al*, 2012). Plants produce these chemicals to protect themselves, but recent research demonstrates that many plant chemicals can also protect human beings against diseases. There are many phytochemicals isolated from herbs and each acts differently. These phytochemicals have various health benefits which are manifested as antioxidant, anti-microbial, anti-inflammatory, cancer preventive effects (Rupasinghe *et al*, 2003; Savithramma *et al*, 2011)

Asteraceae (compositae) is commonly referred to as the aster, daisy or sunflower family. It is one of the largest families of vascular plants having more than 22,750 species. *Verbesina encelioides*, a weed can tolerate wide range of climatic conditions including drought and high temperature (Kaul and Mangal, 1987). Different parts of this plant such as the leaf, stem and flower are useful and important in pharmaceutical industries. This plant possesses antimicrobial, antiviral, anti-tumor, hypoglycemic and anti-implantation efficacies (Jain *et al*, 1988; 2007). In the present work, phytochemical analysis and separation of components of whole plant part was investigated out in *Verbesina encelioides*.

## Materials and Method

### Plant Material

Healthy plants of *Verbesina encelioides* were collected in the month of April, 2013 from the Mansarovar area, Jaipur. The authenticity of the plant was confirmed by renowned taxonomist Prof. S. Kshetrapal, Department of Botany University of Rajasthan, Jaipur, India. The voucher specimens were maintained at The IIS University, Jaipur.

### Preparation of plant extracts

The dried powder of whole plant of *Verbesina encelioides* was extracted successively on each of the selected solvents viz. n-hexane, chloroform, ethyl acetate, methanol and water by soxhlet extraction for relevant time periods. The extracts were concentrated to dryness in the oven. The % yields from various solvents are given in Table 1. All the extracts were stored in a container for further use.

**Table 1. Extractive values of all extract of *Verbesina encelioides* (whole plant)**

S. No.	Solvents	Weight of the extract (g)	W/W % yield
1	n-Hexane	0.144	1.44
2	Chloroform	0.18	1.8
3	Ethylacetate	0.193	1.93
4	Methanol	0.533	5.33
5	Water	1.915	19.15

### Extraction process and phytochemical screening

The dried leaves, stem and flower of plant *Verbesina encelioides* were extracted with various solvents ranging from non-polar to polar solvents viz. N-hexane, chloroform, ethyl acetate, methanol and water at different temperature for defatting purpose, in a soxhlet apparatus. Crude extract of the n-hexane, chloroform, ethyl acetate, methanol and water extracts of *Verbesina encelioides* containing the whole plant part were dissolved in 1:10 ratio of its mother solvents to obtain a stock solution. The extracts thus obtained were subjected to a preliminary qualitative test using standard methods (Harborne, 1998).

### Isolation of bioactive compounds

In recent years chromatography has occupied a very significant position in the studies of biology and chemistry. It consists of separation of a mixture based on the different affinities of the components for a stationary phase such as solid or liquid and their differential solubility in a moving phase such as liquid or gas. In TLC Retention factor (Rf) value refers to the ratio of distance moved by the solute on a thin layer of an adsorbent. Rf value of a compound is its diagnostic feature and can be used to identify the component by comparison with the reference standard. The intensity of the color of spot of the compound under test can be utilized for quantitative estimation of the principle in the drug.

$Rf = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$

A spot of the extract was applied about 1cm from the bottom of the TLC Silica gel (MERCK) 60, 20x20 cm plate. For detection of bioactive compounds like alkaloids, flavanoid, tannin and saponin the TLC plates were used in different solvents (Table 2).

**Table 2. Different solvent ratio of Phytoconstituents used in thin layer chromatography**

S. No.	Phytoconstituents	Solvent	Ratio
1	Alkaloids	Acetone: Methanol: GAA	75:25:5
2	Flavanoids	Ethyl acetate : Methanol	50:10
3	Saponins	Ethyl Acetate: Methanol: Water	5:2:5
4	Tannins	BuOH: HOAc: Water	4:1:5

Solvents in the above ratio were poured into chromatography jar filling about inch from the bottom. The spotted plate was placed vertically in such a manner that the spot was just above the solvent level. Solvent was allowed to move up in a closed jar for two hours after which the plates were removed and dried at room temperature. The plates were then observed in UV light (365nm). Various spot were identified. The distance moved by the solvents and the spots were measured. The Rf of the samples were then calculated.

### Results and Discussion

The result obtained in the present investigation (Table 3) of n-hexane, chloroform, ethyl acetate, methanol and water extracts of the whole plant part of *Verbesina encelioides* showed the presence or absence of various secondary metabolites like alkaloids, flavanoids, tannin and saponins. A variety of several phytochemical such as alkaloids, flavanoids, tannin, saponin and others have been reported in this plant (Jain *et al*, 1988; 2007).

**Table 3. Qualitative phytochemical analysis of crude extracts of *Verbesina encelioides* (Whole plant part)**

Phytoconstituents	N-hexane	Chloroform	Ethylacetate	Methanol	Water
Alkaloids	++++	++++	++++	+++-	+--
Flavonoids	++---	+---	++---	+++-	+-
Tannins	++--	++--	++--	++--	+-
Saponins	+++	+++	+++	++--	+-

+ : Present, - : Absent

The presence of these compounds supports the use of this plant in traditional medicine. The present study tried to evaluate the bioactive potential of partially purified compounds isolated by preparative TLC from the whole plant part of *Verbesina encelioides*. Based on these results the crude extract of various solvent ranging from non-polar to polar solvents of whole plant part were used to detect the bioactive compounds through TLC. The TLC results indicated the presence of various spots under ultra violet light (365nm) illumination

(Table 4; Fig. 1, 2, 3 and 4).

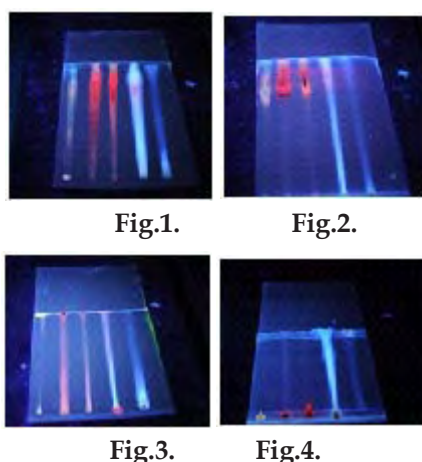


Fig.1-4 : Separation of compound by using different solvent system for thin layer chromatography of whole plant part of *V. encelioides*

Table 4. Separation of compound by using different solvent system for thin layer chromatography of whole plant part of *V. encelioides*

Phytochemical name	Solvent	No of Identified spots	RF value
Alkaloids	N-Hexane	7	0.63,0.74,0.80,0.83,0.86,0.95,0.97
	Ethylacetate	10	0.66,0.70, 0.74, 0.78, 0.82, 0.85, 0.89, 0.91, 0.94, 0.97
	Chloroform	7	0.68,0.72,0.75,0.81,0.84,0.91, 0.95
	Methanol	6	0.72, 0.78,0.83,0.85, 0.89, 0.98
	Water	3	0.070, 0.88, 0.97
Flavonoids	N-Hexane	3	0.17, 0.80 ,0.96
	Ethylacetate	3	0.28,0.63,0.97
	Chloroform	2	0.26,0.97
	Methanol	3	0.37, 0.63, 0.97
	Water	4	0.088, 0.51 ,0.77, 0.98
Tannins	N-Hexane	3	0.083, 0.85, 0.96
	Ethylacetate	3	0.091,0.55,0.96
	Chloroform	5	0.083,0.275,0.56,0.78, 0.98
	Methanol	3	0.041, 0.4, 0.92
	Water		0.04, 0.15, 0.33, 0.44, 0.78
Saponins	N-Hexane	5	0.30, 0.76 , 0.80, 0.91 ,0.94
	Ethylacetate	7	0.2, 0.63 ,0.77,0.80,0.86 ,0.91,0.97
	Chloroform	5	(0.30, 0.56 ,0.80,0.84,0.91
	Methanol	9	0.26,0.51,0.67,0.68,0.72,0.82,0.86,0.88,0.91
	Water	3	0.10, 0.16 , 0.91

Further studies are needed to identify the flavanoids, alkaloids, tannins and saponins in the analyzed extracts

and to isolate and elucidate the structure of the bioactive compounds of the plant which are responsible for the antimicrobial activity and other medicinal value.

## Conclusion

Based on the above results, it is concluded that the crude extract of whole plant part of *Verbescina encelioides* shows the presence of a wide range of phytochemical constituents as observed by Thin layer chromatography technique. Such screening of various natural compounds and identification of natural organic compounds is the need of the hour for successful prediction of drug development.

## Acknowledgment

This work was supported by The IIS University, Jaipur-302020.

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