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Extraction of different parts of *Verbesina encelioides* in various solvents and determination of presence of primary and secondary metabolites in the extracts

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Abstract
The present study investigates the extraction efficiency and phytochemical composition of different parts (roots, stems, leaves, and flowers) of *Verbesina encelioides* (L.) Benth. & Hook.f. ex A. Gray using solvents of varying polarity—water, methanol, and ethanol. The extracts were analyzed for their extractive yields and qualitative presence of primary (carbohydrates and proteins) and secondary metabolites (phenolics, flavonoids, alkaloids, and phytosterols). Results revealed significant variation in extractive values among plant parts and solvents, with the highest yield obtained from the aqueous extract of flowers (222 mg/g dry weight). Phytochemical screening confirmed the widespread presence of carbohydrates, proteins, phenols, flavonoids, and alkaloids across most extracts, whereas phytosterols were confined to ethanol and methanol extracts of specific tissues. These findings highlight the organ-specific and solvent-dependent distribution of metabolites in *V. encelioides*, emphasizing its potential as a source of bioactive compounds for pharmacological and industrial applications. The study provides a scientific basis for targeted extraction and utilization of *V. encelioides* in natural product research.

Keywords: *Verbesina encelioides*, phytochemical screening, extractive value, solvent extraction etc

Introduction

Plants have long been a cornerstone of medicinal research, providing a vast array of bioactive compounds that serve as templates for therapeutic agents. Among these, *Verbesina encelioides* (L.) Benth. & Hook.f. ex A. Gray, commonly known as golden crownbeard, stands out as a member of the Asteraceae family with notable pharmacological potential. Traditionally utilized by indigenous communities for treating ailments such as skin disorders, fever, and inflammation, this plant has garnered attention for its diverse bioactivities. Pharmacological studies have demonstrated its antimicrobial, antitumor, antifungal, antiprotozoal, and hypoglycemic properties (Gorja and Bandla, 2024) [3].

The therapeutic efficacy of *V. encelioides* is attributed to its rich phytochemical composition, encompassing both primary metabolites like carbohydrates, proteins, and amino acids, and secondary metabolites such as flavonoids, alkaloids, terpenoids, saponins, and phenolic compounds. These secondary metabolites are pivotal in plant defense mechanisms and have been linked to various pharmacological activities. The distribution and concentration of these metabolites can vary across different plant organs, making it essential to evaluate each part individually to identify the most potent sources (Sindhu *et al.*, 2010) [8].

Extraction methods play a crucial role in isolating these bioactive compounds. Solvents of varying polarities, such as water, methanol, and ethanol, are commonly employed to extract a broad spectrum of metabolites. The choice of solvent significantly influences the yield and composition of the extracts, thereby impacting the subsequent biological activity assessments (Lee *et al.*, 2024) [5].

Despite the growing interest in *V. encelioides*, comprehensive studies examining the extractive yields and phytochemical profiles across its different organs remain limited. Such investigations are vital for understanding the organ-specific distribution of bioactive compounds and optimizing extraction processes for therapeutic applications. Therefore, this study aims to evaluate the extractive yields and assess the presence of primary and secondary metabolites in the roots, stems, leaves, and flowers of *V. encelioides* using water, methanol, and ethanol as solvents. The findings are anticipated to provide valuable insights into the plant's phytochemical diversity and inform future pharmacological research.

Materials and Methods

Collection and processing of plant material

Plant material (Root, stem, leaves and flowers) of the selected plant were collected. The collected plant was identified at Herbarium, Department of Botany, university of Rajasthan, Jaipur. Those were washed with running tap water and then distilled water to remove dust particles. After that, plant parts were air dried and grinded into mixer grinder to make coarse powder. Those were stored for further work.

Extraction

Plant materials were extracted in different polar and non-polar solvents. Those were water, methanol and Ethanol. For this purpose, 1 gm of each plant part (root, stem, leaves and flowers) were taken dipped into 10 ml of the solvent individually. Total 12 beakers were prepared for this purpose. Those were kept at sonicator at 40°C for 10 minutes. After that, those were filtered. Filtrate were taken into pre-weighed petri-dishes. Those were left for evaporation of solvent. After complete drying, petri-plates were weighed again and weight of extract per gram plant material was calculated. Extracts were collected in glass

vials for further use. Besides these, extract color and consistency were also noted.

Qualitative test for primary and secondary metabolites

Following metabolites were determined qualitatively to confirm their presence in the plant extracts-

Carbohydrate (Fehling method)

Fehling's reagent made up of two solutions: A and B of Fehling's solutions. Aqueous copper sulphate makes up Fehling's solution A, while alkaline sodium potassium tartrate makes up Fehling's solution B (Rochelle salt). The chelating agent in this reaction is provided by the Rochelle salts (sodium potassium tartrate) in the reagent. Before the test, an equal mixture of these two solutions is used. The mixture was heated on a water bath using an equal amount of freshly made Fehling's solution and 2ml of the aliquot. Reddish brown precipitate results from this. The presence of carbohydrates is shown by the red cuprous oxide precipitate that forms.

Protein (Ninhydrin)

Proteins interact with Ninhydrin's pyridine solution, changing it from a deep blue to a violet-pink or, on rare instances, a red colour. Ninhydrin 0.1gm is dissolved in approximately 100ml of distilled water to prepare a solution. However, this Ninhydrin solution is unstable and only lasts for two days. The availability of proteins is confirmed by the violet-colored solution's appearance.

Phenol (FeCl3 test)

The presence of phenols was determined by adding 0.5 ml of FeCl3 (w/v) solution to 2 ml of the test solution.

Flavonoids (Lead acetate test)

1 ml of extract was taken and few drops of 10% lead acetate solution was added. Appearance of yellow coloured precipitate indicates the presence of flavonoid.

Alkaloids (Mayer's test)

Mayer's reagent is an alkaloidal precipitating reagent used for the detection of alkaloids in natural products. Mayer's reagent is freshly prepared by dissolving a mixture of mercuric chloride (1.36 g) and of potassium iodide (5.00 g) in water (100.0 ml). Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's reagent (Potassium mercuric iodide solution) to give a precipitate.

Phytosterol (Libermann-Burchard's test)

Concentrated sulphuric acid is added along the side of the test tube after the extract has been treated with a few drops of acetic anhydride, boiled and cooled. This causes the upper layer to turn green, indicating the presence of sterols, and a deep red colour to form, indicating the presence of triterpenoids.

Results

Extraction of plant parts

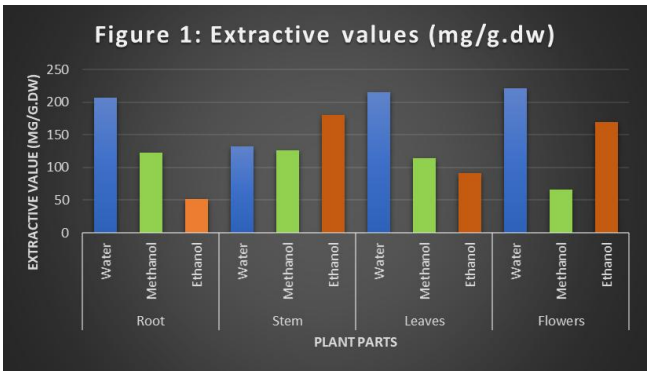
The yield and physical characteristics of extracts varied across plant parts and solvents. The highest yield was observed in the aqueous extract of flowers (222 mg/g dry weight), followed by the aqueous extract of leaves (216 mg/g), indicating the strong ability of water to extract polar compounds, especially from floral and foliar tissues. The aqueous root extract also showed a high yield (207 mg/g), while ethanol generally yielded the least, with the lowest extraction obtained from the root (52 mg/g). This is attributed to the lower polarity of ethanol compared to water, limiting its ability to dissolve highly polar phytoconstituents.

The consistency of the extracts ranged from powder to sticky. Aqueous extracts of root, leaf, and flower were powdery, indicating good drying potential, while methanolic and ethanolic extracts were mostly sticky, reflecting the solubilization of more lipophilic compounds. The drying time for aqueous extracts was the longest, ranging from 7 to 11 days, suggesting that water retained higher moisture content and delayed the drying process.

The color of extracts varied depending on the plant part and solvent. Roots extracted with water appeared dark brown and in powder form, while ethanol extracts were white and sticky. Stem extracts were mostly sticky in all solvents with varying colors from light brown to transparent. Leaf extracts presented a brown powder with water and yellowish-brown sticky material with ethanol. The methanolic flower extract had a distinct dark green color, while the ethanolic extract was green and sticky.

Table 1: Extractive values, extract colour and appearance

Plant part	Solvent name	Yield of extract/g. dw (mg)	Drying time (days)	colour	consistency
Root	Water	207	8	Dark brown	powder
	Methanol	123	4	creamish	sticky
	Ethanol	052	3	white	sticky
Stem	Water	132	7	Dark brown	sticky
	Methanol	126	5	Light brown	sticky
	Ethanol	181	2	Transparent	sticky
Leaves	Water	216	9	brown	powder
	Methanol	114	4	Light brown	powder
	Ethanol	092	4	Yellowish-brown	sticky
Flowers	Water	222	11	Brown	Powder
	Methanol	66	5	Dark green	Powder
	Ethanol	170	3	Green	Sticky



Qualitative test for primary and secondary metabolites

The presence of primary and secondary metabolites was confirmed across all parts and solvents. Carbohydrates were detected in all extracts via the Fehling test, which yielded dark blue to blue precipitates, confirming their presence. Proteins were also universally present, showing positive reactions in all samples with varying intensities of orange to brown color upon Ninhydrin treatment.

Phenolic compounds, essential for antioxidant activities, were evident through the FeCl3 test, with all extracts yielding colored precipitates (yellow to red), with aqueous and methanolic extracts showing more intense coloration, indicating higher phenol content. Flavonoids, detected via the lead acetate test, were abundant in all extracts, with brown to orange precipitates, again more pronounced in aqueous and methanolic extracts of flowers and leaves.

Alkaloids were detected using Mayer's test and were present in water and methanol extracts of all parts, indicated by color changes to orange and yellow. Ethanol extracts, however, showed a negative result (transparent), suggesting poor solubility of alkaloids in ethanol.

Phytosterols were absent in all aqueous extracts, while they appeared in ethanolic and selected methanolic extracts (notably flower and stem) as indicated by brown or yellow precipitates using the Libermann-Burchard's test.

Table 2: Qualitative determination of primary and secondary metabolites in different extracts of various parts

Phytochemical name	Extract type	Root		Stem		Leaves		Flowers	
		Observation	Results	Observation	Results	Observation	Results	Observation	Results
Carbohydrate (Fehling method)	Water	Blue	+	Dark blue	+	Dark blue	+	Blue	+
	Methanol	Blue	+	Dark blue	+	Dark blue	+	Blue	+
	Ethanol	Blue	+	Blue	+	Dark blue	+	Dark Blue	+
Protein (Ninhydrin)	Water	Orange	+	Orange-red	+	Brown	+	Orange	+
	Methanol	Light brown	+	Dark yellow	+	Yellow	+	Yellow	+
	Ethanol	Yellow	+	Orange	+	Brown	+	Orange	+
Phenol (FeCl3 test)	Water	Dark yellow ppt	+	Light yellow ppt	+	Yellow ppt	+	Orange-red ppt	+
	Methanol	Red ppt	+	Orange ppt	+	Orange-Yellow ppt	+	Yellow ppt	+
	Ethanol	Yellow ppt	+	Light yellow ppt	+	Orange ppt	+	Orange-yellow ppt	+
Flavonoids (Lead acetate test)	Water	Dark Brown ppt	+	Light brown ppt	+	brown orange ppt	+	Brown ppt	+
	Methanol	Dark Yellow ppt	+	Light brown ppt	+	yellow ppt	+	Orange ppt	+
	Ethanol	Brown ppt	+	Light brown ppt	+	Orange ppt	+	Yellow ppt	+
Alkaloids (Mayer's test)	Water	Orange	+	Yellow	+	Orange-Yellow	+	Light brown	+
	Methanol	Brown	+	Yellow	+	Light yellow	+	Yellow	+
	Ethanol	Transparent	-	Transparent	-	Transparent	-	Transparent	-
Phytosterol (Libermann-Burchard's test)	Water	Transparent	-	Transparent	-	Transparent	-	Transparent	-
	Methanol	Transparent	-	Ppt	+	Transparent	-	ppt	+
	Ethanol	Dark brown ppt	+	Yellow ppt	+	Light brown ppt	+	Brown ppt	+

Discussion

The current study aimed to investigate the phytochemical profile and biological efficacy of different parts of the plant *Verbesina encelioides.* For this, the research work was divided into different sections. The first section of the study showed results pertaining to extraction of phytochemicals from different plant parts (root, stem, leaves, and flowers) with three solvents: water, methanol, and ethanol. The yield, qualitative phytochemical presence was systematically assessed.

The current study showed variability in yield of extractives from *Verbesina encelioides* plant when different solvents were used. Comparing the different plant parts, the highest number of extractives was obtained in case of flowers, that too when water was used as a solvent. Furthermore, for all the plant parts, the highest yield of extractives was obtained when water was used as a solvent followed by different alcohols (methanol and ethanol). Talking about the color of extractives, the extractives with highest yield were found to be dark brown in color and powdery in consistency.

The yield of extractive values provides crucial insights into solvent efficiency, phytochemical solubility, as well as the biochemical richness of different plant parts. As per the results in this table, water was found to be the most effective solvent as in in terms of extract yield across most plant parts, with the highest yield of extractives recorded in case of flowers (222 mg/g dw) followed by leaves (216 mg/g dw), and roots (207 mg/g dw). As per these findings, the results showcase a predominance of water-soluble phytochemicals in different plant parts, including the polysaccharides, phenolics and glycosides. However, the results were somewhat reversed in stem, where, highest yield of extractives was obtained in case of ethanol (181 mg/g dw), surpassing water and methanol. This points towards the fact that stem tissues may be particularly may possess semi-polar compounds, such as flavonoids or certain terpenoids, which ethanol can efficiently solubilize. Furthermore, highly shortened drying time (2 days) for ethanol extracts leads to better compound preservation as well as comparatively lesser degradation compared to longer drying periods. This highlights the requirement of targeted solvent selection based on tissue type as well as desired phytochemical profile (Ahmed *et al.*, 2024) [1].

Furthermore, this observation is correlated with the color and consistency of the plant extracts. For instance, dark brown color of extractives in highest concentration points

towards presence of phenolic and tannins in the plant extracts. The results showed powdery consistency of aqueous extracts whereas, methanolic and ethanolic extracts were sticky, suggesting presence of resins, sugars, or oils, which are less prone to full desiccation (Mora *et al.,* 2013) [6].

In the next section of the study, the researchers showcased qualitative screening of primary and secondary metabolites, including carbohydrates, proteins, phenolics, flavonoids, alkaloids and phytosterols in extracts of root, stem, leaves, and flowers of the plant. This was performed with the intention to understand the biochemical landscape of the plant, which plays a crucial role in determining its medicinal potential and industrial applications. The results showed that carbohydrates were universally detected in all the extracts using the Fehling's test, as evident from blue precipitates in all the samples. This shows presence of reducing sugars in all the plants parts, no matter what solvent is used for extraction. Also, the results indicate presence of carbohydrates in both primary tissues as well as photosynthetically active parts of the plants such as leaves and flowers. Thereafter, the researchers showed presence of proteins in all the plants extracts, as evident form results of Ninhydrin test. Orangish to brown color was observed in different samples, indicating variability in amount of proteins present in different samples. For instance, methanolic stem extracts and flower extracts yielded dark shades, whereas, other extracts yielded orange shades, which indicates higher amount of proteins in stem and flowers extracts when methanol was used as a solvent.

Results confirmed presence of phenolic compounds, wherein, presence of yellow or red precipitate in the $FeCl_3$ test confirmed presence of phenols. Presence of yellow and red color in methanolic and water extracts of flowers and leaves confirmed presence of antioxidant phenolic compounds in these plant parts. Higher amount of phenols in leaves and flowers is totally justified owing to their ability to be actively involved in plant defense against herbivory. Thereafter, presence of flavonoids identified using lead acetate test was confirmed in different plant parts. The results showed high number of flavonoids in flowers and leaves, as evident from intense coloration. The presence of brown to orange precipitates indicates presence of flavonoid compounds, particularly in water and methanolic extracts. This is increasingly important from the perspective that both flowers and leaves play a crucial role in UV protection, and antimicrobial activity, especially in aerial parts of the plants that are more exposed to environmental stressors. However, in complete contrast to this, alkaloids were found to be readily soluble in aqueous and methanolic extracts. Ethanol extracts failed to show any major color change, confirming the poor solubility of alkaloids in ethanol. The uniform presence of alkaloids in water and methanol extracts across all plant parts is indicative of the fact that entire plant has the capability to serve as potent antimicrobial, analgesic, and anticancer agent (García-Bores AM *et al.,* 2020; Velasco-Ramírez *et al.,* 2022) [2, 9].

Thereafter, we assessed presence of phytosterols in all the aqueous extracts, confirming their non-polar nature. Phytosterols could only be detected in ethanol and methanol extracts—particularly from the stem and flowers. The presence of brown to yellow precipitates confirmed the lipophilic nature of phytosterols, suggesting that ethanol is a more appropriate solvent for the extraction of sterols. This pattern is in perfect agreement with the known solubility properties of sterols as well as their localization in lipid-rich tissues. Several previously published studies have also reported presence of different primary and secondary metabolites in *Verbesina encelioides* (Shehta *et al.,*2022; Kaur *et al.,* 2021; Verma *et al.,* 2019) [4, 10].

Conclusion

The present study demonstrates that *Verbesina encelioides* is a rich source of both primary and secondary metabolites, with significant variations in extractive yields and phytochemical distribution across its roots, stems, leaves, and flowers. Among the solvents tested, methanol and ethanol proved highly effective in extracting bioactive compounds, while water extracts also showed notable activity, emphasizing the versatility of this plant for diverse extraction approaches. The detection of flavonoids, alkaloids, phenolics, saponins, and other bioactive constituents underscores the therapeutic potential of *V. encelioides* and provides a strong foundation for its further pharmacological evaluation.

These findings highlight the organ-specific phytochemical diversity, suggesting that targeted extraction from specific plant parts could maximize the yield of desired metabolites for medicinal or industrial applications. Overall, the study validates *V. encelioides* as a promising candidate for natural product research and reinforces the importance of systematic phytochemical investigations in uncovering plants with potential health benefits.

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