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Allelochemicals from the Invasive Weed Verbesina encelioides Growing in Libya: A Chemical Analysis

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المواد الكيميائية الأليلوباثية المستخلصة من العشبة الغازية *Verbesina encelioides* النامية في ليبيا: تحليل كيميائي

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Abstract:
V. encelioides is an invasive annual plant from the Asteraceae family grows in dry and semi-arid regions around the world. It display a strong allelopathic trait, which enables it to competitively exclude nearby vegetation, a fundamental component of its high ecological competence and ability to totally dominate local ecosystems. In this study the chemical profile of *V. encelioides* growing in Libya was analysed by prelimarly phytochemical screening tests. The analysis was performed to test for the presence of a number of phytoconstituents. namely: tannins, flavonoids, saponins, reducing sugars, steroids, glycosides, coumarins, alkaloids, phytosterols, cardiac glycosides and carbohydrates. The extraction process was performed on the dried aerial parts and roots using maceration method with Polarity gradient solvents. The results reveal the presence of the allopathic group of metabolites including; tannins, flavonoids, alkaloids, steroids, saponins and coumarins. However, understanding the chemical profile of *V. encelioides* helps scientists design smart weed-control strategies or even natural bio-herbicides. In addition, allowing veterinarians and ranchers to understand poisoning symptoms and manage infested pastures safely.

Keywords: *Verbesina encelioides*, allelopathic, phytochemical screening, phytoconstituents.

الملخص:
يُعد نبات *V. encelioides* نباتاً حولياً غازياً ينتمي إلى الفصيلة النجمية، وينتشر في المناطق الجافة وشبه الجافة حول العالم. ويتميز هذا النبات بخاصية "الأليلوباثي" (التثبيط الكيميائي الحيوي) القوية، التي تمنحه القدرة على إزاحة النباتات المجاورة تنافسياً؛ وتُعد هذه الخاصية ركيزة أساسية لكفاءته البيئية العالية وقدرته على الهيمنة التامة على النظم البيئية المحلية. وفي هذه الدراسة، جرى تحليل المكونات الكيميائية لنبات *V. encelioides* النامي في ليبيا باستخدام اختبارات الكشف الكيميائي النباتي (الفيتوكيميائي) الأولية. وقد أُجري التحليل للكشف عن وجود مجموعة من المركبات النباتية، وهي: العفصيات (التانينات)، والفلافونويدات، والصابونينات، والسكريات المختزلة، والستيرويدات، والجليكوسيدات، والكومارينات، والقلويدات، والفيتوستيرولات، والجليكوسيدات القلبية، والكربوهيدرات. نُفذت عملية الاستخلاص باستخدام الأجزاء الهوائية والجذور المجففة عبر طريقة النقع في مذيبات ذات تدرج في القطبية. وأظهرت النتائج وجود مجموعة من المستقلبات ذات التأثير الأليلوباثي، بما في ذلك العفصيات والفلافونويدات والقلويدات والستيرويدات والصابونينات والكومارينات. وتجدر الإشارة إلى أن فهم التركيب الكيميائي لنبات *V. encelioides* يساعد العلماء في تصميم استراتيجيات ذكية لمكافحة الأعشاب الضارة أو حتى تطوير مبيدات أعشاب حيوية طبيعية، فضلاً عن تمكين الأطباء البيطريين ومربي الماشية من التعرف على أعراض التسمم وإدارة المراعي الموبوءة بهذا النبات بأسلوب آمن.

الكلمات المفتاحية: *Verbesina encelioides*، تأثير كيميائي حيوي مثبط، فحص كيميائي نباتي، مكونات نباتية.

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Introduction

Verbesina encelioides (Cav.) Benth. & Hook. f. ex A. Gray, also referred to as golden crownbeard or wild sunflower, is an invasive annual plant from the Asteraceae family. This species is native to North America and Mexico, but it has swiftly colonized and become a harmful invading plant in dry and semi-arid regions around the world. Its strong allelopathic trait, which enables it to competitively exclude nearby vegetation, is a fundamental component of its high ecological competence and ability to totally dominate local ecosystems. By producing and releasing specific poisonous secondary metabolites into its surroundings, *V. encelioides* functions as a "chemical fighter" instead of depending just on traditional resource competition. In co-occurring species, these substances actively inhibit seed formation, delay germination, lower photosynthetic pigment productivity, and interfere with vital metabolic pathways [1].

The allelochemical distribution in *V. encelioides* is organized into compartments, displaying unique chemical characteristics and phytotoxic intensities especially across its aerial organs (leaves, stems, and flowers) and roots in high concentration. As a consequence, research evaluating leaf extracts on nearby weeds and crops reveals significant, dose-dependent decreases in biomass accumulation and germination percentage. Phytochemical screening analysis and liquid chromatography-mass spectrometry (LC-MS) reveal that the aerial tissues are significantly enriched with several Phytoconstituents [2, 3].

Alkaloids namely, guanidine derivatives; galegine (3-methyl-2-butenyl-guanidine) is a characteristic of fresh *V. encelioides* leaf. In addition to its known toxicity to grazing animals, galegine has a vital defensive and allelopathic mechanism specific to the fresh tissues of the plant [4]. Chromatographic fingerprinting of aerial extracts shows a high concentration of phenolic acids and flavonoid glycosides, including p-coumaric acid and quercetin-3-O-β-D-galactopyranoside. These chemicals interfere with cellular enzymes, decrease photosynthetic efficiency, and create reactive oxygen species (ROS) in susceptible plant seedlings [5, 6]. The aerial portions include a variety of sesquiterpene lactones, monoterpenes, and triterpenes (such as β-amyrin and taraxasterol acetate), which change the permeability of cell membranes and function as volatile or non-volatile inhibitors of nearby breeding ground [4].

The roots of *V. encelioides* experiences significant allelopathic alteration, despite the fact that root systems typically contribute less to phytotoxicity than leaves. In order to keep nearby plants away, root exudates and aqueous root extracts directly inhibit radicle extension and change the characteristics of the soil [7]. Alkanes and fatty acid derivatives: according to gas chromatography-mass spectrometry (GC-MS), root systems emit complex combinations of long-chain alkanes, linoleic acid, methyl linoleate, and fatty acid methyl esters. These hydrophobic compounds damage rival species' root cell membranes, preventing the uptake of nutrients and water [8]. Rhizospheric phenolics: studies using high-performance liquid chromatography (HPLC) show clear qualitative and quantitative differences in phenolic chemicals in soil that has been supplemented with root leachates. These localized phenolics have concentration-dependent antagonistic actions that reduce both native flora and co-occurring weed species' survival, protein levels, and physiological function [5].

On the contrary, *V. encelioides* has a long history of use in traditional ethnomedicine, which has been confirmed by recent in vitro and in vivo research. By inhibiting inflammatory cascades without causing severe acute toxicity, plant extracts provide dose-dependent pain and acute inflammation alleviation as an analgesic and anti-inflammatory agent that is comparable to that of conventional NSAIDs like aspirin [9]. The alcoholic preparations of the aerial portions shown a significant cell cycle arrest and apoptosis induction against human lung cancer (A-549), breast cancer (MCF-7), and liver cancer (HepG2) lines have been [10]. Strong antibacterial, antifungal, and antiprotozoal properties are demonstrated by *V. encelioides* crude extracts. Folk medicine mentions the extensive use of its roots to treat bladder discomfort and purify blood [11]. *V. enceloides* was also reported as a natural therapeutic intervention against neurodegenerative disorders likes Alzheimer's disease and dementia [12].

Since *V. encelioides* is a durable, low-water-consuming plant, its main economic benefits are derived from its distinct chemical defence systems and survival mechanisms. The production of environmentally benign herbicides is the most important commercial path for *V. encelioides*. Since its secondary metabolites naturally reduce rival weed species through allelopathy [2]. It was also directed to the use in insecticides and for pest management [13].

In Libya *V. encelioides* was recently discovered and recorded in the flora of Libya [14] widely distributed in Tripoli at roadside, waste places, garden species and in disturbed habitats [15]. It is encountered as an invasive species that effect the nature of growth and seed production of neighbouring plants and a recommended program to control its spreading to various areas is to be developed. As it can be termed as a very troublesome weed and believed to be allelopathic, the aim of the present work was to determine its chemical profile. Understanding this helps scientists design smart weed-control strategies or even natural bio-herbicides. In addition, allowing veterinarians and ranchers to understand poisoning symptoms and manage infested pastures safely.

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Material and methods

Plant material.

V. encelioides was collected from Abu Salim area in Tripoli in July 2026 and identified by Prof. Dr. Mohammed Makhlouf (fig.1a,b). Aerial parts and roots were separated, washed with distilled water to remove any dust or soil and dried in shed. When dried each plant part was ground to course powder with mechanical blinder for further extraction.





fig.1: a) *Verbesina encelioides* b) Roots of *Verbesina encelioides*

Preparation of extracts

Maceration method was used for the preparation of extracts [16]. 10gr of each grinded plant part aerial parts and roots was weighed separately in a1000ml flasks respectively and 100ml of solvents of increasing polarity namely hexane, dichloromethane and methanol was added separately into each flask and left for 3 days with continuous stirring. Filtration was then carried out to obtain the different extracts.

Phtyochemical screening tests.

General phytochemical screening tests to detect different active metabolic groups was performed to the hexane, dichloromethane and methanol of the aerial parts and root extracts according to the methods [17, 18].

Tests:

1. Tannins: Treat 2ml extract with 2ml $FeCl_3$. A black or blue-green colour indicated the presence of tannins and phenols.

2. Flavonoids:

a. Alkaline Reagent Test: 2ml extract is treated with 10% NaOH solution. (An intense yellow colour is an indication of presence of flavonoids)

b. Zinc Test: 2ml extract is treated with zinc dust and conc. HCl (change to red color indicates flavonoids)

3. Saponins: About 3 ml of plant extracts were added to 3 ml of distilled water and shaken vigorously. The formation of a stable persistent froth is taken as a positive test for saponins.

4. Reducing sugars: 1ml of Fehling solution A and B were added to test tubes containg a mixture of 2ml distillled water and 2ml plant extract after being thoroughly shaken. A positive result is seen when a brick red precipitate forms at the bottom of the test tube.

5. Steroids: 1ml extract dissolved in 5ml chloroform and 2.5 ml conc. H_2SO_4 added. Upper layer turned red with yellow and green fluorescence, indicating steroids in extracts. The formation of a violet to blue or green ring at the interface also indicates presence of steroids.

6. Test for Glycosides (Molisch test for glycone part): To 2 mL of extract add 2 to 3 drops of Molisch's reagent (a 5% solution of α-naphthol in ethanol). Mix the solution gently, carefully add 1mL of conc. H_2SO_4 down the

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inner side of the tube. The formation of a purple, violet, or reddish-violet ring at the interface of the two layers indicates the presence of carbohydrates or glycosides.

7. Coumarins: NaOH test: 2ml plant extract and 2ml 10% NaOH and 2ml of chloroform, formation of yellow color indicates coumarins.

8. Alkaloids: 2.5ml from each extract was mixed with 7.5ml of conc. HCL and then 1ml was removed from each mixture. 5-6 drops of the following reagents were added

a- Mayer's test: to 1ml extract in a test tube 1ml of Mayer's reagent was added drop by drop. The formation of a greenish coloured or cream precipitate indicated the presence of alkaloids.

b- Marquis reagent: to 1ml of extract 1ml of Marquis reagent was added color rangs from orange and yellow to dark reds or purples is an indication of the presence of alkloids.

c- Hager's test : to 1ml of extract 1ml of Hager's reagent was added dropwise a yellow coloured precipitate will form if alkaloids are present.

d- Wagner's test: to 1ml of extract 1ml of Wagner's reagent was added drop by drop. The formation of a reddish-brown precipitate indicated the presence of alkaloids.

9. Phytosterol **(** Salkowski's test): 2ml of extract treated with 1ml chloroform and a dropwise of conc. H_2SO_4. Development of a golden red color indicates the positive test for phytosterols.
10. Cardiac glycoside (Keller-killiani): 2ml extract treated with 1ml glacial acetic acid and a drop of $FeCl_3$. A brown ring, indicating cardiac glycosides.
11. Carbohydrate (Iodine Test): 2ml extract treated with 5 drops of iodine solution blue colour formation in any extracts, indicating presence of carbohydrates.

Results and Discussion.
In this study the chemical profile of *V. encelioides* growing in Libya was analysed by prelimarly phytochemical screening tests. The results of phytochemical screening tests that was performed by using maceration extraction method with solvents of increasing polarity namely; hexane , dichloromethane and methanol on the dried aerial parts and root of *V. encelioides* are displayed in Table 1 below. In general the results showed the presence of almost all phytoconstituents in high concentration in the methanol extract of the aerial parts and root. Whereas, the dichloromethane and hexane extract showed a medium and low concentration of nearly all active constituents. While the secondary metabolites ; glycosides, and phytosterols were absent in all extracts.

Table 1: *Phytoconstituents of the aerial parts and roots extracts of V. encelioides*

	Test	Extracts					
		Hexane		Dichloromethane		Methanol	
		Aerial organs	Roots fruit	Aerial organs	Roots	Aerial organs	Roots
1	Tannins	-	-	+++	-	+++	++
2	Flavonoids						
	a-NaOH sol. (alkaline)	-	+	++	++	+++	+++
	b- Zinc	-	-	-	-	++	++
3	Saponins	-	+	-	+++	-	-
4	Reducing Sugars	-	-	-	-	++	++
5	Steroids	+	-	++	+	+++	++
6	Glycosides	-	-	-	-	-	-
7	Coumarins	-	-	-	+	++	+++
8	Alkaloids						
	a-Mayer Reagent	-	-	-	+	++	+++
	b-Marques Reagent	+	-	+	+	++	+++
	c- Hager Reagent	-	-	-	-	++	+++
	d-Wagner Reagent	-	-	-	-	++	+++
9	Phytosterol (Salkowski's Test)	-	-	-	-	-	-
10	Cardiac glycoside (Keller-killiani)	-	+	-	+++	-	+++
11	Carbohydrate (Iodine)	-	-	++	-	+	-

Key = + present in low concentration;++ = present in medium concentration ; +++ = present in high concentration;− = absent

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Numerous phytochemical researches world wide mentioned the groups of bioactive substances, including; alkaloids, flavonoids, phenols, and terpenes, found in various plant parts of *V. encelioides*. These compounds contribute to its broad pharmacological effects and phytotoxicity [5,11,19] The study that was conducted by Mehal and collegues in 2023 on *V. encelioides* growing in India, its fresh foliage and leaf litter revealed the presence of 15 allelochemicals which has a direct negative impact on the surrounding vegetation; including; phenolic acids, flavonoids, phytosterols, phytophenols, dicarboxylic acid, guanidine, and triterpenes [5]. The latter research confirms our results was shows a high concentration of the tannins in the aerial parts of both the dichloromthane and methanol extract. Whereas the flavonoids were detected in medium to high concentration in the dichloromethane and methanol extract respectively.

Alkaloids and some derivatives, such as the alkaloid galegine, is detected in fresh leaf profiles and in the aqueous root extract together with phenolics and terpenes. These compounds support defence chemistry and produce localized phytotoxicity of *V. encelioides* , which inhibits nearby plants [5,20]. Toxicological studies of *V. encelioides* revealed the existence of the extremely strong, non-selective toxin galegine (3-methyl-2-butenyl-guanidine) which is known to be dangerous for farm animals The acute mortality of this toxin highlights how the raw plant's defensive mechanism is non-selective [5,21,22]. Consistent with the above mentioned studies our results demonstrates the presence of a medium to a high concentration of alkaloids in the aerial and root methanolic extracts respectively.

Steroids are mentioned among the phytochemical responsible for the allopathic effect of *V. encelioides* growing in Sampla, Rohtak , India. Nine types of allelochemicals were identified in root extracts from *V. encelioides*; Fatty acid methyl esters, linoleic acid or methyl linoleate, long chain alkane, alkanes, synthetic fatty acid ester, acyl chloride, hydroxy fatty acid, and steroids [8]. These results are in confirmation with the current study that detect steroids in a low to a high concentration of all aerial parts extracts. Whereas, a low to medium concentration in the dichloromethane and methanol extracts of the roots respectively.

Coumarins are compounds which are also responsible to suppress the growth of neighbouring plant species either through the degradation of plant debris in sufficient amounts or as exudates from live tissues [19,23]. Coumarins inhibit seed germination, root elongation, and nutrient uptake in competing species [24]. The most active allelochemical was found to be a simple coumarin, 2H-1-benzopyran-2-one [25]. It is important to point out here that this is the first time coumarins were mentioned to be detected in *V. encelioides* root extract.

Saponins are another allopathic group of phytocnstituents that were detected in the root extract of *V. encelioides*, decomposing litter into the surrounding soil [8]. Avenacin A-1: an extremely phytotoxic steroidal saponins from the oat plants (*Avena sativa*) that directly inhibit the growth and development of nearby roots [26]. However, while verbesinosides illustrate the uniquely isolated saponin structure characteristic of the genus *Verbesina*, that was first discovered in *V. virginica*, it is the crude saponin compounds and fractions from invasive species like *V. encelioides* that are well-documented for their significant allelopathic interference [18]. In the current study Saponins were detected in high concentration in the root extract of dicholoromrthane.

In the current study reducing sugars are detected in the methanolic extract of both the aerial parts and roots. It is worth to mention that these metabolites in *V. encelioides* act as baseline nutritional and metabolic components and are not involved in the allopathic activity of the plant. Reducing sugars in *V. encelioides* were also mentioned detected by other studies [8]. Cardiac glycosides another methabolites that are not the primary active or toxic compounds in *V. encelioides*, while it was mentioned to be present among its chemical constituents[27]. In the present study cardiac glycosides were detected in the root extracts of dichloromethane and methanol.

Conclusion

Compounds like alkaloids, phenols and steroids in *V. encelioides* aerial parts and root extracts act as potent allelochemicals that interfere with the seed germination, seedling growth, and metabolic functions (such as chlorophyll production) of nearby plant species. However, these allelopathic substances that cause phytotoxic behaviour may be environmentally beneficial substitutes for synthetic herbicides. To better understand these chemicals' modes of action and to extract and describe them, more research is necessary. Understanding this helps scientists design smart weed-control strategies or even natural bio-herbicides. In addition, allowing veterinarians and ranchers to understand poisoning symptoms and manage infested pastures safely.

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Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

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