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QUALITATIVE PHYTOCHEMICAL ANALYSIS OF A RECENTLY EMERGING WEED *VERBESINA ENCELIOIDES* IN PUNJAB (INDIA)

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Verbesina encelioides is an exotic wild plant species of family Asteraceae. As per recent survey, it is one of the recently emerging exotic weeds in Punjab state of India and shows various characteristics of establishment, growth and dominance like congress grass (*Parthenium hysterophorus*). The present study deals with phytochemical analysis and allelopathic potential of *V. encelioides*. For this study, cold and hot aqueous extracts of various parts of *V. encelioides* like leaves, stem and flowers were prepared separately to check the presence of phytochemicals which are believed to be mostly involved in allelopathic responses of the plants. Preliminary qualitative phytochemical analysis was carried out to identify the secondary metabolites present in the different parts of the selected plant. The analysis indicates the presence of ample number of phyto-constituents like terpenoids, phenols, saponins, tannins, proteins, amino acids, anthraquinones, alkaloids and reducing sugars in weed extracts.

Keywords: Allelochemical, Allelopathic plant, Exotic weed, Phytochemicals, *Verbesina encelioides*.

Introduction

Verbesina encelioides is commonly known as wild sunflower or golden crownbeard. It is an exotic invasive weed in India and believed to be originated inUnited States and Mexico. Golden crown beard belongs to plant family Asteraceae (or Compositae) and is an erect, annual, wild herb which has wide range of tolerance to climatic conditions and competitive in its growth habit[1].It is a broadleaved, 30-60 cm tall herb having yellow colored, 2-5 cm wide flowers on long peduncles which resembles to sunflower[2].Hence popularly known as wild sunflower.

A field survey was conducted in Punjab from 2008-2017 to enlist the non-native plant species prevailing in the state and during this study, ten new weed species were detected including *Verbesina encelioides*. Since 2015, the survey was directed in diverse places in Punjab to study the existence of *V. encelioides* and it was found to be growing abundantly in various districts of South-west Punjab like Sangrur, Ferozepur, Bathinda, Fazilka and Barnala[2]. On the other hand, it was not recorded in Pathankot, Hoshiarpur and Jalandhar districts of Punjab[3].It is believed thatthe seeds of this plant are introduced into

Punjab from nearby areas of the Rajasthan along with the soilcarrying for construction of roads. Therefore,the plant is mostly growing along the roadsides, especially the newly constructed highways and other pathways.

Verbesina encelioides plant in its natural habitat

Verbesina encelioides infected area

In 1987, while studying the phenology and ecology of this weed, Kaul and Mangal observed that *V. encelioides* shows rapid seedling establishment and growth along with quick vegetative as well as reproductive growth. Various other features which addto the prosperous growth, proliferation and spread of this weed include high phenotypic plasticity, ecological variability, phenological diversity and seed germination in varied soils[2, 4].Inderjit et al. (1999) conducted research on radish (*Raphanus sativus*) seedlings to elucidate the allelopathic interference of*V. encelioides*on its growth. Theresults concluded that roots of *V. encelioides* showed allelopathic potential on radish and researchers viewed it as a mechanism behind the dominance of this weed in certain wild and residential areas[5].

Jain *et.al* (2008) reported the presence of numerous metabolites in *V. encelioides* like sesquiterpenes, flavonoids, essential oils and terpenoids etc. and said that some of these compounds might exhibit antimicrobial, antiviral, antitumor and anti-inflammatory activities[6].*Parthenium hysterophorus,* another member of family Asteraceae, is the best example of allelopathic exotic plant which secretes allelochemicals to its surrounding to inhibit the growth of other plants in its vicinity. *V. encelioides* is also showing similar characteristics and invading in the state with an alarming rate. Therefore, the present study was carried out to qualitatively evaluate the presence of various phytochemicals in thevarious parts of the plant under investigation and to analyze their allelopathic potential.

Materials and Methods

Collection of Weed Plant Material

The fresh plant material of *V. encelioides* needed for phytochemical studies was collected locally from Talwandi Sabo (District Bathinda, Punjab). The healthy and disease-free plants were collected from the areas heavily populated with this weed.

Preparation of Plant Material

For preparation of aqueous extracts of various parts of the plant, firstly the material was washed with tap water for 2 or 3 times to remove the dust and dirt. Afterwards, the plants were spread on the clean surface over the mat in laboratory and were left for about an hour to dry. Later on, all plant parts like leaves, flowers, stem and roots were separated and dried separately in shade for about 15-20 days. After that, the materials were converted into fine powder using a mechanical grinderand the powder of different plant parts was stored separately in polythene bags in the refrigerator for further use. This powder was used for the preparation of aqueous (hot and cold) extracts for phytochemical testing.

Preparation of weed extracts

For the cold aqueous extract formation, 100 grams of powdered plantmaterial was soaked in 200 ml of distilled water at room temperature for 24 hours. Afterwards, the extract was filtered using muslin cloth and then with filter paper to have 50% aqueous weedextract. The extract was poured into glass vials and stored in the refrigerator for further use in phytochemical analysis[7]. The powdered plant samples (10g/ 125ml) were also extracted in hot water using Soxhlet apparatus at 55-60ºC for 8-10 hours.After extraction, extracts were cooled, poured into glass vials and were stored at 4ºC in the refrigeratorforphytochemical testing[8].

Phytochemical Analysis

The hot and cold aqueous leaf, flower and stem extracts of *V. encelioides* were subjected to various tests for the analysis of different phytochemicals. Various tests and their procedures are given below in detail:

Test for flavonoids:

Lead acetate test: 2 ml of hot and cold aqueous extracts were poured into separate well marked test tubes. In these test tubes,

add few drops of lead acetate solution. Formation of yellow color precipitates specifies the existence of flavonoids[9].

Concentrated H_2SO_4 test:

In separate test tubes, 2 ml of hot and cold aqueous *V. encelioides* extracts were treated with few drops of conc. H_2SO_4. Orange color formation indicates the occurrence of flavonoids[9].

Test for terpenoids: Salkowski's test:Add 2ml of chloroform and 2-3 ml of conc. H_2SO_4 carefully in 5-6 ml of plant extracts taken separately in different test tubes.Appearance of reddish-brown colored interface specifies the presence of terpenoids[9].

Test for phenols: Lead acetate test: 2 ml ofhot and cold aqueous extracts of different plant parts were poured in separate test tubes and treated with few drops of lead acetate solution. The formation of yellow colored precipitate designates the presence of phenol in sample[9].

Alcoholic Ferric chloride test: Few drops of ferric chloride solution (prepared in alcohol) were added into the test tubes separately containing 2-2ml of aqueous extracts. The appearance of bluish black color in test tubes depicts the presence of phenol[9].

Test for saponins: Foam test: 2 ml of each aqueous extract was shaken with 5 ml of distilled water for about 15 minutes in separate test tubes. Foam formation shows the presence of saponins[9].

Test for tannins: Ferric chloride test: To 5 ml of plant extract, add about 2-3 ml of water and heat this mixture on a water bath. The mixture was filtered and few drops of ferric chloride were added to the filtrate. Formation of dark green color indicates the presence of tannins[9].

Test for proteins and amino acids:

Ninhydrin test: 5 ml of hot and cold aqueous weed extracts were taken in separate test tubes and to each, 2-3 drops of freshly prepared 0.2% Ninhydrin reagent was added and mixture was heated. The appearance of pink or purple color indicates the presence of proteins, peptides or amino acids[9].

Test for anthroquinones:

Borntrager's Test: 5ml of each plant extract was boiled with 10% aqueous hydrochloric acid (HCl) for about 2-3 minutes in water bath. This extract was filtered and cooled. Then4 ml of Chloroform ($CHCl_3$) was added and mixed. Later on, few drops of 10% Ammonia (NH_3) were added to the above mixture and heated for 30 seconds. Formation of pink color indicates the presence of anthraquinones[9].

Test for alkaloids:

In separate test tubes, add 3 ml of different weed extracts and to these, add 1 ml of 1% aqueous hydrochloric acid (HCl). These extracts were then used for the alkaloids testing by Mayer or Wagner test.

Mayer's test: To prepareMayer's reagent, add 0.18 gm of mercury chloride ($HgCl_2$) in 30 ml of distilled water and 2.5 gm of potassium iodide (KI_2) to 10 ml of distilled water. Mix both the solutions and shake it well before use.In test tubes, add 1 ml of above made plant extracts. Warm the test tubes after adding 1% HCl solution. Filter this solution and treat them separately with 2ml of Mayer's reagent. Formation of a creamy turbidity orgreen colored precipitates indicates the presence of alkaloids in the extracts[10].

Wegner's test: For preparation ofWagner's reagent, add 0.25 gm of Iodine and 1.25 gm of potassium iodide (KI_2) to 25 ml of distilled water. In separate test tubes, 1 ml of aqueous weed extracts were added and thentreated with few drops of Wagner's reagent. Formation of reddish-brown precipitate indicates the presence of alkaloids in the extracts[7].

Test for steroids:

Acetic anhydride test: 5 ml of plant extracts were added to 2 ml of acetic anhydride and 2 ml of H_2SO_4. Boil the mixture and filter it.

If the color of mixture changes from violet to blue or green in samples, it is indicative that steroids are present[9].

Test for carbohydrates:

Molisch's test: For the preparation of Molisch's reagent, add 3.95 gm of 1-naphthol in 25 ml of 99% ethanol. 5 ml of each weed extracts were treated with 1 ml of Molisch's reagent and add few drops of conc. H_2SO_4 to the sides of test tubes to form a layer. If red or dark violet color appears, reducing sugar is present[11].

Test for gum and mucilage:

Alcohol test: To 10 ml of each aqueous extract, add 2 ml of absolute alcohol with constant stirring. White or cloudy precipitate formation indicates the presence of gum and mucilage[12].

Test for phlobatannins:

HCl Test: Few drops of 2% hydrochloric acid were added to 1ml of the plant extract. Appearance of red colored precipitates indicates the presence of phlobatannins.

13) Test for Glycosides: To 2 ml of every plant extract, 3ml of chloroform and 10% ammonia solution was added. Formation of pink color indicates the presence of glycosides.

Results and Discussion

Various phytochemical analysis tests were performed to test the presence of different phytoconstituents in both cold and hot aqueous extracts of various plant parts (leaves, stem and flowers) of *V. encelioides*and results are represented in Table 1.These chemicals or secondary metabolites are a major factor in regulation and organization of the structure of plant communities.

Almost all the phytochemicals analyzed in present study were reported in every plant part under investigation except anthraquinones and glycosides which were absent in leaf extracts. In both cold and hot aqueous leaf extracts, phytochemicals which showed positive results includes flavonoids, terpenoids, phenols, saponins, tannins, proteins-amino acids, alkaloids and reducing sugars. On other hand, few tests for compounds like steroids, glycosides, gum and mucilage were negative in cold as well as hot aqueousleaf extracts. Separately hot andcold aqueous extracts of stem and flowers showed the presence of flavonoids, terpenoids, phenols, proteins-amino acids and alkaloids. It was also observed that, anthraquinones, saponins and reducing sugar were extracted only in cold water extract of stem and hot aqueous extract of flowers. Tannins were not observed only in stem extracts whereas, steroids and gum/resin were totally absent in all parts of the plant.Mora, *et.al* (2013) reviewed the published literature about phytochemical components and biological activities of genus *Verbesina.* The results reviewed that large class of secondary metabolites or phyto-compounds have been isolated from this genus time to time like flavonoids, alkaloids and several terpenes[13]. Secondary metabolites like phenols, alkaloids, terpenoids, benzoxazinoids, glucosinolates and isothiocyanates are some important allelochemicals[14]. Presence of these natural chemicals in *Verbesina*supported their allelopathic potential.In the field it was observed that plant was highly invaded along roadsides and waste areas but not reported in agriculture land. *Verbesina* infested areas showed very little diversity of other plants which again gives clue about their allelopathic activity.

It was detected that cold aqueous extracts showed better results for extraction of phytoconstituents than hot water. Therefore, it is expected that,in nature when water passes through plant litter, these allelochemicals are easily leached out into the soil, which may interfere in the growth of surrounding native plant species.There are various mechanisms through which

Sr. No.	Phytochemical Tests	Aqueous cold leaf extract	Aqueous hot leaf extract	Aqueous cold stem extract	Aqueous hot stem extract	Aqueous cold flower extract	Aqueous hot flower extract
1	**Test for Flavonoids test**						
	Lead acetate test	+	+	+	+	+	+
	Concentrated H_2SO_4 test	+	+	+	+	+	+
2	**Test for Terpenoids**						
	Salkowski's test	+	+	+	+	+	+
3	**Test for Phenols**						
	Lead acetate test	+	+	+	+	+	+
	Ferricchloride test	-	+	+	+	+	+
4	**Test for Saponins**						
	Foam test	+	-	+	-	-	+
5	**Test for Tannins**						
	Ferric chloride test	+	+	-	-	-	+
6	**Test for Proteins**						
	Ninhydrin test	+	-	+	+	+	+
7	**Test for Anthraquinones**						
	Borntrager's test	-	-	+	-	-	+
8	**Test for Alkaloids**						
	Mayer's test	+	-	+	+	-	-
	Wagner's test	+	-	+	+	+	+
9	**Test for Steroids**						
	Acetic anhydride test	-	-	-	-	-	-
10	**Test for Carbohydrate**						
	Molisch's test	-	+	+	-	-	+
11	**Test for Gum and mucilage**						
	Alcohol test	-	-	-	-	-	-
12	**Test for Phlobatannins**						
	HCl test	+	+	+	+	+	+
13	**Test for Glycosides**	-	-	+	-	-	+

Table 1: Phytochemical Analysis of *Verbesina encelioides*
(In the above table, (+) indicates the presence and (–) indicates the absence of phytochemical)

allelochemicals are released into the environment like volatilization from leaves, exudation from roots and leaching from fallen leaves and plant parts[15].Hence, all these properties of wild sunflower are pointing towards the allelopathic nature of this plant.

Conclusion

The phytochemicals analysis in *V. encelioides* leaf, stem and flower extracts (hot and cold) showed positive results to various tests performed, which confirms the presence of certain compounds in this weed which might be responsible for its dominance in areas of its occurrence. This dominance can be co-related to the allelopathic impact of this weed. The speed at which *V. encelioides*is spreading in Punjab, it is viewed as second *Parthenium hysterophorus* of Punjab if left uncheck. Allelopathic impacts of this weed on cultivated crops, native as well as exotic weeds in Punjab offer future perspective in the study of this weed.

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