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## Comparison of Allelopathic Efficacy of Weeds: A New Avenue for Chemotherapeutics

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### ABSTRACT

Weeds are considered troublesome and undesirable component leading to enormous reduction in crop yield, wastages of resources and human energy. An attempt has been made to evaluate the effect extracts of four obnoxious weeds, *Argemone mexicana*, *Parthenium hysterophorus*, *Cyperus rotundus* Lin., *Verbesina encelioides* on the mitotic activity of germination of mung beans. Metabolites were extracted in methanol, acetone, hexane and distilled water using soxhlet extraction and assayed for the presence of bioactive metabolites. Presence of terpenes, flavonoids and quinines were observed in the methanol extract of all the weeds and hexane extracts indicated the presence of glycosides and flavonoids. Based on percentage inhibition studies, it was found water extracts followed by acetone extracts for all the weeds were most potent as a growth inhibitor. *Argemone Mexicana* and *Parthenium hysterophoresus* were found to be most potent in inhibiting mitotic activity and growth although statistical significance ( $P < 0.05$ ) was prevalent only in *Argemone Mexicana*.

**Keywords:** Mitotic inhibitors, seed germination, seedling growth, secondary metabolites

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## INTRODUCTION

Weeds are the most aggressive, troublesome and undesirable element of the world's vegetation and cause enormous reduction in crop yield, wastages of resources and human energy and also a health hazard to human being. Existence of weeds in crop fields causes chemical competition which is referred as 'allelopathy'. Allelochemicals are secondary plant metabolites implicated in influencing physiological functions and biochemical reactions<sup>1,2,3</sup>. These allelochemicals can have beneficial (positive allelopathy) or detrimental (negative allelopathy) effects on the target organisms. Plants have always been source of new therapeutic agents as they provide molecules with complex chemistry, novel mechanisms of action, higher potency and lower toxicity<sup>4</sup>. Many drugs from plants have been found to display significant chemotherapeutic activity but side-effects and emergence of resistances has become a bottleneck<sup>5</sup>.

It has been observed vinca alkaloids, saponins, flavonoids and polyphenols form the major classes involved in plant-derived therapeutics for cancer prevention or chemotherapy<sup>6,7</sup>. It has been also been observed weeds produce varied type of allelochemicals, which can act as a novel source of chemotherapeutics. Till now extensive studies have been done to analyze the effectivity of allopathic chemicals but hardly any studies have indicated the most effective source for such activities. So, in the present work, a preliminary comparison assay has been made to evaluate effect of four easily available obnoxious weeds having medicinal properties, *Argemone mexicana*, *Parthenium hysterophorus*, *Cyperus rotundus* Lin., *Verbesina encelioides* on the mitotic activity of beans.

## MATERIALS AND METHODS

### Materials

Methanol, acetone, hexane, Anthrone reagent, chloroform, sulphuric acid, Sodium hydroxide and hydrochloric acid (all reagent grade) obtained from Rankem, New Delhi, was obtained

### Collection of Weeds

Weeds of *Argemone mexicana*, *Parthenium hysterophorus*, *Verbesina encelioides* and *Cyperus rotundus* Lin. were collected from Amity University Rajasthan, Jaipur (India) campus and area nearby 5 km radius.

### Preparation of Extract

The collected plants were brought into the Research Laboratory of Amity Institute of Biotechnology, Amity University Rajasthan, and were immediately segregated into shoot (stem and branch), leaf, flower and roots. Shoot and root part of the fresh plant was cut into 1-2 cm

pieces. Samples were dried in an oven at 70 °C for 24 hours. After drying, they were ground using mechanical grinder and passed through sieve (425 µm). Soxhlet extraction of the 30 gm of powdered sample, enveloped in whatmann filter paper No. 1, was performed. 300 ml of different solvent systems like acetone, methanol, hexane and distilled water were used separately for the extraction. Extract was stored for further processing.

### **Phytochemical Screening**

The extracts obtained were checked for the presence of different types of secondary metabolites<sup>8,9</sup> Mainly, tests for glycoside<sup>10</sup>, saponin<sup>11</sup>, terpenes, flavanoids<sup>12</sup> and quinines were performed for the extracts obtained.

### **Germination of Seeds**

Extract was dried in oven at 60°C and residue was re-suspended in distilled water to make a stock of 1mg/ml. Mungbean (*Vigna radiata*) was selected for seed germination bioassay due to its short germination period. Seeds of mung bean were surface-sterilized in 1% NaOCI for 5 min and rinsed with running tap water. Healthy seeds of mungbean were soaked in distilled water for 4 hours and then soaked with different sample extract separately for 2 hours at room temperature so that extracts get imbibed in the seeds. After soaking, the seeds were transferred to petri plates containing wet cotton base for providing moisture. The seeds soaked with distilled water were considered as control. Six seeds were uniformly kept in each petriplate. All the set up were maintained in normal daylight conditions with an average temperature of 28C. The experiment was set up in triplicate to avoid error. The seeds showing normal emergence of radical and plumule were considered as germinated, thus indicating a normal mitotic activity in the seed. The fresh solution was added after 48 hours to the above described petri plates. The seeds were incubated for seven days<sup>13</sup>.

### **Statistical analysis**

Analysis of Variance (ANOVA) technique was used to study the effect of growth inhibition due to different plants. P-value < 0.05 was considered to be statistically significant. The effect of each weed was studied with relation to the length of radical generated.

## **RESULTS AND DISCUSSION**

### **Secondary Metabolites Detection in Weed Extracts**

The extract obtained was dried in an evaporator to obtain concentrated exudes of various sources. The precipitate was re-suspended in distilled water to obtain stock of 1mg/ml. In general, five major secondary metabolites were tested. *A.mexicana* was found to contain glycosides and

quinones in the leaf extracts of whereas saponins, terpenes and flavanoids were distributed throughout the weed extracts. The results are summarized in table 1. Comparative analysis showed that *Argemone sp* is a rich source of secondary metabolites as indicated by previous studies<sup>9</sup> Approximately 45 secondary metabolites have already been identified and characterized from *Argemone sp*.<sup>14</sup> Presence of non polar flavonoids was evident in *Argemone*, *Parthenium* and *C. rotundus*.

**Table 1. Secondary metabolite composition in the organic solvent extracts obtained from different weeds**

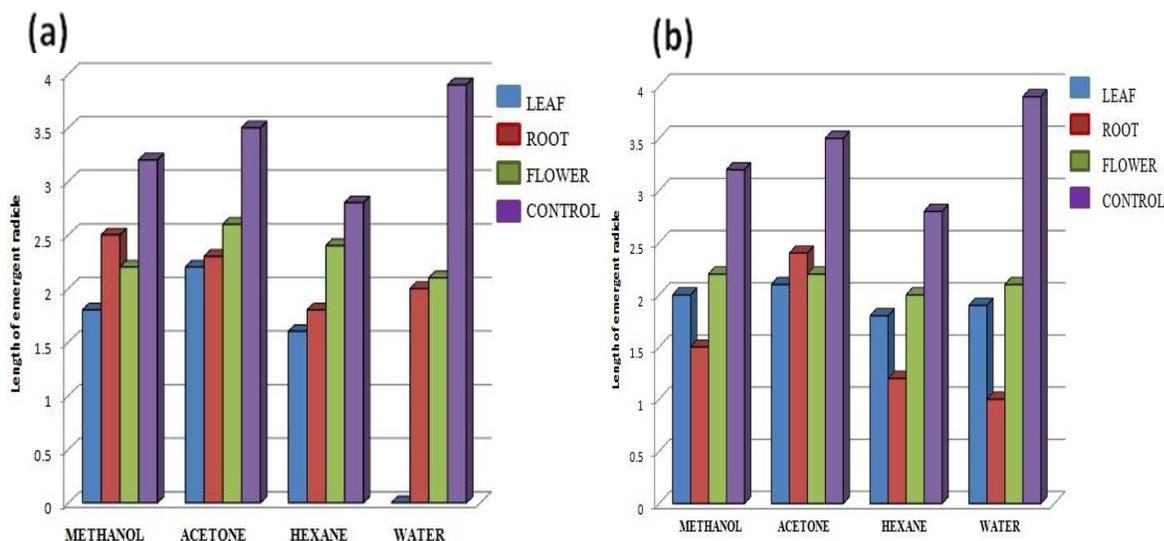
Source	Solvent	Secondary metabolites				
		GLYCO	SAPO	TERP	FLAVA	QUIN
<b><i>Argemone Mexicana Linn</i></b>						
<b>Leaf</b>	Methanol	+VE	-VE	+VE	-VE	+VE
	Acetone	+VE	-VE	+VE	-VE	+VE
	Hexane	+VE	-VE	-VE	+VE	-VE
	Distilled water	-VE	-VE	-VE	+VE	-VE
<b>Root</b>	Methanol	-VE	+VE	+VE	-VE	-VE
	Acetone	-VE	+VE	-VE	-VE	-VE
	Hexane	-VE	+VE	-VE	+VE	-VE
	Distilled water	-VE	+VE	+VE	-VE	-VE
<b>Flower</b>	Methanol	-VE	-VE	+VE	-VE	-VE
	Acetone	-VE	-VE	+VE	-VE	-VE
	Hexane	-VE	-VE	-VE	+VE	-VE
	Distilled water	-VE	-VE	+VE	-VE	-VE
<b><i>Parthenium hysterophorus</i></b>						
<b>Leaf</b>	Methanol	-VE	+VE	-VE	-VE	+VE
	Acetone	-VE	-VE	-VE	-VE	+VE
	Hexane	-VE	-VE	-VE	-VE	-VE
	Distilled water	-VE	+VE	+VE	-VE	+VE
<b>Root</b>	Methanol	-VE	+VE	+VE	-VE	-VE
	Acetone	-VE	-VE	+VE	+VE	-VE
	Hexane	-VE	-VE	-VE	-VE	-VE
	Distilled water	-VE	+VE	+VE	+VE	-VE
<b><i>Verbesina encelioides</i></b>						
<b>Leaf</b>	Methanol	-VE	-VE	-VE	+VE	+VE
	Acetone	-VE	-VE	-VE	+VE	-VE
	Hexane	-VE	+VE	-VE	+VE	-VE
	Distilled water	-VE	+VE	+VE	-VE	-VE
<b>Root</b>	Methanol	-VE	+VE	-VE	-VE	-VE
	Acetone	-VE	-VE	+VE	-VE	-VE
	Hexane	-VE	+VE	+VE	+VE	-VE
	Distilled water	-VE	+VE	+VE	+VE	-VE
<b>Stem</b>	Methanol	-VE	-VE	+VE	+VE	-VE
	Acetone	-VE	-VE	+VE	+VE	-VE
	Hexane	-VE	-VE	-VE	+VE	-VE
	Distilled water	-VE	-VE	-VE	+VE	-VE

<b>Flower</b>	Methanol	-VE	-VE	+VE	-VE	-VE
	Acetone	-VE	+VE	-VE	-VE	-VE
	Hexane	-VE	+VE	-VE	-VE	+VE
	Distilled water	-VE	+VE	+VE	+VE	-VE
<b><i>Cyperus rotundus</i></b>						
<b>Leaf</b>	Methanol	-VE	-VE	+VE	+VE	-VE
	Acetone	-VE	-VE	-VE	+VE	-VE
	Hexane	-VE	+VE	-VE	-VE	-VE
	Distilled water	-VE	-VE	+VE	+VE	+VE
<b>Root</b>	Methanol	-VE	+VE	+VE	+VE	-VE
	Acetone	-VE	+VE	+VE	-VE	-VE
	Hexane	-VE	+VE	+VE	+VE	-VE
	Distilled water	-VE	-VE	+VE	+VE	-VE
<b>Flower</b>	Methanol	-VE	+VE	+VE	-VE	-VE
	Acetone	-VE	+VE	-VE	-VE	+VE
	Hexane	-VE	-VE	-VE	+VE	+VE
	Distilled water	+VE	-VE	-VE	+VE	-VE

### Allelopathic Activity Testing

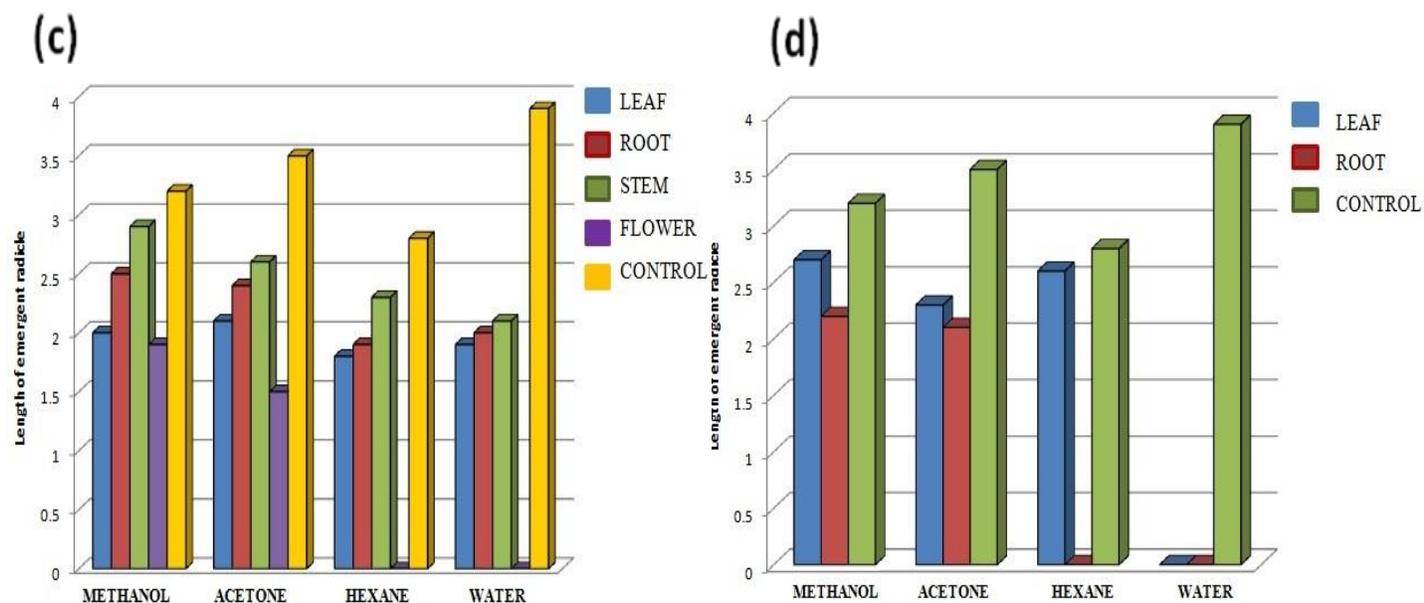
The activity testing of the *Argemone* extract showed maximal affectivity in the leaf extracts (Figure 1). Very few reports have been made indicating presence of flavonoids in the hexane extract<sup>15,16,17</sup> Evidently, hexane extract was found to be most potent in terms of growth inhibitory activity, followed by the methanolic extracts. Additionally, it was observed that the water extract of *Argemone* leaf inhibited the growth of the seedling totally (Figure 1). Based on the phytochemical analysis results, water extract showed the presence of flavanoids (Table 1) and thus it can be proposed, flavanoids from this weed tend to have a major growth inhibitory activity. Many flavonoids have already been reported from *Argemone sp* like exitin, together with 8-methoxydihydrosanguinarine, 13-oxopropine, rutin and quercetrin<sup>18</sup>. It becomes mandatory now to identify the flavanoid responsible for the anti-mitotic activity. Extensive evaluation needs to be done to identify whether the growth inhibitory potential is cumulative.

Extract from root of *C. rotundus* has indicated the maximum potential for growth inhibitory activity. Additionally, n- hexane extracts was observed to be most potent in terms of growth inhibition (Figure 1b). The composition analysis has indicated presence of terpenes, saponins and flavonoids as the major constituents. Based on the data, again flavanoids from the weed seem to be major modulator of activity. Flavonoids like quercetin, kaempferol, catechin and myricetin have been isolated and characterized<sup>19</sup>.



**Figure. 1. Activity assay for (a) *A. mexicana* and (b) *C. rotundus* using mung beans and extract from various parts of weed**

The water extract of *V. encelioides* was found to contain saponin, terpenes and flavonoids. Many sterols, glycosides and terpenes have already been characterized from *V. encelioides*<sup>20</sup>. Although reports of presence of glycosides<sup>21</sup> in this weed are there, we could not detect glycosides in the extracts obtained. The water extract of the stem and flower of *V. encelioides* was found to totally inhibit germination of the seedling (Figure 2a). The growth inhibitory potential was found to be maximal in the water extract which contains flavanoids (Table 2).



**Figure. 2. Activity assay for (c) *V. encelioides* sp. and (d) *P. hysterophus* using mung beans and extract from various parts of weed.**

The phytochemical screening for *P. hysterophorus* was done for leaf and root portions. The water extracts from both root and leaf showed complete absence of radical emergence (Figure 4). The extract indicated the presence of saponins, terpenes and quinines (Table 1). Comparative analysis indicated that saponins and terpenes are playing the growth inhibitory roles in mung beans.

### Statistical Analysis

The control was maintained by soaking in distilled water . Growth obtained in control was taken as standard and percentage inhibition was calculated using the formula:-

$$\frac{\text{Length [C]} - \text{length [T]}}{\text{Length [C]}} \times 100 = \text{Percentage Inhibition}$$

Where C= Control, T= Test

The summarized data is given in Table 2.

**Table 2: Percentage inhibition observed in the extracts obtained from different weeds.**

SOLVENT	PERCENTAGE INHIBITION			
	<i>A. Mexicana</i>			
	Leaf	Root	Flower	
Methanol	44	22	32	
Acetone	38	38	26	
Hexane	43	36	15	
Distilled water	100	49	47	
SOLVENT	<i>C. rotundus</i>			
	Leaf	Root	Flower	
Methanol	38	54	32	
Acetone	40	32	38	
Hexane	36	58	29	
Distilled water	52	75	47	
SOLVENT	<i>V. encelioides</i>			
	Leaf	Root	Stem	Flower
Methanol	38	22	10	41
Acetone	40	32	26	42
Hexane	36	33	18	100
Distilled water	52	49	47	100
SOLVENT	<i>P. hyserophoresus</i>			
	Leaf	Root		
Methanol	16	32		
Acetone	26	40		
Hexane	18	100		
Distilled water	100	100		

In *A. mexicana* , it was observed that maximum percentage inhibition occurred in leaf extracts with distilled water showing 100% followed by methanolic extract at 38%. The percentage inhibition mediated by organic solvent were in the range of 31-33% whereas the distilled water extract

showed 65% inhibition (Table 2). ANOVA analysis of the rate of inhibition with different types of solvent showed a statistical significance.

In *C.rotundus*, compared to aerial parts of the weed, higher percentage inhibition was found in the extracts formed from root. The water extract indicated maximum of 75% inhibition. On an average, it was observed that the range of inhibition was in the range of 36-58% (Table 2). The inhibitory capacity of the plant was found to be statistically insignificant.

In *V. encelioides*, water extracts from all parts of the weed had maximal inhibitory action. The methanolic, hexane acetone and distilled water extracts showed 27%, 35%, 46% and 62% inhibition. The water extract was shown to demonstrate a higher inhibition rate.

In Parthenium, root extracts indicated a higher inhibitory capacity as compared to leaf extracts. The water extracts of both leaf and root showed 100% inhibition and terpenes are the common component of both the extracts. Terpenes have been previously implicated as cell cycle inhibitors<sup>23,24</sup>. It has also been demonstrated that Parthenium has the potential of inhibiting seed germination and seedling growth of soybean and haricot bean<sup>25</sup>. Thus, on the basis of experimental results, it is confirmed that terpenes present in Parthenium play a major role in cell cycle regulation. There have been few scattered reports of terpenes acting as growth inhibitors<sup>23,28</sup>

Based on percentage inhibition studies, it was found water extracts followed by acetone extracts for all the weeds were most potent as a growth inhibitor.

## CONCLUSION

The flavanoids from the weeds have shown major growth inhibitory potential. Many flavanoids from plant sources have already been successfully tested as anticancer agents over the years<sup>26,27</sup> but very few attempts have been made to utilize the obnoxious organisms like weeds as sources of anticancer agents. Additionally, on the basis of experimental results, it was confirmed that terpenes present in Parthenium also play a major role in cell cycle regulation. Further comparative studies need to be done to identify and develop an anticancer agent from weeds.

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