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RESEARCH PAPER

# Assessment of phenology and toxicity of *Prangos* platychlaena Boiss. in Halgurd mountain of Iraqi Kurdistan

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**A B S T R A C T:**

*Prangos platychlaena* Boiss, a wild plant belongs to the Apiaceae family, it is a native plant of Kurdistan Region-Iraq. Root, leaves, stem and flowers of the plant were collected from Halgurd mountain Iraqi Kurdistan region. Its initial growth begin in May, flowering at the beginning of June and reaches fruit formation by the end of June until the last week of July, while the seed formation and dispersal were begun by the end of July until the end of August. Ethanol extract of different parts of the *P. platychlaena* has significant cytotoxcity potential on the human liver carcinoma cell and the activities of cytotoxicity can be classified as root > stem > flowers > leaves. Toxicity of water extract assayed against *Triticum aestivum* L.(wheat) and *Hordium vulgare* L( barley) and *Lolium rigidum* Gaud, results were indicated that the germination percentage and the root elongation decreased gradually with increasing the concentration of extraction until complete inhibition. *T. aestivum* shows higher tolerance ability to root, leaf, stem and flower extracts. As well as there was inhibition of *Lolium rigidum* due to all parts extract. Thus *P. platychlaena* Boiss can be used as a herbicide.

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KEY WORDS: cytotoxicity; liver carcinoma; wheat, barley; Lolium.
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## 1.INTRODUCTION:

In the Iraq especially Kurdistan region is well known for its flora and the abundance of wild plants and their natural products that its people have long used as traditional medicine, food and other uses (Hamad et al., 2017). The family *Apiaceae* is regarded as one of the important famililes for its chemical contents. The genus of *Prangos* belongs to the family of Apiaceae, under the order of apiales, having thirty species distributed from the Mediterranean to central Asia, fourteen of them found in Turkey (Ahmed et al., 2011). While, seven of them found in the different areas of Kurdistan region of Iraq, which are *P.platychlaena* Boiss., *P.ferulacea* L, *P. uloptera* DC., *P.asperula* Boiss., *P.pabularia* Lindl., *P.peucedanifolia* Fenz, and *P.carymbosa* Boiss. The *P. platychlaena* Boiss is a perennial plant, length can reach up to (1-1.5m), naturally growing in the high mountains in the Kurdistan region of Iraq, on rocky slopes at the altitude of 2200-2800m above sea level. Their species were distributed more in the Halgurd mountain (Ghazanfar and Edmondson, 2013).

The phenomenon of phenology is a biological process that involves a series of cyclical steps such as vegetative growth, flowering, fruit formation and seed formation and dispersal (Valdez-Hernández, 2015). Toxicity assay is an important aspect in the investigating processes of neighboring plant's inhibition growth and studies of cytotoxicity. Such processes were encouraged by natural plant products that have an effect on certain plants. Part of this study was concerned about the extracts from the roots, leaves, stems and flowers of *P. platychlaena* Boiss with regard to their cytotoxicty on human liver carcinoma

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cells and toxicties toward other plants. Our survey on the availblity of the literature on a plant, as yet, no study covered the scientific information about the phenology and toxicity of this plant species.

## 2.MATERIALS AND METHODS

### 2.1Description of studied location

Halgurd mountain in Kurdistan region well-known for its various flora species. Its located in the north east of Erbil city. The studied area in the mountain is of altitude; 36:42.30758N, longitude; 44:52.29358E and about 96 km distant from Erbil city. Location and topography map of the area was measured by using the global positional system (GARMIN/GPS 72), (Figure 1).

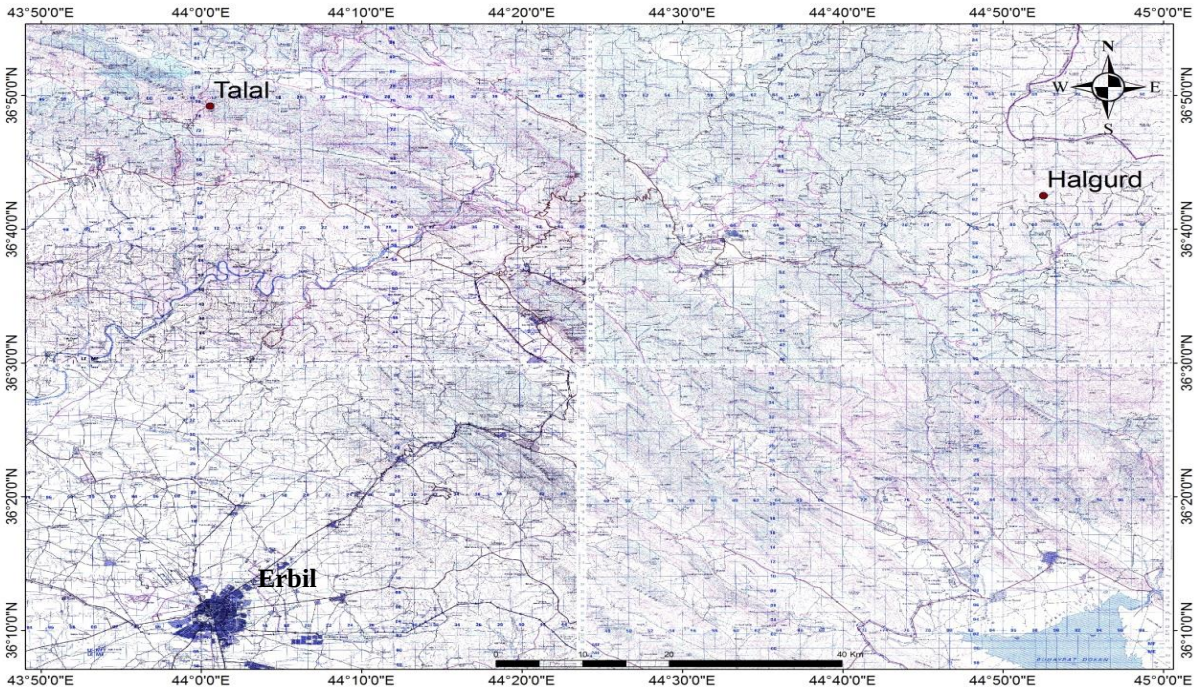



Figure 1: Location and topography map of Halgurd mountain.

### 2.2 plant material collection and treatment

Different plant parts of *P. platychlaena* Boiss *were* collected during May to July of 2017 from the Halgurd mountain in Kurdistan region of Iraq, at the altitude of 2170m. The different plant parts root, leaves, stem and flowers of the *P. platychlaena* Boiss were collected, cleaned and shed dried at the room temperature near 38-40°C till the constant weight obtained. The dried parts grinded slowly sieved to obtain a fine powder and preserved in tight closed bottles according to (Yeo et al., 2014).

### 2.3 Extraction method

Dry powder of each plant part (10.0g) mixed with petroleum ether (40-60%), ethanol (99.9%) and distilled water, for three days at room temperature (30-40 °C) and repeated for three times with stirring a regular interval. The extracts solution was filtered throw double layer of muslin cloth and Whatman NO.1 filter paper and concentrated using a rotary evaporator to obtain the crude of plant extracts (Yeo et al., 2014).

### 2.4 Assessment of life cycle and Phenological stage of *Prangos platychlaena* Boiss plant

Regular visit were arranged every week during 2017-2018 to monitor the life cycle of *P. platychlaena* Boiss in Halgurd mountain and follow the phenological stages during the life cycle; vegetative growth, flowering, fruit formation and seed formation and dispersal stage (Mirinejad, 2015).

### 2.5 Cytotoxicity of *P. platychlaena* Boiss against human liver carcinoma cells

The cytotoxicity of *P. platychlaena* was evaluated by the determination of their $IC_{50}$ using

the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium) test, a colorimetric method for monitoring cell survival in vitro assay. The basic of this test is change the yellow color of Tetrazolium salt to the violet crystals in the culture medium. The liver cell line ( HepG2) was collected from the Pasteur Institute of Iran. The ninety six well plates were used, and $1x10^4$ cells/well were incubated with 90µl of Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS) containing different concentrations of ethanol extracts that ranging from 1 to 500µg/ml, and incubated over night at 37 ºC in a CO2 incubator (5%). Then, 10µl of MTT solution (5mg/ml) was transferred into each well and the mixture was incubated for 4h at 37 ºC. After incubation time, 100µl of Dimethyl Sulfoxide (DMSO) was added into each well to dissolve the formazan from the MTT and incubated at 37 ºC for 24h. ELISA reader (BioTech, USA) used for measuring the absorbance at a wavelength of 570nm. Untreated cell (<1%DMSO) was used as control (Chanda and Nagani, 2013), the percentage of the reduction of viability was calculated as follow:

Viability % = $OD_e / OD_c * 100$…..(1)

Where: $OD_e$: is the mean value of the measured optical density of the extract of the sample, $OD_c$: is mean value of the measured optical density of the control. For each concentration tested, five replicated wells were used and 50% inhibition of cell growth ($IC_{50}$) used as an analysis parameter. The lower value of viability % was indicated the sample has a higher potential of cytotoxicity. The sample was considered cytotoxicity if the percentage viability was <70% and non cytotoxicity if the value of percentage viability was > 70% (Cannella et al., 2019).

### 2.6 Assessment of toxicity of *P. platychlaena* Boiss on seed germination and root elongation of some plants

#### 2.6.1 Choose the plant seeds

The decision to choose the crop *Triticum aestivum* L. ( wheat), *Hordium vulgare* L ( barley) and wild plant *Lolium rigidum* Gaud seeds for this assay depends on the availability of the weed and uses of the crops in our area which plays an important role in the economics of the region. Seeds of all of them were obtained from the Directorate of Research Center of plants in Erbil. In this experiment we choose *Triticum aestivum* L., *Hordium vulgare* L. and *Lolium rigidum* Gaud. seeds to evaluated the effect of *P.platychlaena Boiss* parts extracts on their germination and root growth.

#### 2.6.2 Preparation of plant stock solution and bioassay

The effect of plant extract on the germination and root elongation of tested plants were studied in the laboratory**.** The obtain of a stock solution (w/v) , weight (1gm) of extracted plant parts such root, leaves, stem and flowers were dissolved in distilled water, 2.5 % DMSO and 2 % DMSO, respectively. The stock solution of each extract (10mg/ml) were further diluted with the distilled water, 2.5%DMSO and 2%DMSO, respectively, to different concentration (0.5, 1.5, 2.5, 3.5, 4.5 and 5.5 mg/ml). From each treatment taken 10 seeds were placed in a petri dish of 9.0cm in diameter, lined with Whatman No.1 filter paper, moistened with 7ml of each extract at different concentrations. All petri dishes were incubated in the growth chamber at $22\pm2^0$ C. Germination % (G %) and root elongation, were measured after 7 days and statistically analyzed (Javaid et al., 2006, Kapur and Govil, 2000)**.** Germination % was calculated according to (Kanimarani, 2018), with the following equations**.**

**Germination = (Total no. of germinated seeds /Total no. of tested seeds) *100….(2)**

The seed inhibition percentage of the *P.platychlaena* against the *T. aestivum* L., *H. vulgare* L. *and L. rigidum* Gaud were calculated as per the formula described by (Hoque et al., 2003), and used in the $IC_{50}$ calculation. The value of $IC_{50}$ was determined on the bases of the equation used by (Ali and Taha, 2016)**.**

**I =C - T*100/ C**…… (3)

Where: I = seed inhibition percentage, C= Response of control plant, T= Response of treated plant

### 2.7 Statistical analysis

The cytotoxic activities, germination rate and root elongation of the *P. platychlaena* Boiss were statisticaly analyzed using ANOVA table, by Dunnett's multiple comparision tests, by Graph Pad-Prism version 7. All results arranged as mean ± standard error for each data and *P* value less than 0.05 considered as statistically significant differences among them.

## 3. RESULTS AND DISCUSSION

### 3.1 Assesment of the phenology of *P. platychlaena* Boiss in Halgurd mountain

The results in Figure 2 showed the stage of growth and development of *P. platychlaena* Boiss started from the 1st week of May, then 2nd stage the vegetative growth which initiated in the 2nd week of May, and continued until the end of May, while the flowering stage started from the beginning of June and continues until the end of June. Also, the fruit formation is initiated at the end of June and continues until the last week of July. On the other hand, some plants started with seed formation and dispersal after the month of July and continue until the last week of August. Finally, the degradation of the vegetative growth gradually occurs and the plant died, by the end of September (Castro-Diez et al., 2003).

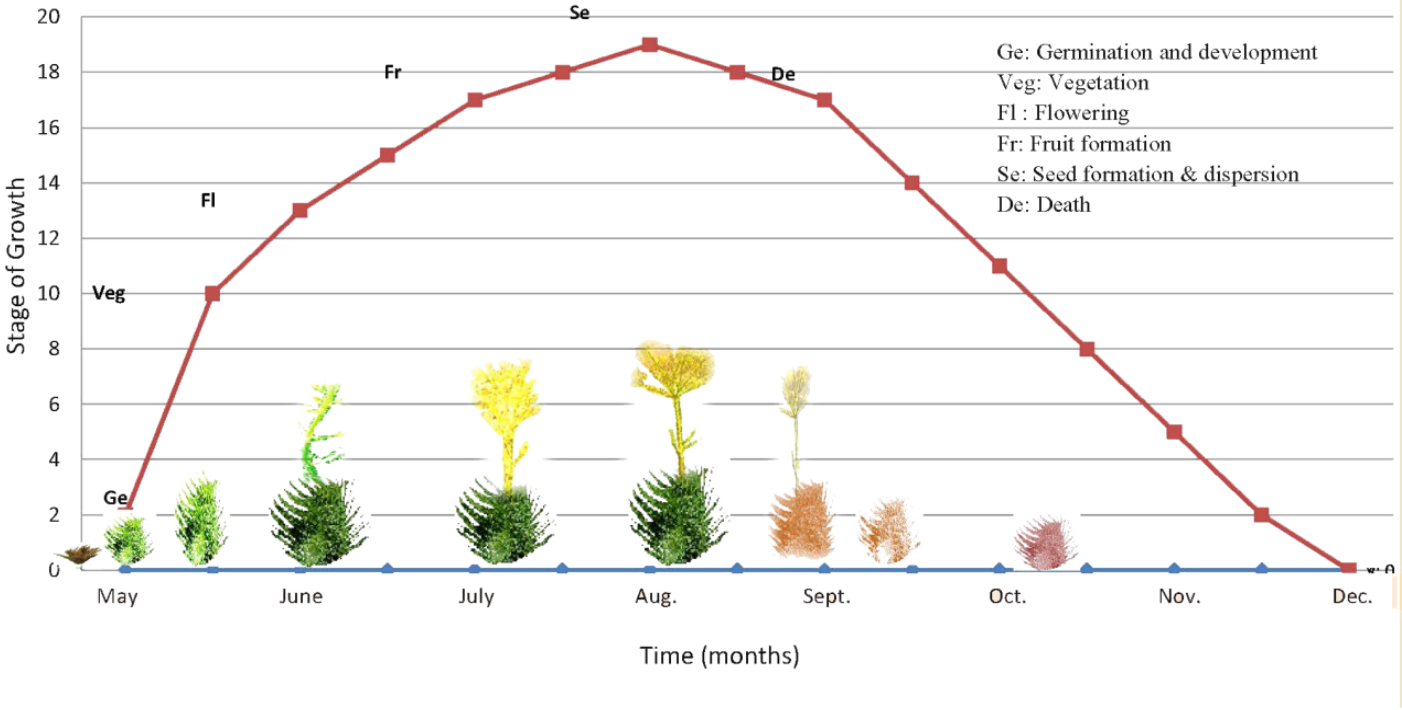



**Figure 2:** Growth and phenological stages of *Prangos platychlaena* Boiss from May to September.

### 3.2 Assessment of cytotoxicity of *P. platychlaena* Boiss against human liver carcinoma cells

In order to determine the rate of inhibition of the cell growth, the cytotoxicity of *P. platychlaena* Boiss extracts were carried out against liver carcinoma cell line (HepG2 ) at different concentrations. Statistical analysis showed that the plant extracts have a potential inhibitor effect on the growth of liver carcinoma. The concentration of the extracted of plant parts as root, leaves, stem and flowers have significantly affected against the growth of liver carcinoma cells were 50,100,10 and 10µg/ml respectively. The results showed the cell viability percentage decrease with an increase in the concentration of plant extracts (Table 1 ). While, the $IC_{50}$ values of the of the root, leaves, stem and flowers extracts were 94.0, 299.0,108.0 and 208.0 µg/ml, respectively (figure 3). The activities of extraction of the plant parts against liver carcinoma cells can be classified as root > stem > flowers > leaves. This finding indicated that the root part has higher cytotoxicity potential than the other parts due to containing the chemical compounds may have more potential activities against the growth of liver carcinoma cells. In comparison the cytotoxicity of *P. platychlaena* Boiss with other *Prangos* species, that partial agreement with results showed by (Shokoohinia et al., 2014), root extract by acetone of *P.ferulacea* L. showed the cytotoxicity effect on the human ovarian carcinoma. According to (Farooq et al., 2014), the phytochemical analysis of methanol: dichloromethane (1:1) for root extract of *P. pabularia* led to isolation of osthol compound, which have potential of cytotoxicity against epidermoid carcinoma (A431), lung (NCI-H322), prostate (PC-3) and colon (HCT-116) cell lines.

Generally, natural chemical compounds found in the plant such as phenolic acid, tannins,

coumarins, flavonoids, terpenes, ..etc, have a major role in the prevention and treatment of cancer. The bioactivities of these compounds were responsible for their anti-inflammatory effects, anti-carcinogenic, inhibition proliferation and contribute to their apoptosis by stopping the cell cycle (Huang et al., 2010, Booth and Bohlmann, 2019).

**Table 1: Effect of *P. platychlaena* Boiss root, leaf, stem and flower extract concentrations on human liver carcinoma cell viability.**

| Concentration of extracts & their control | Cell viability (%) | | | |
|---|---|---|---|---|
| | Root | Leaf | Stem | Flower |
| Control | 100±7.4 | 100±7.4 | 100±7.4 | 100±7.4 |
| 1.0 µg/ml | 91.5±5.8 | 92.9±2.82 | 90.9±2.89 | 87.1±7.8 |
| 10.0µg/ml | 87.2±5.23 | 89.2±4.2 | 79.1±6.8* | 81.1±2.4* |
| 50.0µg/ml | 73.9±5.3* | 90.4±1.67 | 74.6±4.7 | 67.6±3.6* |
| 100.0µg/ml | 57.2±5.3* | 68.6±2.7* | 36.69±6.2* | 55.2±3.0* |
| 200.0µg/ml | 21.0±3.49* | 59.0±2.4* | 27.78±3.9* | 51.8±3.7* |
| 500.0µg/ml | 25.3±3.7* | 38.6±1.46* | 20.3±3.5* | 39.3±2.8* |

Values was mean ± standard error, Symbol * refer to the significant differences among treated cell and control cell line

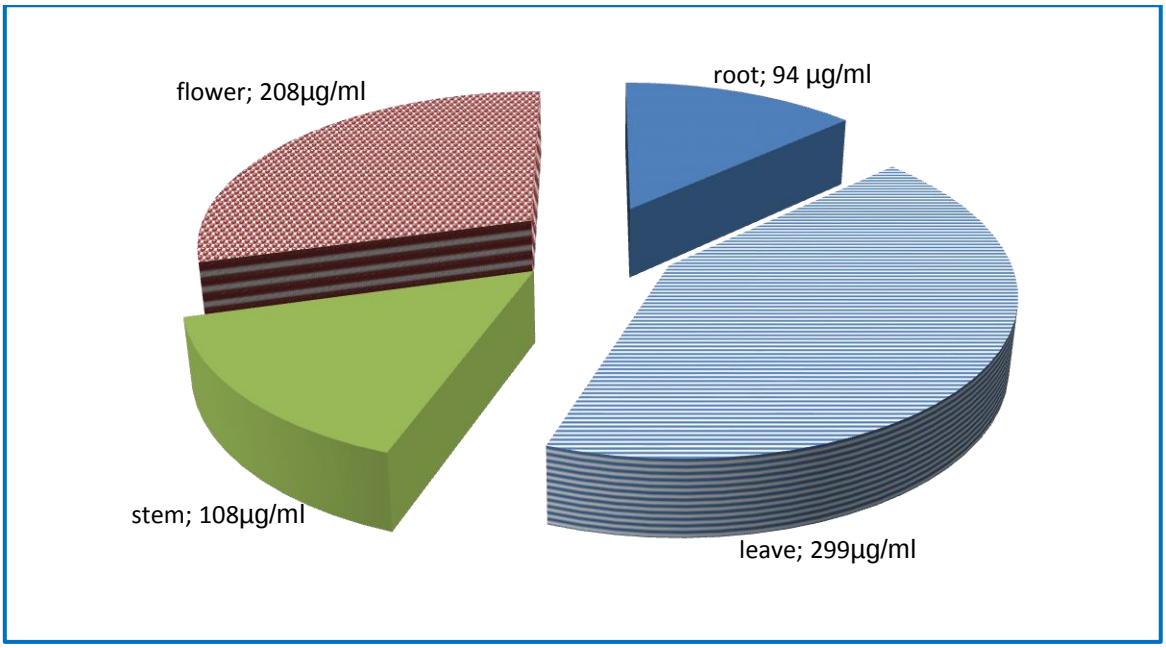



**Figure 3: The values of $IC_{50}$ of *P. platychlaena* Boiss root, leaf, stem and flower extracts on liver carcinoma cell line growth**

### 3.3 Assessment the toxicity of *P. platychlaena* Boiss on seed germination and root elongation

Seed germination and root elongation (radicals) of *Triticum aestivum*, *Hordium vulgare* . and *Lolium rigidum* seeds, during the 7days of treatment with different plant parts extraction of *P. platychlaena* Boiss, depends on the type of treated seeds and the phytochemical compounds in a part of the *P. platychlaena* Boiss plant used. The results revealed that the minimum percentage of germination rate for *T. aestivum* , *H. vulgare* and *rigidum seeds* were 27±3.3, 7±3.3 and 13±3.3% respectively, found in the water root extracts at 4.5, 2.5 and 2.5 mg/ml respectively, while the leaves water extracts showed an adverse effect on the percentage germination rates of *T. aestivum*, *H. vulgare* and *L. rigidum* seeds, in which the minimum value were 10±0.0, 10±0.0 and 10±5.7% respectively, which obtained by using 3.5, 2.5 and 2.5 mg/ml of extract, respectively. On the other hand, the minimum value of the germination percentage of all three plant seeds against stem water extract was 20±5.7, 10±0.0 and 7±3.3%, respectively, by concentrations the amount of 4.5, 2.5 and 2.5 mg/ml respectively. Finally, the water extraction of the *P. platychlaena* Boiss flowers showed the minimum percentage of germination rate which recorded10±0, 7±3.3 and 7±3.3% against *T. aestivum* L, *H. vulgare* and *L. rigidum*, respectively, found at the level of 2.5mg/ml respectively. The statistical analysis showed significant differences ($P<0.05$) among the treatment data at different concentrations with control data. While the germination percentage rate of each of *T. aestivum*, *H. vulgare* and *L. rigidum* were don't detected when ethanol and petroleum ether extract were used, those results were shown in Table 2.

The results in Figure ( 4, 5,6 and 7) indicated that the minimum value of root elongation for *T. aestivum* L., *H. vulgare* and *L. rigidum* seeds when treated with water root extract at 4.5, 2.5 and 2.5 mg/ml, were 3.83±0.44, 4.3±0.33 and 1.1±0.1 cm/plant, respectively. On the other hand, water extracts of leaves showed an adverse effect on the root elongation of *T. aestivum* L., *H. vulgare* and *L. rigidum* seeds, in which the lowest value was 1.33±0.06, 1.33±0.33 and 1.6±0.16 cm/plant, respectively, which obtained by concentrations 3.5, 2.5 and 2.5 mg/ml of extract, respectively. While, the minimum value of root elongation of all three plant seeds against stem water extract was 4.5±0.28, 5.3±0.33 and 1.3 ±0.15 cm/plant respectively, by using the amount of 4.5, 2.5 and 2.5 mg/ml respectively. Finally, the water extraction of the *P. platychlaena* Boiss flowers showed the minimum

value of root elongation which recorded 1.66±0.33, 2.43±0.29 and 1.16±0.08 cm/plant against *T. aestivum* L., *H. vulgare and L. rigidum,* respectively, recorded at the level of 2.5mg/ ml, respectively. The statistical analysis of data revealed the significant differences ($P<0.05$) among the treatments compare with control data at different concentrations of plant extraction. As well as, root elongations of each of *T. aestivum* L., *H. vulgare* and *L. rigidum* were don't detected when ethanol and petroleum ether extract were used.

In general, seed germination and root elongation were wide and rapidly used toxicity tests because of sensitivity and suitability for unstable chemical compounds (Lin and Xing, 2007). The results indicated that the germination percentage and the root elongation development decreased gradually with increasing the concentration of extraction until complete inhibition will occur due to increasing the accumulation of the phytochemical compounds which showed adverse effects on the growth of a seed . The data revealed the *T. aestivum* plant seedling was more resistance toward the extracts of root, leaf, stem and flowers of the *P. platychlaena* Boiss plant than that of *H. vulgare* and *L. rigidum* seedling, this may be related with the chemical compounds and physiological structure of their seeds and their genetically performance (Lin and Xing, 2007). This phenomenon allows *T. aestivum* to grow in the areas covered with the *P. platychlaena* Boiss plant. While the toxicity of ethanol and petroleum ether extracts don't detect because the extracts by both solvents don't soluble in water and it requires to add DMSO to increase solubility, thus the later solution prevented seeds germination (Hung et al., 1992).

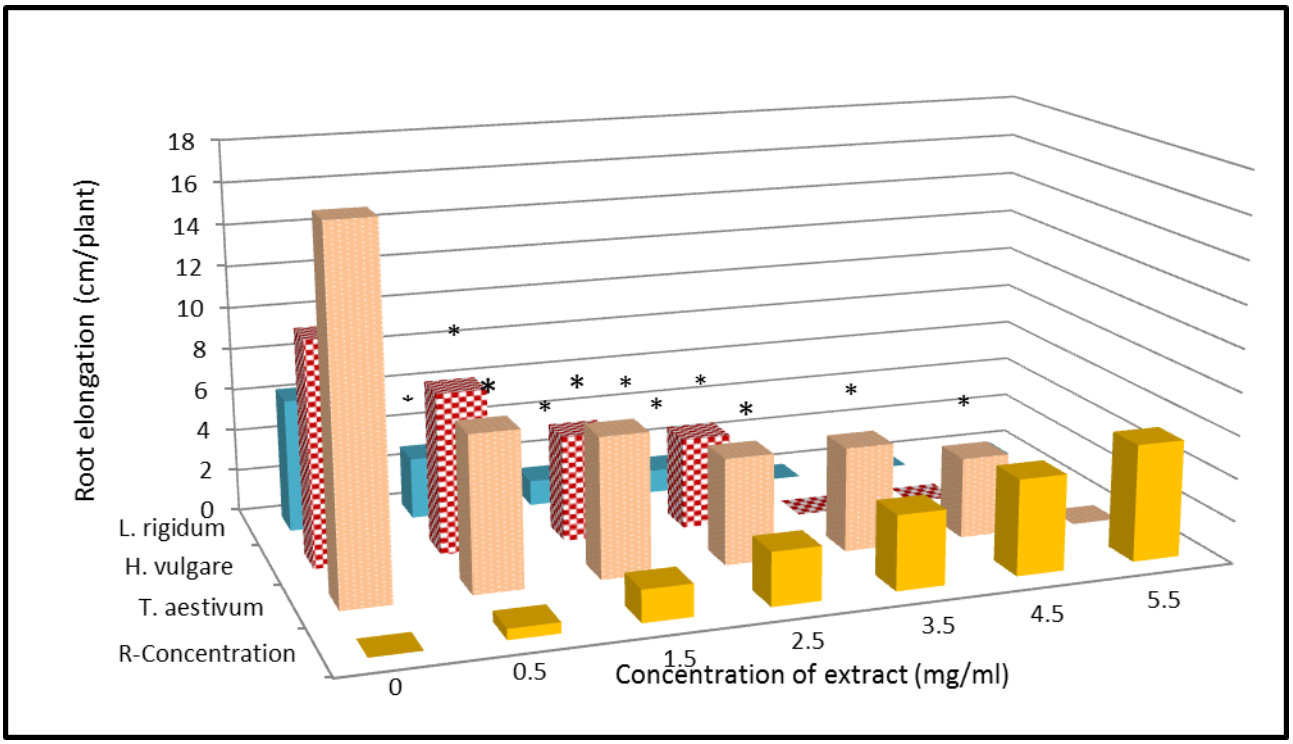
The results showed that the range of variation in the $IC_{50}$ values of the water extracts of root, leaves, stem and flowers of *P. platychlaena* Boiss was 0.48- 2.62mg/ml, Figure 8. This finding showed that the part of the flower of this plant has a lower value of $IC_{50}$ and higher toxicity potential, the other parts. Therefore, toxicity level can be arranged as: flowers > leaves > stem > roots, this may be due to the proportion of the amount and type of phytochemicals produced in the flowers of this plant.

**Table 2: Germination percentage of seeds incubated with different extracts from different plant parts of *P. platychlaena* Boiss**.

| Extracts | Plant parts | Concentration (mg/ml) | Germination rate (%) | | |
|---|---|---|---|---|---|
| | | | ***T. aestivum*** | ***H. vulgare*** | **L. rigidum** |
| **Water** | **Root** | **Control** | 90±0.0 | 92±1.66 | 97±1.6 |
| | | **0.5** | 83±8.8 | 60±5.7* | 80±5.7 |
| | | **1.5** | 60±5.77* | 22±1.6* | 40±5.7* |
| | | **2.5** | 33±3.3* | 7±3.3* | 13±3.3* |
| | | **3.5** | 30±0.0* | 0.0±0. | 0.0±0. |
| | | **4.5** | 27±3.3* | 0±0. | 0±0. |
| | | **5.5** | 0.0±0.0 | 0.0±0.0 | 0±0.0 |
| | **Leaf** | **Control** | 93±3.3 | 90.0±0.0 | 93±3.3 |
| | | **0.5** | 55±2.8* | 50±0.0* | 47±3.3* |
| | | **1.5** | 30±0.0* | 30±5.7* | 37±8.8* |
| | | **2.5** | 20±5.7* | 10±0.0* | 10±5.7* |
| | | **3.5** | 10±0.0* | 0.0±0.0 | 0.0±0.0 |
| | | **4.5** | 0.0±0.0 | 0.0±0.0 | 0.0±0. |
| | | **5.5** | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| | | **Control** | 93±3.3 | 92±1.6 | 93±3.3 |
| | | **0.5** | 90±5.7 | 60±5.7 | 63±3.3 |

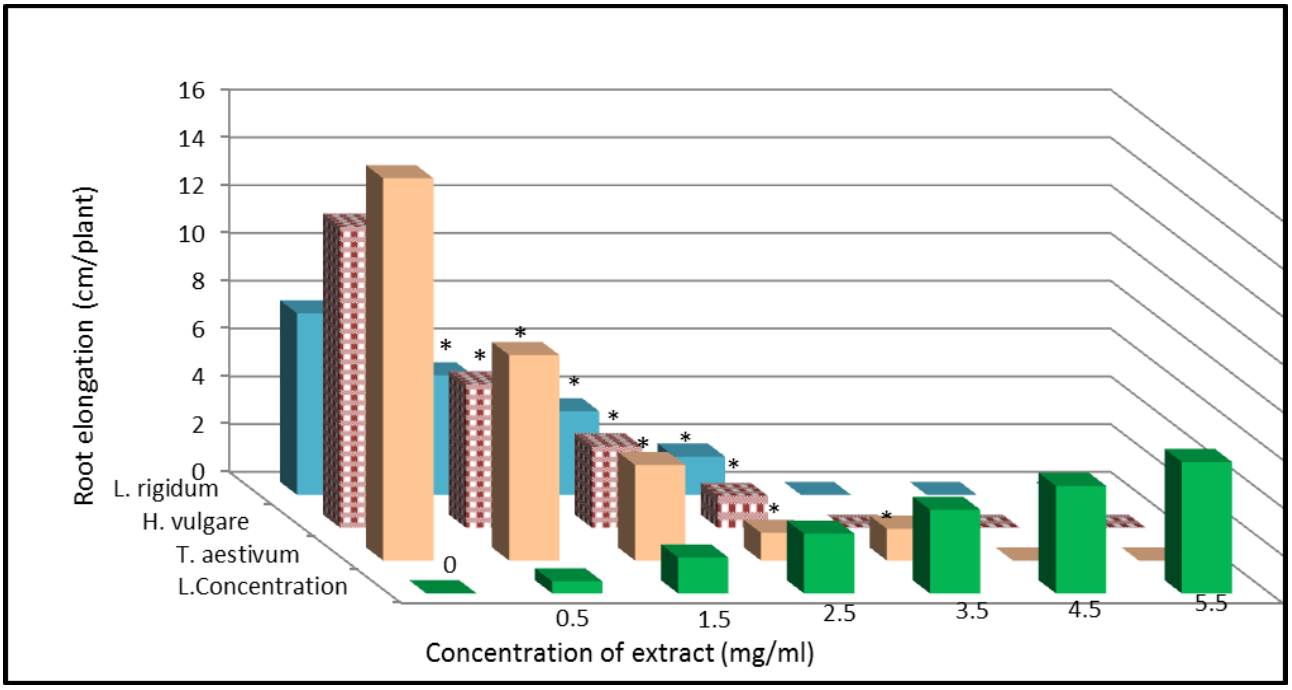
| | | | | | |
|---|---|---|---|---|---|
| | **Stem** | **1.5** | 50±5.7* | 17±3.3* | 30±0.0* |
| | | **2.5** | 27±3.3* | 10±0.0* | 7±3.3* |
| | | **3.5** | 23±6.6* | 0±0.0 | 0.0±0.0 |
| | | **4.5** | 20±5.7* | 0.0±0.0 | 0.0±0.0 |
| | | **5.5** | 0.0±0.0 | 0.0±0.0 | 0±0.0 |
| | **Flower** | **Control** | 97±3.3 | 91.0±1.6 | 91±0.5 |
| | | **0.5** | 53±3.3* | 45±2.8* | 48±1.6* |
| | | **1.5** | 23±3.3* | 30±5.7* | 25±2.8* |
| | | **2.5** | 10±0.0* | 7±3.3* | 7±3.3* |
| | | **3.5** | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| | | **4.5** | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| | | **5.5** | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| **Ethanol** | **All parts** | **Control** | N.D. | N.D. | N.D. |
| | | **all concentration** | N.D. | N.D. | N.D. |
| **Petroleum ether** | **Al parts** | **Control** | N.D. | N.D. | N.D. |
| | | **all concentration** | N.D. | N.D. | N.D. |

The symbol * significantly treated data with control data at (P<0.05), Values are expressed as mean ± standard error, N.D.: Not Detect



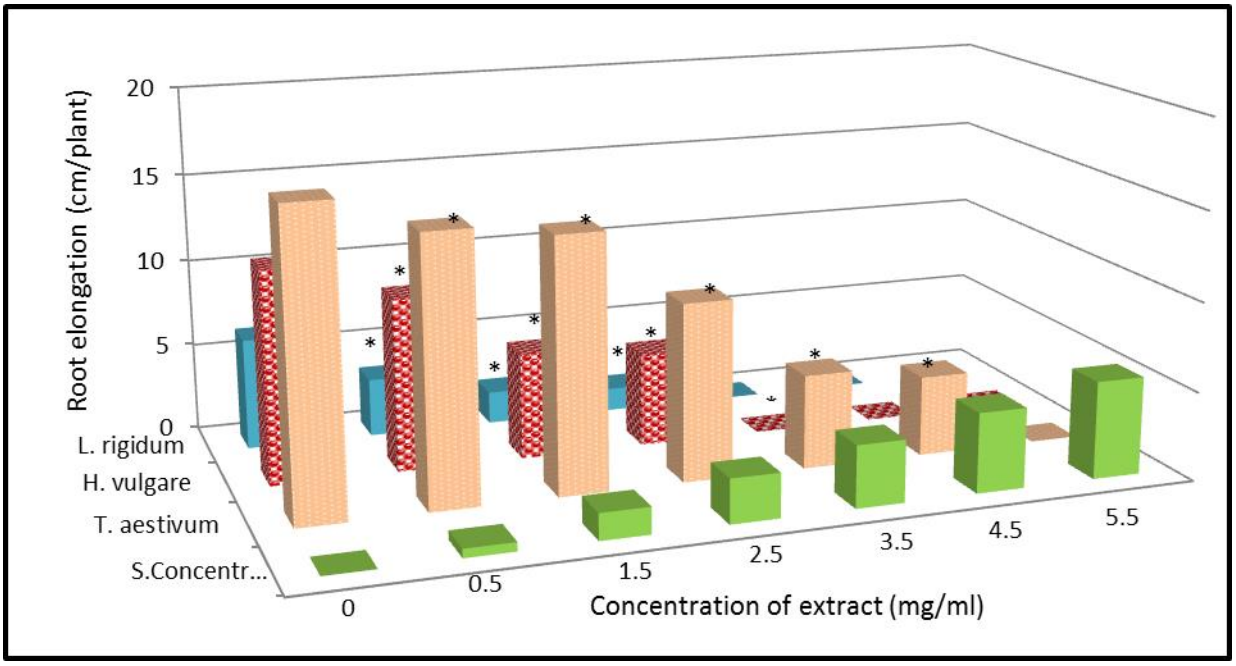

Values are mean, Symbol *: it means significantly among treatment and control data

**Figure 4: Effect of *P. platychlaena* Boiss root extract concentration on root length (cm)**



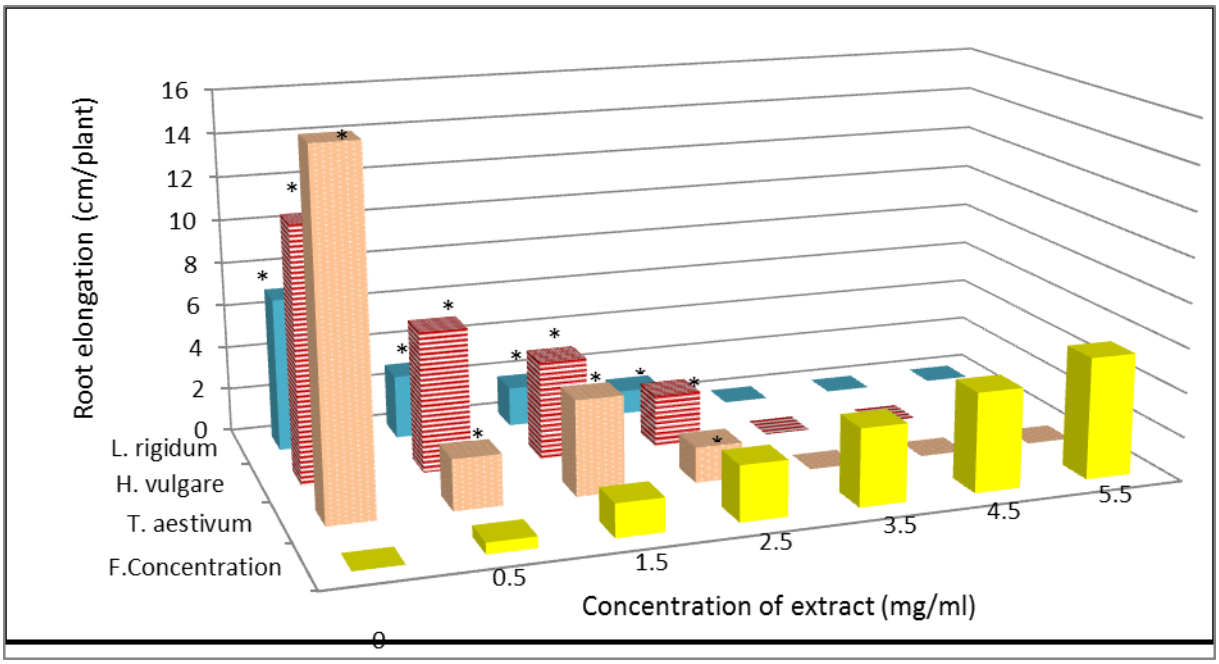

Values are mean, Symbol *: it means significantly among treatment and control data

**Figure 5: Effect of *P. platychlaena* Boiss leaf extract concentration on root length (cm)**




Values are mean, Symbol *: it means significantly among treatment and control data

**Figure 6: Effect of *P. platychlaena* Boiss stem extract concentration on root length (cm)**




Values are mean, Symbol *: it means significantly among treatment and control data

**Figure 7: Effect of *P. platychlaena* Boiss flower extract concentration on root length (cm)**

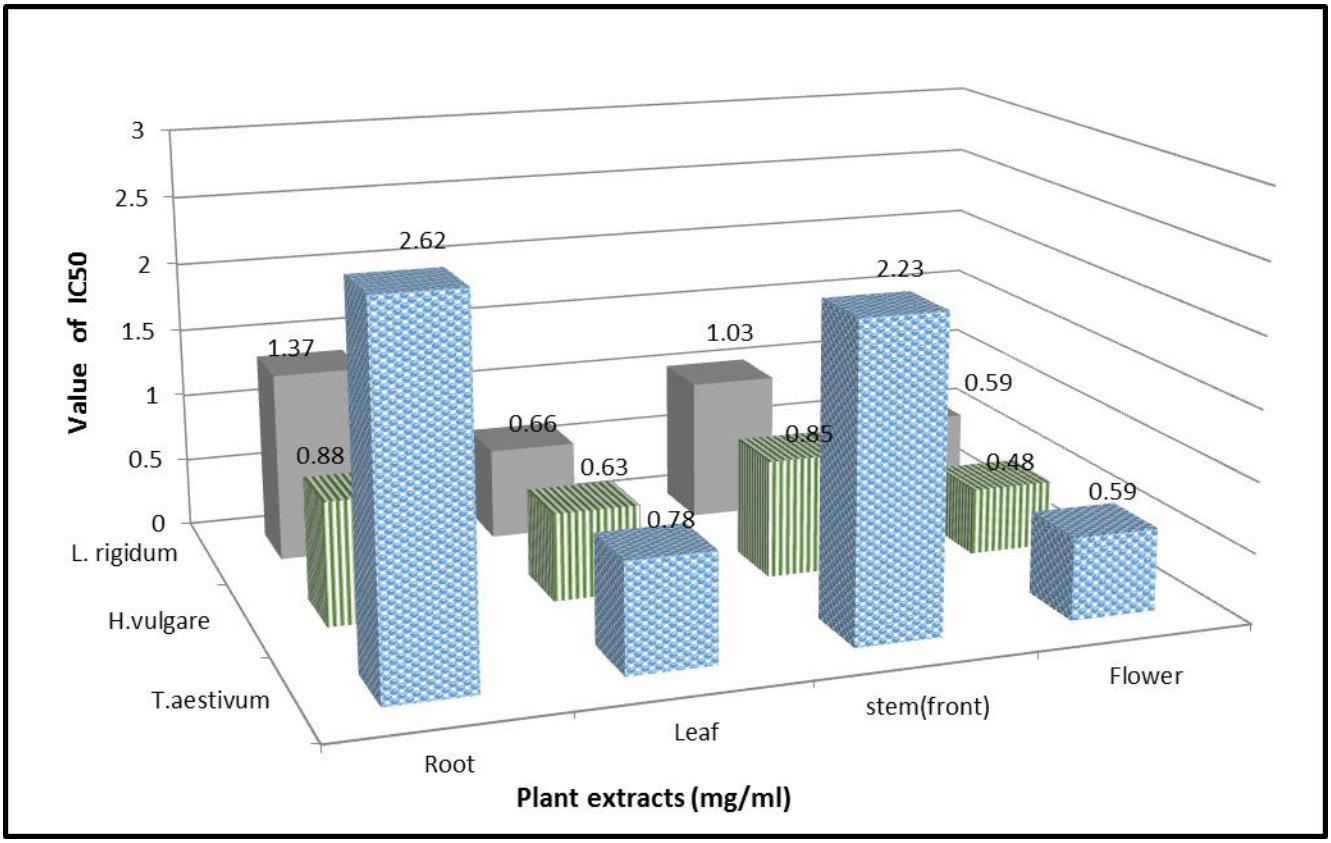



**Figure 8: Value of $IC_{50}$ of *P. platychlaena* Boiss root, leaf, stem and flower extracts against *Triticum aestivum* L, *Hordium vulgare* L and *Lolium rigidum* Gaud .**

## 4. CONCLUSIONS

Summary of the results showed that the phenological characters of *Prangos platychleana* Boiss started from June to August. Root, stem, leaf and flower extracts showed significant cytotoxic potential on human liver carcinoma cell. As well as flower water extract have more effects to inhibit both germination percentage and root elongation of *Triticum aestivum* L., *Hordium vulgare* L and *Lolium rigidum* Gaud.

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**Conflicts of Interest**

The authors declare no conflict of interest.

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