VARIATION IN PHYTOCHEMICAL, PHYSIOCHEMICAL CONTENTS AND TOXICITY OF PRANGOS PLATYCHLAENA BOISS IN HALGURD MOUNTAIN OF IRAQI KURDISTAN

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ABSTRACT

The wild plant *Prangos platychlaena Boiss* belonged to the family *Apiaceae*, is a native plant of Kurdistan-Iraq. The root, leaves, stem and flowers of the plant were collected in the Hlgurd mountain of Kurdistan region of Iraq, and extracted by ethanol solvent to obtain the crude extracts. Our results showed that the phytochemical contents are more concentrated in the initiated growth stage than the other stages and the phytochemical concentration posatively correlated with temperature and negatively correlated with humidity.The physiochemical contents such as total carbohydrate, protein, coumarin and dry weight more concentrated in the leaves and flower than other parts.This experiment was conducted to reveal the toxicity of leaves of the *P.platychlaena Boiss* on the rat. The results revealed that leaves cause toxicity of rats and its lethal dose was 3.95g/kg this is due to the leaves part containing large amount of coumarin than other aerial parts. These finding sugested that this plant, specially their leaves must not be used as the fooder for animals

Key word*:* crude extracts, protein, coumarin, lethal dose.

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تباين في المحتويات الكيمونباتية والكيموفيزيائية والسمية لـ *PRANGOS PLATYCHLAENA* BOISS في جبل هةلكورد في كوردستان عراق

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المستخلص

ان نبات البرنقوس المسطح *(Prangos platychlaena)* نبات برّي ينتمي الى العائلة الخيمية Apiaceae ويعتبر من النباتات الأصيلة في مختلف مناطق كردستان العراق خاصة المناطق الجبلية. تم جمع مختلف الأجزاء النباتية منها الأوراق، السيقان، الأزهار، و الجذور كلاً على حدة للنبات المذكور من جبل هلكورد, ثم استخدم مذيب الإيثانول للحصول على المستخلصات النباتية. أظهرت نتائجنا أن المحتويات الكيمونباتية تواجدت بتراكيز عالية عند مراحل النمو البدائية مقارنة مع بقية مراحل النمو، كما وأثبتت النتائج أن التركيز الكيمونباتي لمختلف الأجزاء النباتية كان يتناسب طردياً مع درجات حرارة الهواء وعكسياً مع نسبة الرطوبة. هذا وأن المحتوى الكيموفيزيائي كالكربوهيدرات الكلّية، البروتينات، الكومارينات والوزن الجاف تواجدت بتراكيز أعلى في الأوراق والأزهار مقارنة مع الأجزاء النباتية الأخرى.أجريت هذه الدراسة للكشف عن مدى سمية أوراق هذا النبات على الفئران (*Rattu norvegicus*). تبيّنت النتائج أن الأوراق تسببت في سمية الجرذان المختبرية مع الجرعة القاتلة بقيمة 3.95 غم/كغم، والسبب يعود إلى الحقيقة التي تؤكد بأن الأوراق تحتوي على كمية أعلى من الكومارينات مقارنة بالأجزاء الهوائية الأخرى. على ضوء نتائج الدراسة يقترح بأن هذا النبات خاصة أجزاءه الورقية لا يصلح استخدامه كعلف للماشية.

الكلمات المفتاحية: الاستخلاص الخام، بروتين، كومارين، الجرعة القاتلة.

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INTRODUCTION

Mountain region of north Iraq, specially the Halgurd mountain is famous for the diversity of wild plants and their natural products used for a long time by its habitants as traditional medicine, fooder and other purposes (1). The *Apiaceae* family is regarded as one of the important famililes, with its different medicinal species. The genus of Prangos belongs to the family of Apiaceae, under the order of apiales, having 30 species distributed from the Mediterranean to central Asia, 14 of them found in Turkey (2), while seven of them are distributed in the Kurdistan region of Iraq are *P.platychlaena Boiss., P.ferulacea (L.), P. uloptera DC., P.asperula Boiss., P.pabularia Lindl., P.peucedanifolia Fenz,* and *P.carymbosa Boiss . P. platychlaena Boiss,* is a perennial plant, length can reach up to (1-1.5m), This plant is naturally growing in the mountain of Halgurd, it contains different quantities of phytochemical compounds, which cause to toxic to other animals (3). The environmental factors quantitatively effect the plant metabolic processes and the phytochemical compounds through their effects on the growth rate, plant development and partitioning of the assimilates into vital metabolites. These factors were temperature, moisture,..etc. that causes to change the qualitative and quantitative phytochemicals production in plants (4). Toxicity assay is an important aspect of the investigating process of the death of animals. This process promoted by plant natural products, have possessed the effect on some animals. Part of this study is concerning the extracts from the root, leaf, stem and flowers of *P. platychlaena Boiss* which deals with the chemical compositions, and toxicity of the of *P. platychlaena Boiss*. Our survey on the availitiy literature concerning *P. platychlaena Bioss,* no study as yet,covers the investigations about the toxicity of this plant species.

MATERIALS AND METHODS

Description of studied location

Kurdistan region of Iraq is consist of a foothill and mountains land. Halgurd mountain was regarded as one of the important locations for the flora our country. Its located at the north east of Erbil area. The altitude, longitude and distances of the studied location from Erbil area were 36:42.30758N and 44:52.29358E and 96 km, respectively. This figure was measured by using the global positional system (GARMIN/GPS 72). (Figure 1)

Determination of air temperature (°C) and humidity (%) in the Halgurd location.

The air temperature and humidity of the studied location were measured daily in different times: 6 am, 12midday, 6 pm and 12 midnight, by using digital Thermo Hygro meter clock (Thermo Hygro clock-KTJ /TA218) All data were recorded from May to July of 2018 and tabulated (Table 1)

Variation in phytochemical contents of *P. platychlaena* Boiss in Halgurd mountain

Determination of phytochemical compounds and growth parameters in the vegetative parts of *P.platychlaena Boiss* during different periods of the growing stage in Halgurd mountain was according to the standard methods. After determination of studied area, we chose three individual plant of *Prangos platychlaena Boiss*, at the beginning of growth, the distance among them are about 30.0-40.0m, each of them labeled taking the samples after full leaves developed, during (10, 20, 30 and 40) days of growth stages: growth initiation, vegetative stage, pre-flowering stage and flowering stage, respectively. During this work the air temperature and humidity were recorded and the following parameters, such as total phenol, flavonoid, chlorophyll and carotene were analyzed according to standard methods.

Preparation of plant extracts

The different plant parts of the *P. platychlaena* Boiss were collected, cleaned and shed dried at the room temperature near 38°C until to gain constant weight, and grinding by grinders slowly, and were passed through a 25-mesh diameter sieve to obtain fine powder after that stored in the special bottle. Dry powder of each plant part (10.0g) was extracted by using ethanol solvent for three days at room temperature and repeated for three times with stirring a regular interval. The extracts solution were filtered throw double layer of muslin cloth and Whatman NO.1 filter paper and concentrated using rotary evaporator to obtain the crude extracts (5).

Total phenol content (mg/g): Determination of total phenol content in the leaves by Folin-reagent method (6)

Total flavonoids content (mg/g): Total flavonoids content in the leaves was determined by using the aluminum chloride colorimetric method (7)

The chlorophyll and carotenoids content (mg /g F.wt. leaves): Both chlorophyll and carotenoids were determined according to the method used by (8). The following formulas were used

Chlorophyll a = (15.65) (A 666nm) -(7.34) (A 653nm)

Chlorophyll b = (27.05) (A 653nm)-(11.21)(A 666nm)

Total chlorophyll = chlorophyll a + chlorophyll b

Carotenoids = 1000A470-2.860 Chl.a – 129.2 Chl b / 245

2.5 Physiochemical analysis of *P. platychlaena* Boiss

The physiochemicals analysis of different plant part of *P. platychlaena* was determined by different standard methods, as the following:

Total soluble carbohydrate (mg / g dry weight): The total soluble carbohydrate was estimated by using the anthrone method according to that employment by (9) **Total protein (mg/g dry weight):** The total protein contents were calculated by multiplying the value of total nitrogen by (5.7) according to (10)

Dry weight (g) : The fresh leaves (100g) were dried at 70ºC until constant weight, then the dry weight of leaves were measured according to the that employment by (11)

Total Coumarin contents (mg/g)

Determination of coumarin contents in different plant parts of *P. platychlaena* Boiss following the method outlined by (12), by using lead acetate solution and coumarin compound uses as a standard solution.

Toxicity assessment of *P. platychlaena Boiss* extract on rat and determination of lethal dose

This experiment is conducted at the department of Biology, the University of Salahaddin, the experiment procedure was performed according to the EPA(2002)test guideline (13). The overnight fasted female rats (*Rattu norvegicus*), (200-230)g, were housed under standard conditions (12hr.light, 12hr.dark) photoperiod, at the humidity of 60 ± 5 %, temperature 22± 3º C, having standard diets and free access to tap water. All animals were housed in standard plastic cages (38× 18×30)cm, bedded with the chip of wooden which sterilized with UV light about 1.5 hr and randomly divided into different groups (n=5). The 1st group (control group) received DMSO only, the other groups were orally treated with a single dose of ethanol leaves extract of *P. platychlaena Boiss* at 0.5,1, 2, 4,4.5 and 5 g/kg, the volume of extract solution that can be administered orally depends on the size of the animals and not exceed 1ml /100g of their body weight. After the solution of extracts has been administered, food may be withheld for a 3-4hr. Throughout the acute toxicity study signs of toxicity observed the first 24hr and rat body weight were recorded daily during 7 days and 14days after treatments. The value of the LD_{50} was calculated to depend on the mortality of the rats which recorded after 24hr.

Calculation of Median lethal dose (LD_{50})

The mortality of each rat was recorded after 24hr of the treatments and the LD_{50} was calculated by using Karber's method (14) as follows:

$LD_{50} = LD_{100} - \Sigma (a*b) /n$

LD_{100}= lethal dose causing the 100% death of all animals

a = the difference between two successive doses of administrating extract

b = the average number of dead animals in two successive dose

n =total number of animals in a group

Statistical analysis

1- 2- The physiochemical content, and acute toxicity of *P. platychlaena Boiss* were statisticaly analyzed using Kruskal- Wallis test and Dunn's multiple comparision test by Graph Pad-Prism version 7. While analysis data of phytochemical contents of the *P. platychlaena Boiss* were determined using ANOVA table and Tukey's multiple comparision test, by Graph Pad-Prism version 7. All results arranged as mean ± SE for each data and *P* value less than 0.05 considered as statistically significant differences among them.

RESULTS AND DISCUSSION

Air temperature (°C) and humidity (%) in the Halgurd location

The air temperature and humidity data of the studied location were measured and tabulated in Table (1).

Variation in phytochemical contents *P. platychlaena* Boiss

The results in a table (2) shows, that the concentration of total phenol significantly ($p<0.05$) decreased gradually from the growth initiation stage to the flowering stage. The highest value 23.5± 2.07 mg/g is found in growth initiation stage, while the lowest values in the pre flowering stage were 14.9 ±0.27 mg/g. As well as, the amount of flavonoids is gradually decreased from 4.32±0.10mg/g to 3.2±0.08 mg/g which found in the growth initiation and pre-flowering stage, respectively. The significant differences were found among the periods of growth of this plant. While the concentration of total chlorophyll and carotenes were not significantly different at ($p> 0.05$). wherease, the air temperature gradually increases and the humidity percentage was decreasing during the experiment period.

Correlation between phytochemical compounds in *P. platychlaena* Boiss and environmental factors

The environmental variable such as temperature and humidity were represented in (Table 1) can influence the level of phytochemical in the *P. platychlaena*

Figure (2, 3, 4 and 5) showed that the concentration of phenol, flavonoid, chlorophyll and carotene were negatively correlated with temperature, with r = -0.92, -0.87, -0.92 and -0.99, respectively, while with the humidity were positively correlated, with r = 0.75, 0.64, 0.94 and 0.94, respectively.

physiochemical properties of *P. platychlaena* Boiss

The contents of total soluble carbohydrate, total protein, coumarin and dry weight of different plant parts of *P. platychlaena* Boiss. reported and calculated in (Table 3). The results revealed that the concentration of total carbohydrate, total protein, coumarin and the amount of dry weight were showed significantly($p<0.05$) differences among the plant parts. The highest value of total carbohydrate is found in plant leaves and its 632±9.97, while the flowers containing the highest amount of protein, 55±0.65 which is more than the other three parts. In deed, the leaves showed high value of dry weight (43±0.33). While the highest value of coumarin from aerial part was 450±7.6 mg/g found in the leaves.

Assessment toxicity of *P. platychlaena* Boiss on lab animals (Rat) and determination of lethal dose

The toxicity or lethal effects were recorded during the treatment periods in the treated groups given single doses orally of 0.5, 1, 2, 4,4.5 and 5 g/kg of leaves extraction of *P. platychlaena* Boiss. during 14days of an experiment. The treated rats showed some signs of toxicity such as hypoactivity, piloerection, bloat and lacrimation, in a dose dependent manner. Most of the signs remained with them until death, while some of the rats were recovered to a normal state. The number of rats mortality were 2, 3 and 5 recorded in the groups which received 4, 4.5 and 5 g/kg, respectively, and the value of LD_{50} was 3.95g/kg.(Table 4). The oral toxicity of the plant showed significant differences between the treated rats groups in increasing their body weights after 7 and 14 days ($p<0.05$). While the weight of rat was significantly increased at 4.0g/kg during 14days was 248± 1.66(Table 5). Based on the available literature, this is the first study on the phytochemical contents and toxicity of the *P. platychlaena* Boiss. Variation of phenols and flavonoids concentration during the growth of the plant may be related to the climatic factors and the phenology stages of this plant. The accumulation of phenols and flavonoids in the leaves during growth initiation might be due to the fact, that during this stage, the protection of plant is mainly by phenolic compounds, which are accumulated during this stage [15].While the concentration of chlorophyll and carotenes were not significantly changed, this might possibly due to the physiological changes during the growth period of the plant. In general, these results indicated, that the high amount of phytochemical compounds were present in the growth initiation stage which might be the cause to increase the ability of toxicity in this stage when consumed by other

organisms specially goats. Due to the lowering of temperature and high humidity led to reduce the evaporation rates and the amount of water increased in plant, this might lead to stimulating the high metabolic activity in the plant, led to increase the level of phytochemical contents and viceversa with the high temperature and low humidity (1). These results indicated that the environmental change may change the phytochemical concentration and degree of toxicity of the plant. The result indicated that leaves and flowers of this plant have an important role in the nutritional value of the plant, which may have direct relationship with protein, carbohydrate contents and their dry weight (17). While the highest value of coumarin from aerial part found in the leaves of plant, may be the causes of increasing toxicity during consumption by animals. In this toxicity study, the leaves extraction of *P. platychlaena* showed significant effects on treated rats. Thus, when a single oral dose of the leaves extraction applied it produces some signs of toxicity such as hypo activity this changes may be related to the decreases of the locomotor activity controlled by central nervous system, lacrimation sign may be a result of the muscarinic effect of cholinergic poisoning (13),and bloating may be result of physical obstruction or damage to the esophagus which affects breathing and potentially results in death (18).The LD_{50} value of the leaf extract of *P. platychlaena* Boiss. after oral administration was 3.95g /kg, according to [14] toxicity scale, the *P. platychlaena* Boiss extract is slightly toxic, this change may be due to the establishing of poisoning in these rats because our studied on chemical analysis showed that the leaf of this plant contains a large number of coumarin compounds than the other aerial parts . Coumarin compounds produced their anticoagulant effect by interfering with the cyclic inter conversion of vitamin K because the structure of coumarin is similar to that of vitamin K, which is necessary to normal blood clotting. Coumarin decreases blood clotting by blocking vitamin K epoxide reductase in liver, an enzyme that the recycles oxidized Vitamin K. As well as, the vitamin K is an activator of coagulating factors (factors II, VII,IX and X) require γ-carboxylation for biological activity, so by decreasing of vitamin K, the synthesis of these factors are decrease and coagulation process will increase in the body of treated animals. (19). The oral toxicity of the *P. platychlaena* Boiss. showed a significant increases in the body weight of rats at 4.0g/kg during 14days this might be due to the rats ability to recover after the first week of treatment and also might be due to increases of their appetites as a result of the leaf extraction of the mentioned plant (20). These results showed different parts of *P. platychlaena Boiss* containg a large number of phytochemicals and more concentrated in the initiated growth stage, also phytochemical contents posatively correlated with temperature and negatively correlated with humidity. The results revealed that leaves cause toxicity of animals (rat), this is due to the leaves part containing large amount of coumarin than other aerial parts. The data indicated the degree of toxicity of *P. platychlaena* may be strong in the initiated growth stage of plant and early of season of spring

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Conflicts of Interest

Authors declare no conflict of interest.

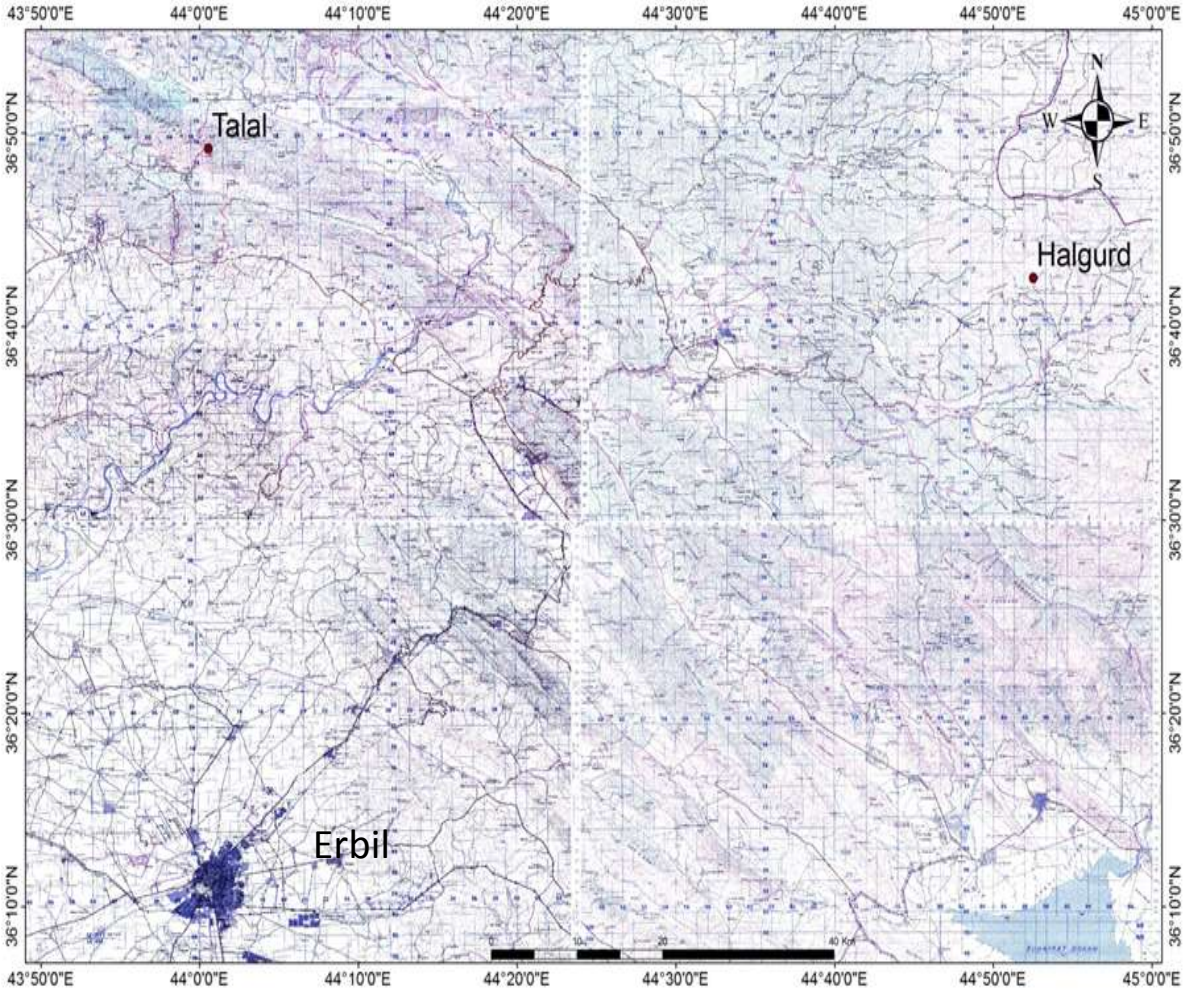

Figure 1: Map of studied location and distance from Erbil area

Table 1. the measure of temperature (°C) and humidity (%) in the Halgurd location during May – July 2018

12.0		6.0pm		12.0		6.0am		date	
humidity	Tem.	humdidy	Tem.	humidity	Tem.	humidity	Tem.		
28	16	28	16	30	15	26	15	01/05/2018	
31	15	29	15	28	14	27	13	02/05/2018	
29	15	30	14	27	17	28	15	03/05/2018	
28	16	31	13	31	16	31	14.8	04/05/2018	
30	20	29	14	31	14	32	15	05/05/2018	
29	18	30	15	29	14	30	16	06/05/2018	
30	15	30	14	28	17	29	15.2	07/05/2018	Growth initiation
28	14	30	19	31	18	29	14	08/05/2018	
30	16	30	14	32	16	31	16	09/05/2018	
29	15	29	17	31	19	31	15	10/05/2018	
292	160	296	151	298	160	294	149	total	
29.2	16	29.6	15.1	29.8	16	29.4	14.9	mean	
27	19.2	29	20.1	25	23.1	32	14.1	11/05/2018	
28	19.3	30	19	28	24.1	29	16	12/05/2018	
26.9	14	28	19	28.2	25	28	14.2	13/05/2018	
27	14.2	26	18	29	22	28	15	14/05/2018	
26	19	27	21	28	21	29.2	15	15/05/2018	
29.2	14.2	26.8	18.8	31	24.2	29.6	16.4	16/05/2018	Vegetative stage
28	19.7	28	17.2	29	23	27	14.2	17/05/2018	
29.9	19.1	29	19.4	27	23.4	27	14.5	18/05/2018	
28	19.2	29	19.2	27	24.1	28	14.1	19/05/2018	
25	14.4	31	20.8	29	25.5	27.3	14.2	20/05/2018	
275	172.3	283.8	192.5	281.2	235.4	285.1	147.7	total	
27.5	17.23	28.38	19.25	28.12	23.54	28.51	14.77	mean	
19	23	15	22	21	29	18	20	21/05/2018	
23	22	16	21.2	22	29.2	22	21	22/05/2018	
22	23	17	19	20	28	27	20.1	23/05/2018	
23	19	15	22.7	22	29.5	30	19	24/05/2018	
24	19	18	23	18	32.8	15	20	25/05/2018	
21	23	20	22	19	31	24	20.1	26/05/2018	Pre flowering stage
22	20.2	21	21.1	19	30	16	20.3	27/05/2018	
21	22	22	21.4	23	31	17	21	28/05/2018	
20	24.4	21	23	25	28.9	29	21.6	29/05/2018	
21	24	21	24.6	10	32.2	28	21.1	30/05/2018	
216	219.6	186	220	199	301.6	226	204.2	total	
21.6	21.96	18.6	22	19.9	30.16	22.6	20.42	mean	
17	22	10	23.4	10	34	13	16.4	31/05/2018	
16	21	10	29.4	10	34.5	11	23.4	01/06/2018	
15	23	11	29	10	31	10	22.5	02/06/2018	
16	22	10	26.2	10	29.8	11	23.1	03/06/2018	
17	23.4	14	24	10	31.2	17	23.4	04/06/2018	
15	21.6	12	23	10	30.6	17	21.3	05/06/2018	Flowering stage
15	22	11	25	15	27.3	31	19.4	06/06/2018	
14	22	10	24	10	34	17	18.8	07/06/2018	
17	23.1	11	22	11	31	20	18.9	08/06/2018	
16	27	10	23	11.1	35	15	27.5	09/06/2018	
158	227.1	109	249	107.1	318.4	162	214.7	total	
15.8	22.71	10.9	24.9	10.71	31.84	16.2	21.47	mean	

Table 2. Variation in phytochemical contents in *P. platychlaena* Boiss. during a different period of growth

Parameters / Periods	Tem. ◦C	Humdity %	Phenol mg/g	Flavonoid mg/g	Chlorophyll mg/g	Carotene mg/g
Growth initiation	16	30	23.5±2.07*,**,***	4.32±0.10*,**,***	1.66±0.006	0.22±0.0
Vegetative stage	17	28	17.7±0.66*	3.58±0.06*	1.63±0.004	0.20±0.004
Pre flowering stage	22	22	14.9±0.27**	3.21±0.08**	1.62±0.008	0.17±0.004
Flowering stage	23	16	15.4±0.16***	3.46±0.16***	1.59±0.03	0.16±0.015

Values are mean ±SD, Symbol *, **, *: it means significantly with data caring the same symbol among the periods of growth**

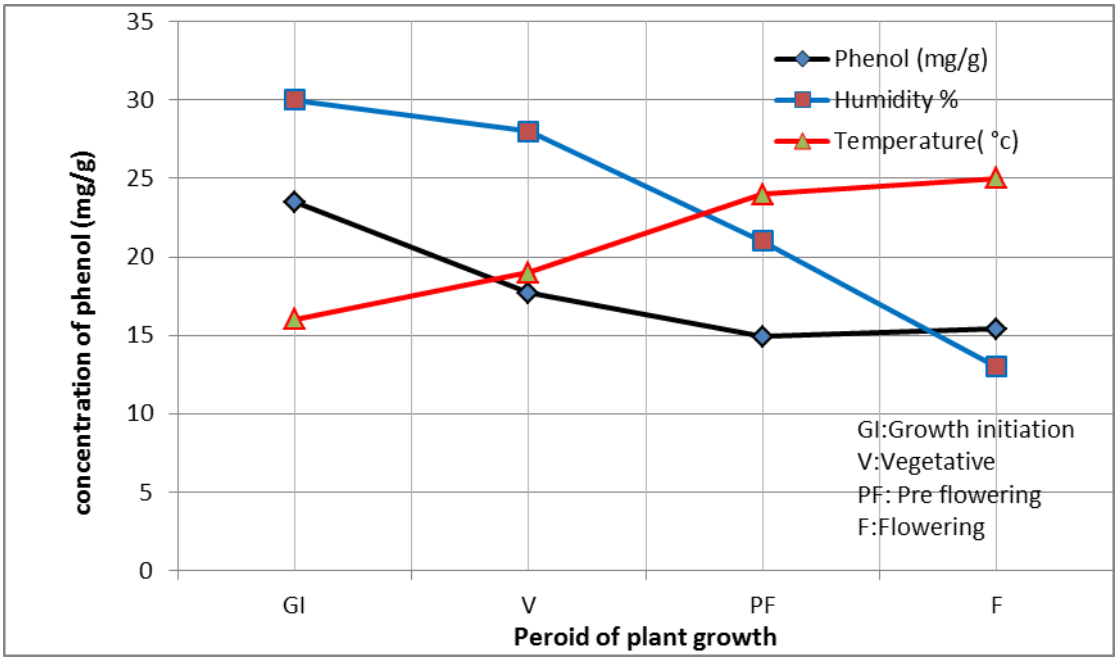

Figure 2. Variation of phenol content (mg/g) in leaves of *P. platychlaena* during a change of temperature and humidity.

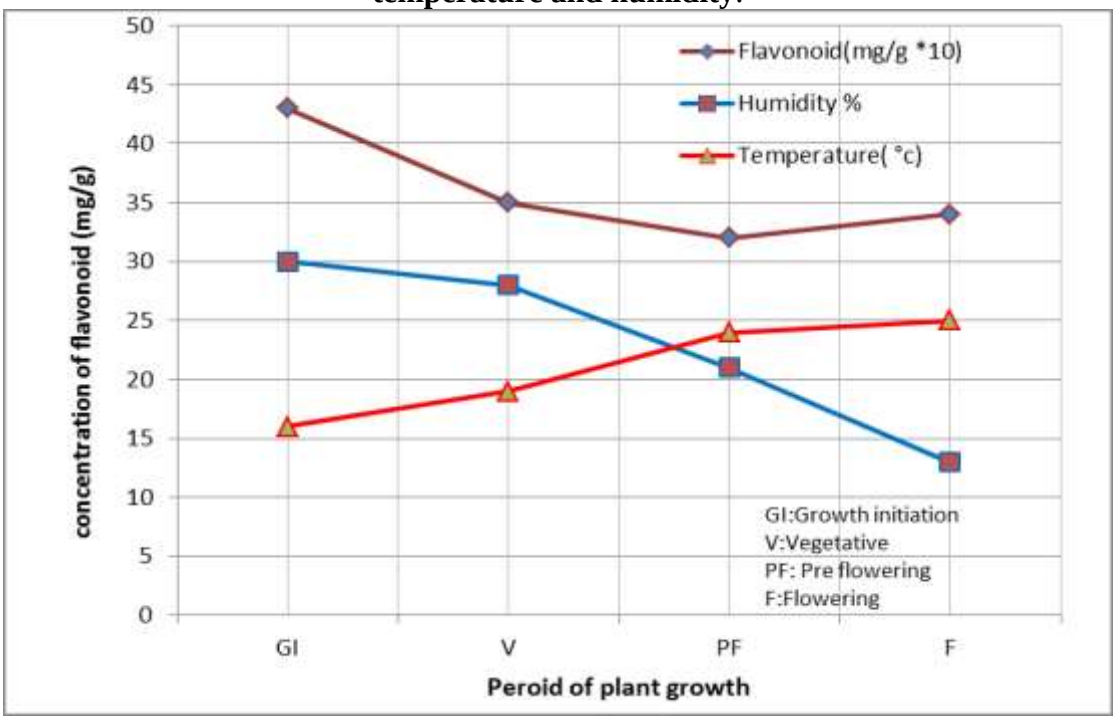

Figure 3. Variation of flavonoid content (mg/g) in leaves of *P. platychlaena* during a change of temperature and humidity

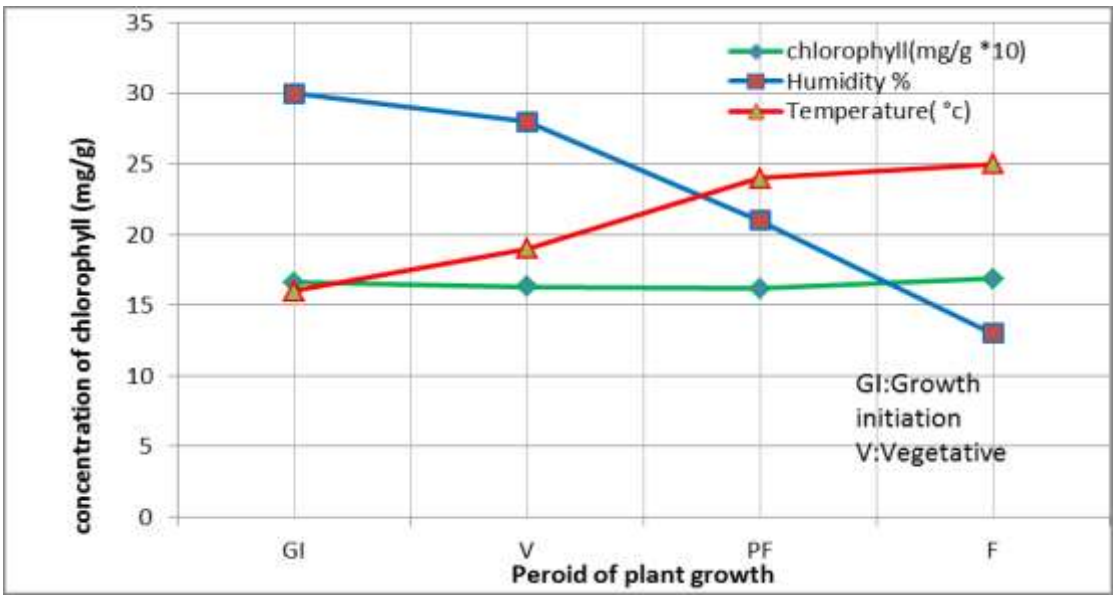

Figure 4. Variation of chlorophyll content (mg/g) in leaves of *P. platychlaena* during the change of temperature and humidity

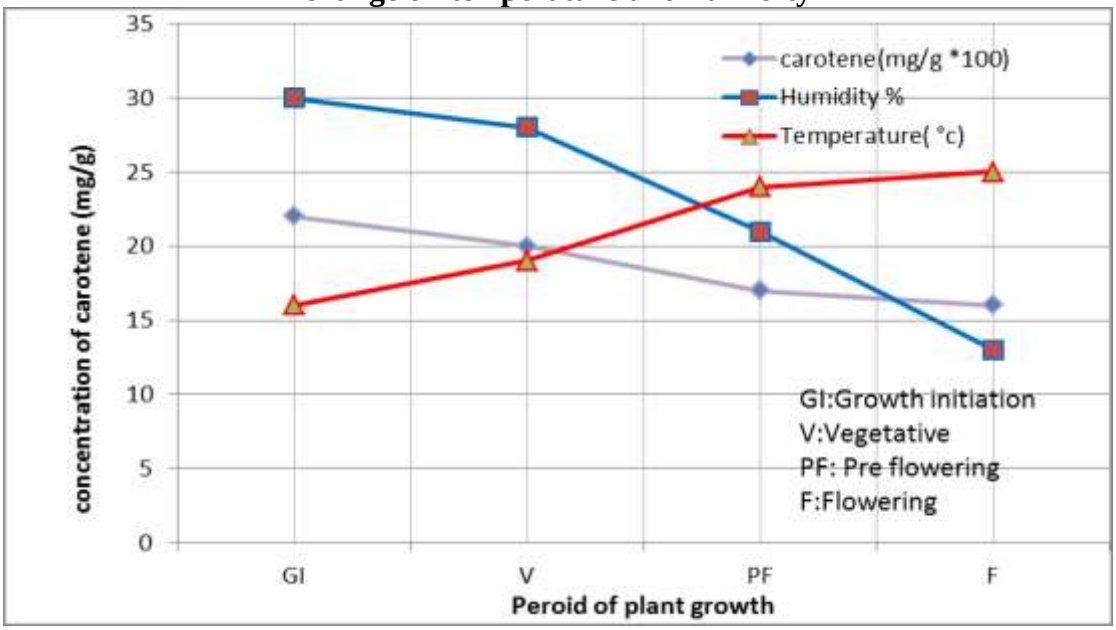

Figure 5. Variation of carotene content (mg/g) in leaves of *P. platychlaena* during the change of temperature and humidity

Table 3. Physiochemical properties of different plant part of *P. platychlaena* Boiss in studied location

Some properties	root	leaves	stem	flower
Total soluble carbohydrate(mg/g)	621±10.02*,	632±9.97**,	305±10.02***,	107±10.27*,**,***
Total protein(mg/g)	30±0.5	25±0.61*	34±2.08	55±0.65*
Dry weight (g)	41±0.57	43±0.33*	39±0.33	36±0.57*
Coumarin(mg/g)	595±13.2*	450±7.6	286±1.66*	410±7.63

Values are mean ±SD, Symbol *: it means significantly with data caring the same symbol in each property among plant parts

Table 4. Effect of a different oral single dose of *Prangos platychlaena* Boiss. leaves in rat

Concentration (g / kg)	Treated rat	Dead rat	symptoms
0.5	5	0	none
1.0	5	0	none
2.0	5	0	Few Hypoactive
4.0	5	2	Hypoactive,piloerection, lacrimation, bloat
4.5	5	3	Hypoactive,piloerection,lacrimation, bloat,
5.0	5	5	Hypoactive,piloerection, lacrimation, bloat

LD_{50} = 3.95g/kg

none: no toxicity symptom

Table 5. Body weights of rat in the toxicity study in groups treated with different dose of *P.*

groups	Change in body weight(g)		
	0 day	7 days	14 days
Control	214±7.4	227±3.3	237±2.18*
0.5g/kg	205± 5.5	207± 8.8	220± 1.15
1.0g/kg	209± 4.09	215± 2.08	219± 6.04
2.0g/kg	218± 9.1	223±8.5	224±8.4
4.0g/kg	212± 6.0	215±6.3	248±1.66*
4.5g/Kg	216	D	D
5.0g/kg	211	D	D

D: The rats died during 24h- 48h after treatments, Values are expressed as mean ±SD, * P<0.05 for mean compared to 0days

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