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INFLUENCE OF ARID CLIMATE ON THE CONTENT OF THE PLANT "CYNODON DACTYLON (L) PERS" IN FLAVONOÏDS

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ABSTRACT

During its evolution, plants have developed substances (called here active principles) with different functions, this can be a defense against parasites or other aggressors (micro-organisms), a technique to prevent the growth of other nearby plants and thus ensure good nutrition, as a means of growth or renewal of the species.

The concentration of active principles of a plant varies according to the age of the plant, the season, the climate and the environment in general (drought, pollution, etc). This is why it is important to know the best time of the year, and even of the day (morning, day, evening, night) to harvest the plant. According to our study, it was found that the plant Cynodon dactylon "L" Pers has a different composition and content of flavonoids between two periods.

Keyword: Cynodon Dctylon “L” Pers; active principles ; flavonoids; the periodic content; climate influence; medicinal plant.

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1. INTRODUCTION

For millennia, man uses plants to provide for his basic needs and including food, clothing and medical needs. More than 80% of the world's population uses medicinal plants to heal themselves accordingly, partly because of their effectiveness, and on the other hand by the lack of access to medicines prescribed by modern medicine besides these modern drugs [11].

The climatic conditions favored or disadvantaged the development of active principles in medicinal plants [30]. Temperature and precipitation are climatic factors of any primary importance because they control all metabolic phenomena and condition the distribution of all species and communities to be living in the biosphere

Algeria, because of its geographical position, enjoys several factors of pedogenesis and large climatic variations to which are added hydric resources, all favorable to the development of intensive crops of aromatic and medicinal plants (PAM) [4].

In this direction; The present work consists in carrying out the comparative study between the composition of the extracts of the plant Cynodon dactylon on different class flavones is its content in two different periods or months of December and or July.

Cynodon dactylon Poaceae is a perennial very diffused, very dense. Its stems are straight and very branched. It is widespread in the south of France, Italy and North Africa [21], as it is in the Sahara of Algeria,

The stems of Cynodon dactylon a grey-green colour and are short, usually 2–15 cm (0.79–5.91 in) long with rough edges.[19] The erect stems can grow 1–30 cm (0.39–11.81 in) tall. The stems are slightly flattened, often tinged purple in colour.

The seed heads are produced in a cluster of two to six spikes together at the top of the stem, each spike 2–5 cm (0.79–1.97 in) long [19].

It has a deep root system; in drought situations with penetrable soil, the root system can grow to over 2 metres (6.6 ft) deep, though most of the root mass is less than 60 centimetres (24 in) under the surface. The grass creeps along the ground and roots wherever a node touches the ground, forming a dense mat. C. dactylon reproduces through seeds, runners, and rhizomes. Growth begins at temperatures above 15 °C (59 °F) with optimum growth between 24 and 37 °C (75 and 99 °F); in winter, the grass becomes dormant and turns brown.

C. dactylon is seed-propagated but propagates mainly by cuttings, discards and underground stems.

According to the previous studies realized by Miller [22], 100 grams of the dried plant contains: protein (11.6 g); fatty compounds (2.1 g); total carbohydrate (27.9 g); fiber (25.9 g); ash (10.4 g); calcium (530 mg); phosphor (220 mg); iron (112 mg) and potassium (1.63 mg). In addition to that, *Cynodon dactylon* contains also: hydrocyanic acid, triticine, sugars and antibiotic gasoline.

Cynodon Dactylon (L) Pers is a kind of Quackgrass invasive grass, considered a weed by farmers, however the medicinal point of view it is full of virtues by its active principles, what it owes its name to its instinctive use as a purgative from dogs and cats [27 Furthermore, it is considered anti-inflammatory, antidiabetic and antimicrobial, hypolipidemic [23-28-29], diuretic decongestant urinary tract and recommended in cases of nephrolithiasis “kidney or bile” [13-24] with potassium salts and essential oil it contains.

2. CHEMICAL PRODUCTS

All the chemicals used were of analytical quality. Reagents were purchased from Sigma-Aldrich, Biochem Chempharma, Chromanorm and Riedel-de Haen.

- Hydrochloric acid (37%).
- Ethyl acetate (99.5%).
- Methanol (99.7%)
- Ethanol (96%).
- n-Butanol (99.5%).
- Diethyl ether (98%).
- Acetic acid (100%).

3. MATERIALS

A / apparatus: we carried out the determination of the flavonal classes with the U.V spectrophotometer of UNICAM type.

B / Materials used:

1 / A quartz tank of 1cm X 4 cm.

2 / Ethanol for the aglycone extract.

3 / Methanol for the anthocyanin extract and that of the c-glycosides.

4. The climatic conditions of the region of Ouargla

Table 1 follows presents the climatic data of the region of Ouargla for the period from 2006 to 2015.

Table 1: Climatic data of the region of Ouargla

Settings / Month	*Min Temp (C°)*	*Max temp (C °)*	*Average Temp (C °)*	*Precip (mm)*	*Hum (%)*	*Insol (Hour / month))*	*Evapo (mm)*	*S of wind (M / s)*
January	6,36	**19,95**	13,16	**9,42**	58,77	244,77	90,70	5,79
February	6,25	21,48	13,87	3,16	52,50	241,84	129,15	5,34
March	10,00	26,46	18,23	2,93	46,11	259,09	204,51	5,87
April	14,48	31,11	22,80	1,78	38,95	280,90	254,53	**7,08**
May	19,51	36,11	27,81	1,61	34,18	301,03	327,61	6,62
June	24,24	41,05	32,65	0,79	26,24	253,20	399,75	5,24
July	**27,61**	**44,10**	**35,86**	**0,35**	29,94	327,18	**464,44**	6,41
August	27,25	43,32	35,29	0,56	**29,35**	**330,68**	414,58	5,81
September	23,03	38,79	30,91	3,73	37,89	269,05	299,57	5,41
October	16,96	32,74	24,85	4,14	44,27	265,28	230,60	4,89
November	9,79	25,19	17,49	1,16	53,52	249,68	124,89	4,59
December	**5,50**	20,02	**12,76**	3,78	**59,78**	**223,28**	**88,80**	**4,34**
Average	**15,915**	**31,693**	**23,804**	**2,784**	**42,625**	**270,498**	**252,428**	**5,616**

Source: taken from O.N.M. Ouargla

The climate of Ouargla is characterized by the following parameters:

4.1. Temperature (Temp)

Temperature is a very important climatic factor that reacts directly, interacting with other meteorological factors (precipitation, humidity, etc.), vegetation development and the phenomenon of evapotranspiration. [3].

The evolution and the permanent change of the atmospheric temperature during the seasons of the year act directly on the temperature of the superficial water and thus on its quality and its habitats. The temperature parameter is a function of the altitude, the distance from the sea,

and the topographic position. [15].

The average annual temperature is 23.804C ° with 12.76C ° for the coldest month (December) and 35.86 ° C for the hottest month (July), for the period 2006/2015.

4.2. Precipitation (Precip)

It is irregular and rare, their distribution is by a drought almost absolute (from February to December), and by a maxima, in January and it is more intense in July. Average height of precipitation in the year and sending of 2,784 mm.

***** Ombrothermal diagram of Bagnouls and Gaussen:

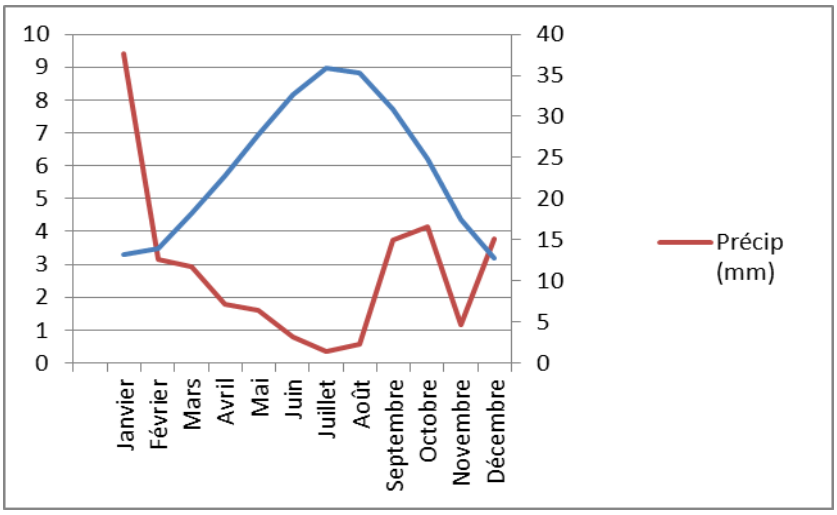

Fig.1. Ombrothermal diagram of Bagnouls and Gaussen from Ouargla region

4.3. Humidity (Hum)

Because of the high temperature and low precipitation in the region of Ouargla note that the relative humidity of the air is very low, it is of the order of 29.35% in August and 59.78 % of maximum in December, the annual average is 42.625%.

4.4. Evaporation (Evapo)

It should be noted that the means of evaporation are very high, especially when they are reinforced by hot winds. It is of the order of 252 428 mm in year, the maximum evaporation in the month of July equal to 464.44 mm and the minimum equal to 88.80 mm in the month of December.

4.5. Insolation (Insol)

The region of Ouargla is characterized by its strong sunshine with a minimum of 223.28 hours in December and a maximum of 330.68 hours in August.

4.6. Wind (wind speed)

According to studies by Dubief, J (1963) [7] in the region of Ouargla found that the strongest winds blow from the northeast / southwest. The most dominant winds in winter are the west saddle; while in spring dominate the prevailing north and west winds, in summer the winds are north-east and south-west in autumn.

5. THE CLIMATE EFFECT ON THE PRODUCTION OF ACTIVE PRINCIPLE

The formation of active principle may or may not be increased by climatic conditions and the arid climate may be favorable or unfavorable to this formation.

The production of active principles by the plant is depends on two groups of factors are as follows:

a. Internal factor:

All plants have hereditary characteristics. Other by the manufacture of an active principle is limited to certain species which they inherit by its character, and this property may be potential at well-specified climatic conditions [16].

b. External factors

It has two conditions act on the production of the active priciple it is the edaphic condition (concern the ground) and ecological (the temperature, the precipitation, the winds ...) [16].

6. MATERIALS AND METHODS

We harvested the plant on 20/12/2012 and 02/07/2012 to study the influence of climate conditions on flavonoids.

The plant was dried in the oven at the temperature of 80 °C (because s stay stable at this temperature) during 03 days with a stirring to obtain a homogeneous dry plant. After drying for the different parts of the plant, grinding and stored away from light in clean and dry containers made of smoked glass, closed with airtight lids according to the method described

elsewhere [20].

For this reason, an extraction was carried out on the three parts of the plant that were harvested during this period, as well as the frontal resolution of the compounds and the content of flavonic class were calculated.

6.1. Extraction of aglycones and anthocyanins and c-glycosylflavones

2 g of plant material powder was added to 200 ml of cold hydrochloric acid HCl (2N), then dipped in a boiling water bath for 40 min with air flow, the solution was stirred regularly every 10 minutes. After cooling and filtration, the acid solution was transferred to funnel. The acid hydrolysis allows the transformation of proanthocyanidins to anthocyanins and the release of flavonoids aglycone of their o-glycosides forms. Extraction was done according to the protocol described elsewhere [10].

Diethyl ether (3 x 70 ml) was used to extract phenolic compounds except anthocyanins and c-glycosides. The extracts was evaporated under ventilated hood, then transferred in 10 ml of ethanol, an aliquot was used for differential assay by aluminum chloride (AlCl3) at wavelength equal to 420 nm.

n-butanol (3 x 70 ml) was used to extract anthocyanins and c-glycoflavones. This solvent causes the red colored anthocyanins from oxidation proanthocyanes. Then proceeds to a determination of anthocyanins at 520 nm, the phase is then extracted with *n*-butanol, is carried out after a determination of c-glycosylflavones at 340 nm, concentrated the previous phase by confrontation with 2N HCl until 2 3 ml, this volume is used to identify anthocyanins and c-glycosyl-flavones by thin layer chromatography (TLC).

For extraction of glycosides compounds (c-glycoside and o-glycoside), 2.5 g of plant material was macerated in 250 ml ethanol for 48 hours, after filtered the alcoholic phase, a second maceration of vegetable powder was conducting for a few minutes in 100 mL of ethanol. The two alcoholic phases were joined and was evaporated by the evaporator rotatory. 100 ml of boiling water was added to the dry residue, after cooling the glycosides was extracted by 100 ml of *n*-butanol. Then butanol was evaporated. 5 ml of ethanol was added to the dry residue. The total heteroside classes (c-glycoside and o-glycoside) were identified by thin layer chromatography (TLC) [17].

6.2. The separation conditions for thin-layer flavon classes (TLC)

For the thin-layer separation, a one-dimensional separation in a chromatographic tank has been carried out with the different types of supports (stationary phase φs) and several types of solvents (mobile phase φm) are:

- The supports are the commercial silica gel 60 plates of size 10 * 10 cm with a thickness of 0.375 mm.
- the elution solvent for the aglycones is the mixture of dichloro ethane, ethanol (95/5).
- the elution solvent for anthocyanins and glycosides is the mobile phase of ethyl acetate; Methanol; Water (10 / 1.5 / 1) [1] [13].
-

7. RESULTS AND DISCUSSIONS

7.1. Separation results on CCM

7.1.1. for aglycones

Table 2: Rf of the two separate silica gel extracts

$C_2H_4Cl_2$/MeOH (95/5) December extract				*$C_2H_4Cl_2$/EtOH (95/5) July extract*			
color	***Rf leaves***	***Rf Stems***	***Rf roots***	***color***	***Rf leaves***	***Rf Stems***	***Rf roots***
Purple	0,90	0,93	0.94	***Purple Blue***	0,96	0,97	0,97
Yellow	0,82	0,84	0,83	***Yellow***	0,92	0,93	-
Y-Green [b]	0,72	0,76	0,75	***P-clair*** [a]	0,86	0,88	0,88
B-Green [c]	0,66	0,70	0,69	***Y-green***	0,79	0,79	0,78
Mauve	0,42	0,49	0,45	***B-Green***	0,72	0,72	0,73
Dark orange	0,38	0,39	0,31	***Yellow***	0,64	0,66	0,68
Blue	0,21	0,24	0,20	***Purple***	0,57	0,57	0,59
Y-shine	0,11	0,12	0,10	***J-dark***	0,49	0,47	0,48
Brown	0.07	0,07	0,05	***Purple***	0,36	0,37-	0,38
	0,00	0,00	0,00	***Blue***	0,27	0,16	-
				Y-shine	0,20	0,09	0,14
				Brown	0,08	0,00	0,07
					0,00		0,00

[a]: P = Purple; [b]: Y= Yellow; [c]:B = Blue

7.1.2. for anthocyanins

Table 3: Rf Separated Compounds on Silica Gel

EtOAc/MeOH/H_2O (10/1.5/1) ***December extract***				***EtOAc/MeOH/H_2O (10/1.5/1)*** ***July extract***			
Couleur	***Rf leaves***	***Rf Stems***	***Rf roots***	***Couleur***	***Rf leaves***	***Rf Stems***	***Rf roots***
Brown	-	0,97	0,98	***Brown***	0,94	0,89	0,92
Purple	-	0,83	0,82	***Blue***	0,74	0,66	0,75
Blue	0,69	-	0,70	***Y-dark***	0,58	-	0,62
Y-green [a]	-	0,64	0,65	***Yellow***	0,43	0,44	0,49
Yellow	0,4	-	0,41	***B-Green*** [b]	0,38	0,40	0,43
Green	0,22	-	-	***Y-green***	0,32	0,29	0,33
Blue	0,07	0,072	0,06	***Purple***	0,19	0,15	0,19
Y-green	0,00	0,00	0,00	***B-Clear***	0,06	0,05	0,05
				Yellow	0,00	0,000	0,00

[a]: Y= Yellow; [b]:B = Blue

7.1.3. Discussion

According to Tables 2 and 3 It is noted that the aglycone extract in July contains more products compared with that of December, in the leaves there are 13 products, in the stems we have 12 and in the roots are found 11.

Similarly, for the anthocyanin extract, the richest parts are the leaves and roots, where a product is missing in the dark yellow for stems extract and Rf between 0.58 and 0.62 in the other two parts.

7.2. The flavonoid content in two periods

Quantitative analysis of the different flavonoid classes was performed in two different periods, as follows:

7.2.1. Determination of aglycones

The differential assay of flavones and flavonols is performed based on the chelating properties of 1% AlCl3 dissolved in 96% EtOH (v/v). After standing for 10 min, the spectrum was scanned between 380 and 460 nm and the maximum absorbance was identified.

The content expressed as quercetin aglycone (flavonol) was calculated by the formula (2) proposed by Ouafi [17].

$$T\left(\frac{mg}{g}\right) = \left(\frac{\Delta(DO)}{\varepsilon}\right) \times M \times V \times \left(\frac{d}{P}\right) \quad (2)$$

Δ (D.O): optical density of differential peak (D.OAl3+ - D.OEtOH)

D.O(Al3+): optical density of the aglycones in the AlCl3 1% in EtOH solution

D.OEtOH: optical density of the aglycones in ethanolic solution.

ε: quercetin molar absorption coefficient of the differential peak (ε = 23000).

M: molar mass of quercetin = 302 g.mol-1

V: volume of the ethanol phase of aglycones form.

d: dilution factor equal 6.

P: dry weight of hydrolysed vegetable matter =1 g

7.2.2. Determination of anthocyanins

Because of the rapid degradation of anthocyanins compared to aglycones, we first proceed to the determination of anthocyanins as follows:

The Determination of anthocyanins was done by the scan of UV spectrum from 480 to 600 nm and identifies the maximum absorbance. The anthocyanin content is given using the formula (1) proposed by Lebreton [10].

$$T\left(\frac{mg}{g}\right) = \eta.\left(\frac{DO}{\varepsilon}\right) \times M \times V \times \left(\frac{d}{P}\right) \quad (1)$$

Where :

η = 6 (factor taking into account the equivalent transformation of proanthocyanidins on anthocyanidins performance).

DO**:** optical density at the maximum wavelength between 515 and 540 nm in the aqueous phase

ε: molar absorption coefficient = 34700 for cyanidin

M: molar mass of the procyanidin = 306 g.mol-1

V**:** volume of the aqueous phase after hydrolysis.

d**:** dilution here factor equal to 1.

P: dry weight of plant material (1 g).

7.2.3 Determination of c-glycoflavones

The content of the c-glycoflavones was calculated by the formula (3):

$$T\left(\frac{mg}{g}\right) = \left(\frac{DO}{\varepsilon}\right) \times M \times V \times \left(\frac{d}{P}\right) \qquad (3)$$

DO: optical density at the maximum wavelength (340 nm)

ε : absorption coefficient of the Orientin (ε =18850)

M : molar mass of the Orientin (M= 448 g.mol-1).

V: volume of the butanol phase measured after hydrolysis.

d: dilution here factor equal to 1.

P = dry weight of plant material (P =1 g).

7.2.4. Quality assurance and quality control

The quality assurance and quality control in this study was performed by the determination of some statistical parameters, which are: arithmetic average, standard deviation and coefficient of dispersion through the relationship given by Abedessamie [18] as follow:

The average T was given by the formula (4): $\bar{T} = \sum \frac{Ti}{n}$

The standard deviation S was given by formula (5): $S = \sqrt{\sum \frac{(Ti-\bar{T})^2}{n}}$

The Coefficient of dispersion CD was given by formula (6): $CD = \frac{S}{\bar{T}}$

7.2.5. Résults

1/ for the aglycones the following results were obtained:

Table 4: Aglycone content

THE ORGAN / THE PERIOD	*leaves (mg/g)*	*stem (mg/g)*	*Root (mg/g)*
December	*0,274*	*0,09*	*0,16*
July	*0,031*	*0,006*	*0,013*
Average $\bar{T}$	*0,153*	*0,048*	*0,087*
Standard deviation S	*0,12*	*0,042*	*0,074*
Dispersion coefficient CD	*78 %*	*87 %*	*85 %*

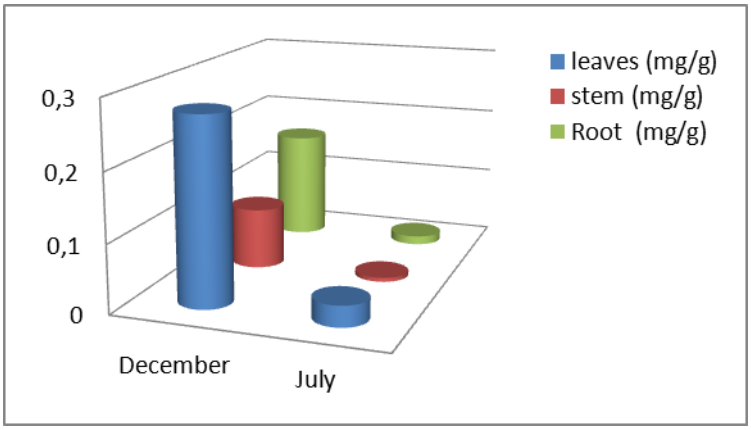

Fig.2. The Aglycone content of different part of *Cynodon Dactylon* in two different periods

Table 5: Anthocyanins content

THE ORGAN / THE PERIOD	***leaves (mg/g)***	***stem (mg/g)***	***Root (mg/g)***
	HCl 2N	***HCl 2N***	***HCl 2N***
December	*1,381*	*0,99*	*0,307*
July	*0,72*	*0,34*	*0,24*
Average $\bar{T}$	*1,05*	*0,67*	*0,27*
Standard deviation S	*0,33*	*0,33*	*0,034*
Dispersion coefficient CD	*31 %*	*49 %*	*12 %*

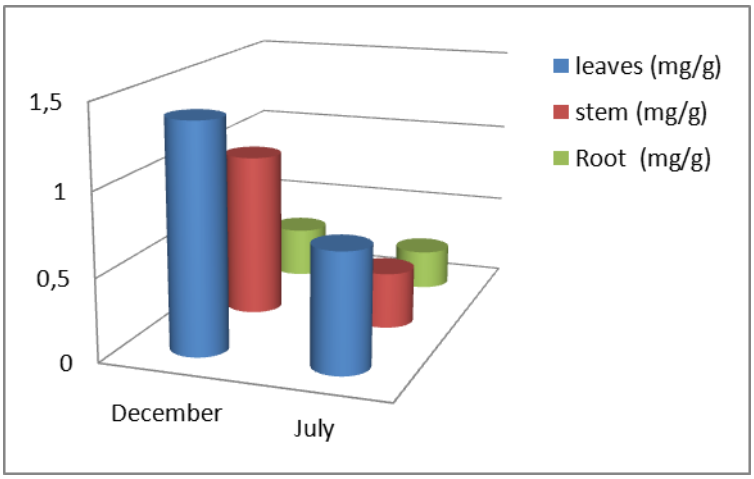

Fig.3. The Anthocyanins content of different part of *Cynodon Dactylon* in two different periods

Table 6: c-glycosides content

THE ORGAN / THE PERIOD	*leaves (mg/g)*	*stem (mg/g)*	*Root (mg/g)*
	BuOH	***BuOH***	***BuOH***
December	*8,058*	*7,466*	*7,616*
July	*6,58*	*4,54*	*3,029*
Average ∓	*7,319*	*6,003*	*5,323*
Standard deviation S	*0,739*	*1,46*	*2,29*
Dispersion coefficient CD	*10 %*	*24 %*	*43 %*

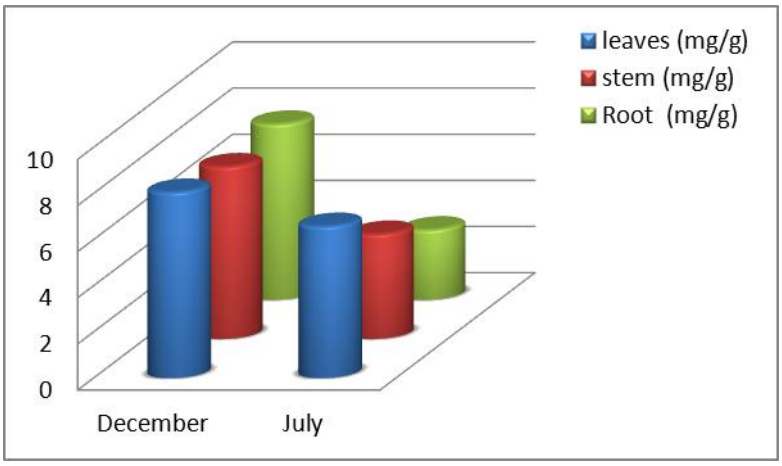

Fig.4. The c-glycosides content of different part of *Cynodon Dactylon* in two different periods

7.2.6. Discussion

We noted that the differential content of aglycones equal or 0.153 ± 0.12 mg / g for the leaves, 0.148 ± 0.042 mg / g for the stems and 0.087 ± 0.074 mg / g for the roots.

Especially since the absolute content of anthocyanins in the acid phase of each part of the plant material varied from 0.72 to 1.381 mg / g with an average of 1.05 ± 0.33 mg / g for leaves, from 0.34 to 0.99 mg / g with an average of 0.67 ± 0.33 mg / g in the stems and 0.24 to 0.307 mg / g with an average of 0.27 ± 0.034 mg / g.

The content of C-glycosides in the leaves varied from 6.58 to 8.058 mg / g with a mean of 7.319 ± 0.739 mg / g, in the stems ranging from 4.54 to 7.466 mg / g with a mean of 6.003 ± 1.46 mg / g and the root content ranging from 3.029 to 7.616 mg / g with a mean of 5.323 ±

2.29 mg / g.

From the results, it can be noted that the chemical composition of the plant in July is totally different from that of December, due to the climatic conditions that have an effect on the plants, for example the high temperature of the arid zones where July increases the evaporation rate of the soil water[31].

According to Benzyane M; and al **[5]**, the water is the first agent used in the production of chlorophyll and we know that the production of flavonoid compounds depends on it. On the other hand, very strong light (strong) decreases the production of chlorophyll, thus the production of certain active principles. The photosynthetic efficiency of the plants being higher before flowering [25-26], the flowering period of the Cynodon Dactylon "L" Pers plant is between July and September.

In case of severe drought, the very important transpiration is regulated by a progressive closure of the stomata, characteristic of the Mediterranean species, in order to avoid an excessive drying of the tree [2] which influences the rate and the composition of the extracts of flavonoids.

The water available in the soil in July is lower; the water supply in the tracheids can never be restored. In this case, the trunk retracts [9].

According to the bibliography, edaphic conditions also have an effect on the production of the actives principles, , for example the content of the soil in different elements such as the nitrogen that enters into the production of alkaloids. And the water that decreases the temperature of internal organs and participates in the synthesis of chlorophyll.

As can be said that, the Cynodon Dactylon plant from July to September is where fluorescence period and according to TERRAK (1987) [12] the plant decrease the production of phenolic compounds at the fluorescence stage.

According to Tissut (1968) [14] the end of leaves life is characterized by a strong production of flavonoids, and as Cynodon dactylon from October to December is a maturing stage, majority operations in the mature stage is the concentration of nutrients from parts of plants into grains, like sugar, amino acids, mineral salts transfer, then transform them into materials embedded in the grain tissues and in the stored image reserves during the silence for use in the

germination phase, At this point, photosynthesis continues to produce food as long as there is a green part of the plant tissue.

Especially since the plant produces certain compounds in specific periods and this may be due to hereditary factors, because the plant in the fluorescence stage of this period (climatic conditions such as temperature, precipitation, etc.) influences on the enzymes responsible for the production of flavonoids, which in turn attract insects for dispersal and pollination.

8. CONCLUSION

The results obtained give us an idea that the phenolic content of plants and influenced not only by extrinsic factor such as temperature, light, precipitation, soil etc… mais également affecté par des facteurs internes à la plante, tels que la floraison ou la fructification.

The same phenomenon of evolution of the phenolic content in the course of seasons was already by TERRAK 1987 [12] in the date palms.

Much research has been conducted on various medicinal plants to determine the most favorable conditions for obtaining a maximum yield of active principle.

We refer in this question to the report of the Wageningen Symposium in 1957 partially devoted to the influence of external factors as well as criticisms of Fluck, (1955) concerning the influence of soil and climate on the active principles of medicinal plants [6-8], in particular water stress which may affect the ratio of roots to aerial parts [32-33].

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