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# Quantitative analysis of different class of flavonoids content in *Cynodon Dactylon (L) Pers* growing at Ouargla region in the South-East of Algeria.

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**ABSTRACT**

Flavonoids compounds including: aglycones, anthocyanidins and c-glycosides where identified and quantified in *Cynodon Dactylon (L) Pers* contents. The material plant was extracted according to the method developed by Lebreton and collaborators and analyzed by both thin layer chromatography and UV-Visible spectrophotometer In addition to that, several chemical test were performed. The s content in material plant, showed that: leaves, stems and roots were reached of c- glycosides compounds. The c-glycosides were the most abundant compounds, where it's account about 83% of the total and more than 35 % of the c-glycosides were found in the leaves part of the *Cynodon Dactylon (L) Pers*.

**Keywords**: *Cynodon Dactylon (L) Pers*, flavonoids, aglycones, anthocyanidins, c- glycosides, TLC.

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## INTRODUCTION

The flora study is very important, where it allows knowing the main biological traits of plants and their biogeographical distribution [1]. However, several aspects of a considerable number of plant species remain unknown in some ways: biological, taxonomic and ecological [2-3]. The Sahara, which occupies 10% of the total Africa surface, is the largest hot desert in the world [4] and including many eco-regions. Among these eco-regions, who is in the northern Sahara, where rainfall occurs during the winter permitted the feeding a variety of plants that bloom before the hot and dry summer season. The flora of the northern Sahara is very poor because of the vastness of this area [5]. Algerian Sahara represent about 80% of the total surface, however a very little work related to the development of biological resources were recorded, where these latter are very original and representative of arid environments [6]. Sahara plant resources represent a flora of approximately 500 species of higher plants [5], which remains nowadays used by populations such as medicinal plants.

*Quack Grass*, also called in arabic "*Ael Kazmir*" consists of many styles House, showing a difference in the characteristics of plant flowering, wheat creeping, de grass two extremes, known by modern medicine, an effective analgesic for pain, and the liquid extracted from it is often used in the treatment of dysuria and inflammation in cases of relapsing to venereal diseases.

Three species of *Quack Grass* are knowing [7], the first specie is the *Repent Agropyrum*, the second is the *Triticum Repens* and the last specie is the *Cynodon Dactylon "L" Pers*, this latter is widespread in the south of France, Italy and North Africa [8], as it is in the Sahara of Algeria, the wadi beds and cosmopolitan cultures. *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 ingredients, what it owes its name to its instinctive use as a purgative from dogs and cats [9]. Furthermore, it is considered anti-inflammatory [10] diuretic, decongestant urinary tract and recommended in cases of nephrolithiasis "kidney or bile" [9-12] with potassium salts and essential oil it contains.

It is a great succor in cases of renal colic, especially if the treatment is started at the first signs of the crisis. It is recommended in the treatment of Diabetes and Rheumatism [13-14]. The plant extract also has significant application in dropsy and secondary syphilis, wounds and cardio protective [15-16], several compounds have been identified and from different morphological part of the *C. Dactylon*.

*Cynodon Dactylon (L).* as antiviral, antimicrobial [17], immunomodulatory activity [18] and has significant application in treating dysentery, dropsy [19], hypolipidemic and act as hypoglycemic agent [20].

According to the previous studies realized by Miller [21], 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* contains also: hydrocyanic acid, triticine, sugars and antibiotic gasoline.

Other important constituents reported in the previous studies for this plant were the flavonoids, which includ many compounds such as: apigenin, luteolin, orientin and vitexin [22-24]. Compounds are present in the plant as glycoside forms, when the saccharide part of this latter can be present as mono-, di- or tri-saccharidique forms. Generally, there are two types of glycosides form: the former is the o-glycoside form (Figure 2, compound number 8) and the latter is the c-glycoside [25].The connection between the genin (the aglycone) and the ose can be done by one of the phenolic hydroxyl of the genin but, generally mainly hydroxyl in position "7" of flavones (Figure 2, compound number1), and hydroxyl in position "3" of flavonols (Figure 2, compound number 5) which are involved, and that in the case of o-glycosides.

For The c-glycosides compounds, more than 300 compounds are knowing, the connection is established between the anomeric carbon of the sugar and carbon at position 6 or 8 of the genin [26].

Aglycones are the result of degradation of the o-glycosides, from this point we have studied the various existing classes in the plant *Cynodon dactylon "L" Pers.*

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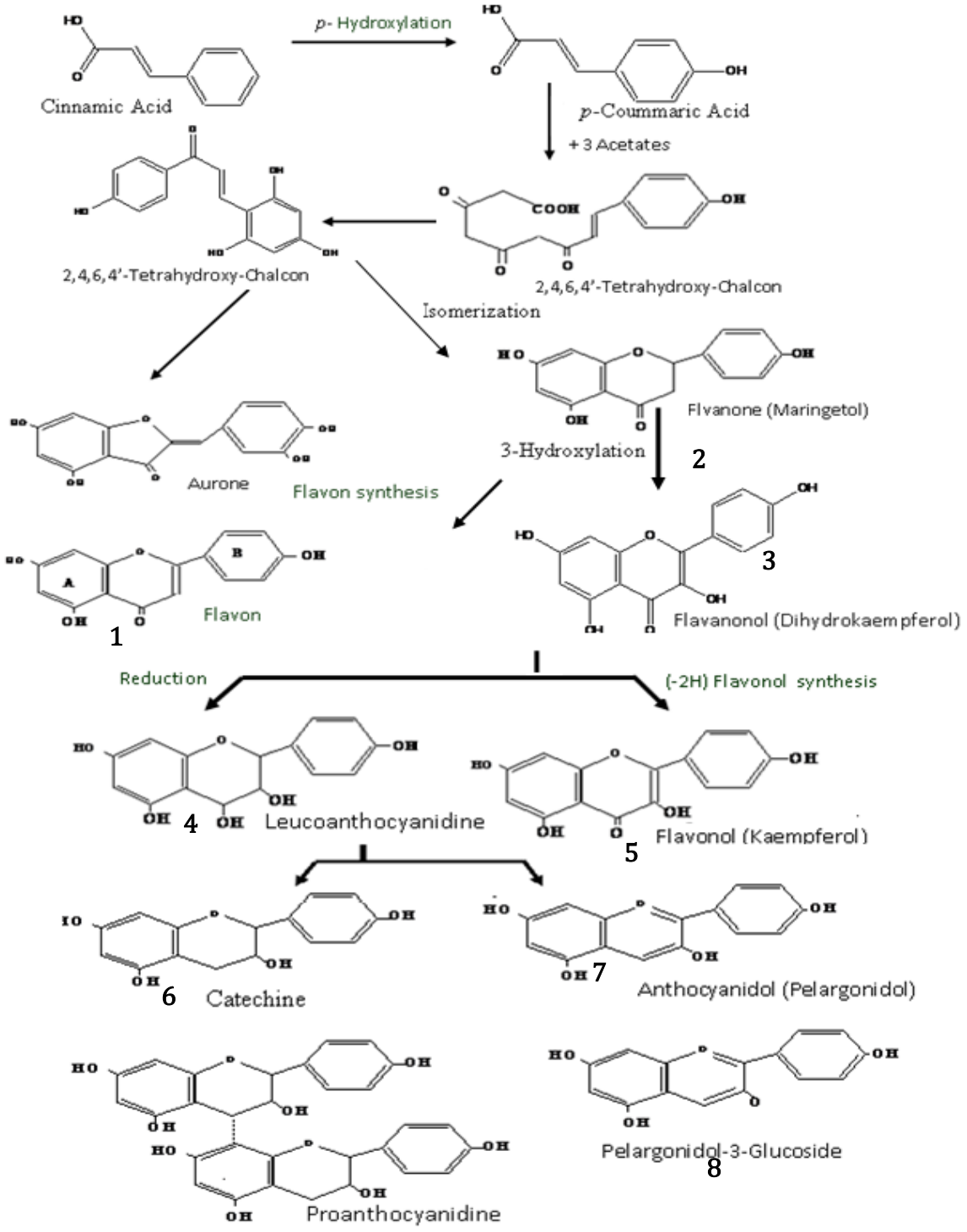
**Figure 2: Bio-synthesis way of flavonoids**




In addition to being more of the medicinal herb, Cynodon dacylon may be used alone as a substrate or co-substrate for aerobic digestion to produce biogas and then increase the recyclability of this biomass [27].

Due to the wide spread of this plant across the country, we have expressed interest in phenolic compounds (flavonoids and anthocyanins) that are most important compounds of this plant, where this study is focused on the quantitative analysis of the active substance.

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## MATERIALS AND METHODS

### Collection of plant material

The species has been collected in December 2009 from a big farm located at about three kilometers North-East of Ouargla City, this latter covers area of 360 $m^2$, and contains over 60 palm, the area is irrigated two hours by week. 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 [28], (see Figure 1).

**Figure 1: *Cynodon Dactylon (L)* growing at the region of Ouargla**

### Chemicals

All chemicals used were of analytical reagent grade. All reagents were purchased from Sigma-Aldrich, Biochem chempharma, Chromanorm and Riedel-de Haèn. hydrochloric acid (37%), acetic acid (100%), ethyl acetate ( 99,5%), methanol (99,7%), ethanol (96%), n-butanol (99,5%), diethyl ether (98%), aluminum trichloride (99.99%), quercetin (≥98%), sulfuric acid (99,5%), chloroform (99%), ferric chloride (99%), antimony trichloride (≥ 99), magnesium (99%), ammoniac (25%), sodium sulfate (99%), mercury chloride (≥99.0%), potassium iodide (≥99.5%) cyanidin (98%), orientin (97%) and procyanidin (90%).

### Materials

Analyses of flavonoids were performed using a UV-Visible spectrophotometer UNICAM with wavelength varies between 190 and 800 nm and a quartz cellule (1 x 4 cm), the ethanol was used for aglycones compounds and methanol for both anthocyanins and c-glycosides compounds.

Thin layer chromatography (TLC) was also used in this study for separation of different flavonoids classes. The stationary phase used was a commercial silica gel plate 60 with size of 10 x 10 cm and 0.375 mm thickness.

Mixture of dichloro ethane and ethanol (95/5: v/v) was used for elution the aglycones fraction and mixture of ethyl acetate, methanol and water (10/1.5/1: v/v/v) was used as mobile phase to elute the glycosides fraction. The revelation was made under the UV lamp at two wavelengths: λ= 254 nm and 366 nm were used for aglycones classe and λ = 366 nm was used for anthocyanins and glycosides.

### Extraction

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 [29].

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 ($AlCl_3$) 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) [30].

**CHEMICAL ANALYSIS**

**Chemical tests**

Several chemical tests were realized in order to prove the existence or not of different active ingredients inspected to be present in the different parts of plant (leaf “L”, stem “S” and root “R”) according to the methods described elsewhere [31-35].

For essential oil test, 50 g of each parts of the vegetable matter powder (L, S and R), were put into 30 containers, about 250-300 mL of distilled water was added, after that the filtrate was extracted with diethyl ether, then the ethereal solution was filtered with addition of dehydrated $Na_2SO_4$ and then the ether was evaporated at a room temperature, the residue was dissolved in water. The remaining solution was let stand until the appearance of yellow layer on the water surface indicates the presence of essential oils.

Two methods were applied for the cardenolides test, the farmer consist to the tedrose test, which involves taking 1 g of powder of each parts of the plant material, soaked in 20 ml of distilled water and filtered. 10 ml of the filtrate was extracted with 10 ml of chloroform and ethanol, the organic phase was evaporate and the precipitate was dissolve in 03 ml of glacial acetic acid, one drop of Ferric chloride solution was added followed by addition of 1 ml of sulfuric acid, the appearance of the green-blue color in the acid phase indicates the presence of cardenolides. the farmer method is the antimony trichloride test, which is realized by taken of 10 g of each part of the plant and extracted with 70% of ethanol, the alcoholic extract evaporated at low pressure in a water bath, heating a small amount of residue with trichloride of antimony, the absence of the deep purple color indicates the absence of cardenolides. The saponosids test, consist to take 2 g of dry powder of each part of plant, heated in 80 ml of distilled water, after filtration and cooling, the solution was agitated, the appearance of foam indicates the presence of saponosids.

**Tannins chemical test**

10 g of dry plant material (L, S and R) was taken for testing the presence of tannins compounds, after extraction with ethanol and filtration of the extract, few drops of ferric chloride solution was added, the appearance of the green color indicates the presence of tannins.

The unsaturated terpenes and sterols test was realized by taken 5 g of dry powder plant (L, S and R), after dissolved in 20 ml of chloroform, the results was filtered. The filtrate was added to 1 ml of sulfuric acid carefully on the tube walls. The appearance of the green color that turns thereafter in red or appearance of two phases of intersection indicates the presence of unsaturated sterols and terpenes.

For alkaloids test, 10 g of powder dry plant (L, S and R) was extracted by addition of 50 ml of hydrochloric acid diluted make the basic extract with ammoniac ($NH_3$) and then extracting the mixture three times with $CHCl_3$, 20 ml each time extraction was evaporate the organic layer, and then dissolve the precipitate in 2 ml of dilute HCl acid solution to add three drops of Mayer Reagent, the appearance of a white precipitate indicates the presence of alkaloids.

**Flavonoids chemical tests**

10g of the dry matter of the three parts of the plant (L, S and R), soaked in 150 ml of HCl (1%) overnight, after filtration, an aliquot of 10 ml of filtrate react with a basic solution of $NH_4OH$, the bright yellow color indicates the presence of flavonoids.

Another aliquot of 5 ml of filtrate was react with 2.5 ml of amyl alcohol, if the alcohol phase was turn to yellow color, it indicates that the presence of free compounds (aglycones), the aqueous phase was separate and evaporate under vacuum pressure, then dissolved the precipitate in 3 ml of HCl, two way are possible; first of all make to solution at hot temperature slightly, after cooling and added 2.5 ml of amyl alcohol, the appearance of the yellow color indicates the presence of flavonoids (glycosides). Secondly add some seed of magnesium to the acid extract, the absence of the red color indicates the absence of flavonoids (glycosides).

**Determination of different class of compounds**

**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 [29].

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

Where :

$\eta$ = 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).

**Determination of aglycones**

The differential assay of flavones and flavonols is performed based on the chelating properties of 1% $AlCl_3$ 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 [30].

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$$T\ (mg/g) = \left(\frac{\Delta(D.O)}{\varepsilon}\right) \times M \times \left(\frac{V}{P}\right) \times d \qquad (2)$$

Δ (D.O): optical density of differential peak ($D.O_{Al}^{3+}$ - $D.O_{EtOH}$)
$D.O(Al_3^+)$: optical density of the aglycones in the $AlCl_3$ 1% in EtOH solution
$D.O_{EtOH}$: 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

**Determination of c-glycoflavones**

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

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

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).

**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 [36] as follow:

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

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

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

**RESULT AND DISCUSSION**

The different color emitted by different flavonoids classes under the UV domain according to the results obtained by Ikan [37] are listed in the Table 1

**Table 1: Colors of the different flavonoids classes under the UV domain according to Ikan works [37].**

| Solution | Domain | Color | Flavonoid classes |
|---|---|---|---|
| No reagent | Visible | Orange, Purple | Anthocyanes |
| | | Yellow Shone | Chalcones, aurones |
| | | Light Yellow | Flavones |
| | UV (253 nm) | Black | Iso-flavones, flavone |
| | UV (300 nm) | Low Brown | Flavonol-3-glycosides, flavones |
| | | Yellow Shone | Flavonol with the 3-OH free |
| | | Yellow Green | Flavonol without OH at the position 5 |
| | UV | Blue | 3,5-methoxy flavonols |
| | | Mauve | The absence of free OH at the position 3 |

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The Table 2- shown the results of different chemical test determination of different flavonoid class which regrouped in the Tables 2 and 3.

**Table 2: Results of different chemical tests applied in this study**

| Chemical tests | Parts of the plant | | |
|---|---|---|---|
| | Leaves | Roots | Stems |
| Flavonoides | +++ | ++ | + |
| Aglycones | +++ | ++ | + |
| Glycosides | +++ | + | ++ |
| Alkaloids | + | ++ | +++ |
| Saponosides | - | - | - |
| Cardenolides | + | - | - |
| Tanins | +++ | + | ++ |
| Insatured sterols and terpenes | ++ | + | +++ |
| Essential oil | + | + | + |

**Table 3: Determination of different flavonoid class**

| Samples | Parts of the plant | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Leaves (mg/g) | | | Stems (mg/g) | | | Roots (mg/g) | | |
| | Aglycons | Anthocyanin | C-glycoflavones | Aglycons | Anthocyanin | C-glycoflavones | Aglycons | Anthocyanin | C-glycoflavones |
| Sample I | 0,21 | 1,85 | 9,00 | 0,07 | 1,16 | 7,91 | 0,19 | 0,27 | 7,31 |
| Sample II | 0,37 | 0,79 | 7,92 | 0,05 | 0,56 | 8,62 | 0,12 | 0,24 | 8,47 |
| Sample III | 0,24 | 1,50 | 7,24 | 0,14 | 1,25 | 5,85 | 0,15 | 0,41 | 7,05 |
| Average $\bar{T}$ | 0,27 | 1,38 | 8,05 | 0,09 | 0,99 | 7,46 | 0,16 | 0,30 | 7,61 |
| Standard deviation S | 0,07 | 0,44 | 0,72 | 0,03 | 0,30 | 1,17 | 0,03 | 0,07 | 0,62 |
| Dispersion coefficient CD | 25% | 31 % | 08% | 41 % | 30 % | 15% | 18% | 24% | 08 % |

While the Table 3 shown the results of determination of different flavonoid class which

The quantitative study of different flavonoid classes within parts of Cynodon dactylon shown that the leaves are the richest part of flavonoid compounds, it represent 0.27 ± 0.25, 1.38 ± 0.31 and 8.05 ± 0.08 for the aglycons, anthocyanin and C-glycoflavones, respectively (Figure 3).

**Figure 3: Flavonoid composition of different part of *Cynodon Dactylon***

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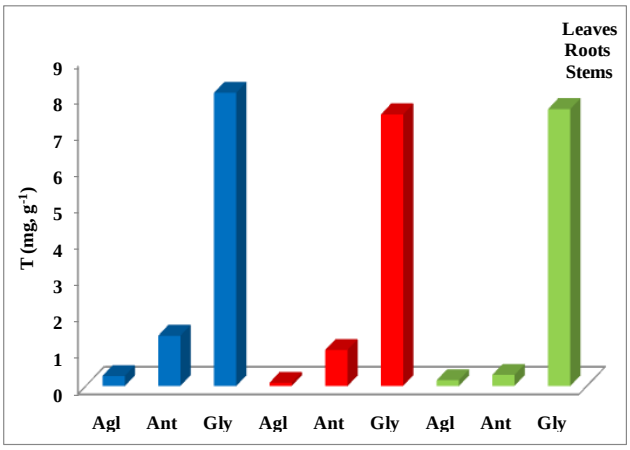


According to the previous study realized by Delaveu [38], the content of s is increasing in the same direction as the mass of vegetable matter, indeed the synthesis of s depends on photosynthesis and consequently is a function of the surface exposed to light.

As far as the determination of these classes leads us to conclude that the predominant part are C-glycosides, in agreement with the fact that most of the phenolic compounds are in the form of glycosides or esters in plants.

The retention factor ($R_f$) of eluted flavonoid compounds, separated by TLC in silica gel medea as stationary phase of different favonic classes are grouped on the Table 4. The mobile phase used is this study are mixture of dichloroethane-ethanol (95/5; v/v) for the aglycones classe, ethylacetate-methanol-water (10/1.5/1; v/v/v) for the anthocyanins and glycosides classes.

**Table 4: The retention factor ($R_f$) of different flavonoids classes**

| The extract aglycones[a] | | | | The extract anthocyanins | | | | The extract of the (c-, o-glycosides) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Color | $R_f$ (L) | $R_f$ (S) | $R_f$ (R) | Color | $R_f$ (L) | $R_f$ (S) | $R_f$ (R) | Color | $R_f$ (L) | $R_f$ (S) | $R_f$ (R) |
| Purple | 0.90 | 0.88 | 0.92 | Brown | — | 0.96 | 0.89 | R-Orange[b] | 0.97 | 0.97 | —0.96 |
| Y-Green | 0.72 | 0.71 | 0.73 | Purple | 0.80 | 0.83 | 0.82 | Yellow | — | —0,85 | 0.88 |
| Yellow | 0.53 | 0.51 | 0.52 | Yellow | — | —— | 0.75 | Yellow | 0.88 | —0.77 | 0.82 |
| Purple | 0.41 | 0.39 | 0.42 | Blue | 0.65 | 0.52 | 0.63 | Y-Green[c] | — | 0.74 | 0.76 |
| Orange | 0.36 | 0.34 | 0.33 | Y-Green | — | — | 0.50 | Purple | 0.77 | 0.69 | —0.69 |
| Yellow | 0.20 | 0.19 | 0.21 | Y-Blade | 0.32 | — | 0.33 | Y-clear | 0.73 | 0.63 | 0.59 |
| Blue | 0.11 | 0.12 | 0.10 | Green | 0.25 | 0.07 | — | Y dark | 0.68 | — | 0.49 |
| Y-Shone | 0.07 | 0.07 | 0.05 | Blue | 0.06 | 0.00 | 0.06 | Yellow | 0.62 | — | 0.42 |
| Brown | 0.00 | 0.00 | 0.00 | Y-Green | 0.00 | | 0.00 | B-Clear[d] | 0.54 | 0.29 | 0.31 |
| | | | | | | | | Yellow | 0.45 | —0.19 | 0.16 |
| | | | | | | | | B-Dark | 0.30 | 0.08 | — |
| | | | | | | | | Yellow | 0.17 | —0.00 | — |
| | | | | | | | | Purple | 0.13 | | 0.04 |
| | | | | | | | | Yellow | — | | 0.00 |
| | | | | | | | | Blue | 0.05 | | |
| | | | | | | | | Yellow | 0.00 | | |

[a]: For the aglycones we note that all organs of the plant contain almost the same chemical composition; [b]: R= Red; [c]: Y= Yellow; [d]:B = Blue

.

Roots are the richest part in anthocyanins, it contains 08 with colors and different $R_f$ product was observed that the leaves considered the richest part by the (c-glycosides and o-glycosides), contains 14 compounds with different frontal resolution and colors.

We can see the spots that appear in yellow, which are considered to be flavonols, light yellow spots for flavones, blue are flavonols methoxy and spots in purple are considered to be iso flavones, but we cannot obtained all separated flavonoids compounds by TLC.

In the previous study conducted by Benzahi [39] for the Cynodon Dactylon «L» Pers analysis by the high performance liquid chromatography chromatography (HPLC), showed the existence of some products in two extracts considered. The extract of aglycones contains products such as hesperetin, fisetin, luteolin and the extract of the heterosides contains: isorhamnetin and hesperidin. This result are also confirmed by several researchers [22- 24, 40], when they found that the plant of Cynodon Dactylon «L» Pers contains several compounds among them, the luteolin.

## CONCLUSION

This study concern the qualitative and quantitative analysis of Cynodon Dactylon (L) Pers growing at Ouargla region in South East of Algeria.

Three parts of Cynodon Dactylon (L) Pers such as: Leaves, stem and root have been testing by several physico-chemical tests and analyzed by spectrophotometer UV-Visible and thin layer chromatography.

Chemical test realized in this study shows that:

The plant content many different chemical classes of compounds such as: flavonoids, alkaloids, cardinoloids, tannins, unsaturated sterols, terpenes and essential oil.

Among these chemical compounds, flavonoids classes were the most abundant and the highest concentration was obtenaid for the leaves part of Cynodon Dactylon (L) Pers. Spectrophotometer UV-Visible and thin layer chromatography analysis has been confirmed that: aglycones, anthocyanins and c-glycosides were the most abundant flavonoids classes in the leaves part of the Cynodon Dactylon (L) Pers.

The Spectrophotometer UV-Visible and thin layer chromatography analysis shows that c-glycosides was the most abundant in different part of the plant (leaves, stem and roots), this fact was confirmed by Delaveu.

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