*Plant Archives* Vol. 20 Supplement 1, 2020 pp. 2160-2168 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

# USING LABORATORY METHODS TO DETECT SOME OF THE ACTIVE COMPOUNDS IN THE LEAVES AND ROOTS OF SENNA PLANT GROWING UNDER THE INFLUENCE OF WATER STRESS AND TREATING WITH SOME ANTIOXIDANTS

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## Abstract

The experiment was conducted in the lath house covered by saran belonging to preparatory school of agriculture in Abu Gharaq for the spring season 2017/2018. To detecting of some the active compounds in the leaves and roots of the growing Senna plant under the influence of water stress with irrigation periods (2, 4, and 6 days) and sprayed three antioxidants (vit. E 150 mg /L, salicylic acid 200mg/L , and selenium 173 mg/L).as well as the control treatment. The experiment was conducted as a factorial experiment (3×4) according to Randomized Complete Block Design (RCBD) with three replicates, thus each replicate contained 12 treatments with six plants per experimental unit and the averages were compared according to the Dunkin test. The results of the study showed an increase in the amount of caffeine in the leaves and roots of the Senna plant when Dehydration plants 4 days, while the spraying salicylic acid led to an increase in caffeine in the roots of the plant Senna. As for Dehydration plants to water them every 6 days, they led to an increase in caffeic acid in the leaves and roots of Senna plants, while salicylic acid helped increase the concentration of caffeic acid in the plant roots. Some methods of laboratory detection have also been studied to know the plant contents of the active substances.

***Key words:*** laboratory methods, Senna, water stress, antioxidants

## Introduction

Most plants are an important food source, in addition to their high nutritional value because they contain energy source of carbohydrates, proteins and fats [Khasawneh *et al.,*1980] It has a therapeutic medical benefit as it has a role in treating the most pathological conditions [Ajagbonna *et al.,* 2000], including asthma, bronchitis, and skin sensitivity [Alamery *et al.,* 2009] and there are many plants such as black seed, ginger, cumin and Ricinus [Shihab *et al.,* 1978] that have been studied as medicinally active plants because they contain chemical compounds It has a clear biological activity against various pathogenic bacteria and fungi. Senna (*Cassia occidentalis*) is a Herbaceous plant belonging to the Fabaceae family, The genus Cassia contains about 500 species, most of which are used for medicinal purposes or to decorate streets and parks due to their abundant yellow flowers. Senna leaves, fruits and roots contain anthraquinone glycosides and their derivatives that consist of Aloe-emoidin and Rhein (resins), both of which are a free or linked form, they form various glycosides forms or images. Commercially sold leaves contain 2-3% of glycoside A and B together and 2-4% of glycoside C. Senna leaves and fruits also contain resinous materials, To which the mild colic accompanies the work of Senna. In general, the Senna is used as a stimulant of the muscular layer of the intestinal wall, so it is used as a Laxative, and the Senna is one of the preferred laxatives for the treatment of chronic constipation. Senna differs from other plants such as Ricinus in its less trace of headache or colic when used [Al-Zubaidi *et al.,*1966].

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## Meterials and Methods

The experiment was conducted in the lath house covered by saran belonging to preparatory school of agriculture in Abu Gharaq for the spring season 2018-2017 where ten leaves were taken before flowering and their fresh weight was measured first, then dried in the electric oven at a temperature of $70^0$C for 48 hours and until the weight stabilized.

**Table 1:** components of the chromotographic separation system.

| | Component | Model or version | Company and origion |
|---|---|---|---|
| 1 | Binary high pressure gradient pump | P6.1L | Knuaer, Germany |
| 2 | Diode array detector | DAD 2.1L | Knuaer, Germany |
| 3 | Sample loop (20 µl) and injector | D1357 | Knuaer, Germany |
| 4 | Analyses and system control software | Claritychrom , V 7.4.2.107 | Dataapex, Czech Republic |

The separation was achieved on a C18 column with a length of 250 mm and a diameter of 4.6 mm and a size of 5 µm

## High-performance liquid Chromatography (HPLC)

Chromatography is the most important technique in the chemical separation of materials, the most common in different industries and different fields of research. Where the chemical compounds to be separated are dissolved in a solvent, and this mixture is inserted into the carrier phase and depending on the nature of the particles, they interact more or less with the fixed phase in the tube called the chromatography column.

High-performance liquid Chromatography(HPLC) It is a method that mixes what is physical and what is chemical and depends mainly on the difference and diversity in the interactions between the solute, the carrier phase and the stable phase. As a result of these interactions the required separation occurs.

## Chromographic system:

The chromatography is separated by a system consisting of the components described and their specifications in table 1.

## Plant extraction preparation

The aqueous methanol extract of Liquorice was extracted according to method [Shihata.1951] with some modification as follows:

The roots and dry leaves of the Senna plant were milled separately and taken a certain weight from them and mixed with the solvent consisting of (20% absolute methanol: 80% distilled water) Mix in an electric mixer for half an hour, filter the resulting solution with filter paper, and put the filter in a 50$^0$C electric oven for 24 hours to obtain the dry extract. Keep in a dark bottle at 4$^0$C until use.

**Table 2:** the effect of water stress and some stress resistors on caffeine (µg.g$^{-1}$) in Senna leaves.

| (A) stress resistorsMg.L$^{-1}$ | | | | | |
|---|---|---|---|---|---|
| **Average (W)** | **selenium 173(A3)** | **vitamin E 150(A2)** | **salicylic acid (A1)200** | **Distilled water (A0)** | **water stress (W)** |
| 0.87B | 0.97C | 0.87CD | 1.31B | 0.76CD | irrigation every two days(W0) |
| 0.76B | 0.97C | 0.76CD | 0.68CD | 0.61D | irrigation every 4 days(W1) |
| 2.20A | 1.30B | 0.65CD | 1.42B | 5.42A | irrigation every 6 days(W2) |
| | 0.59C | 0.77C | 1.14B | 2.26A | Average(A) |

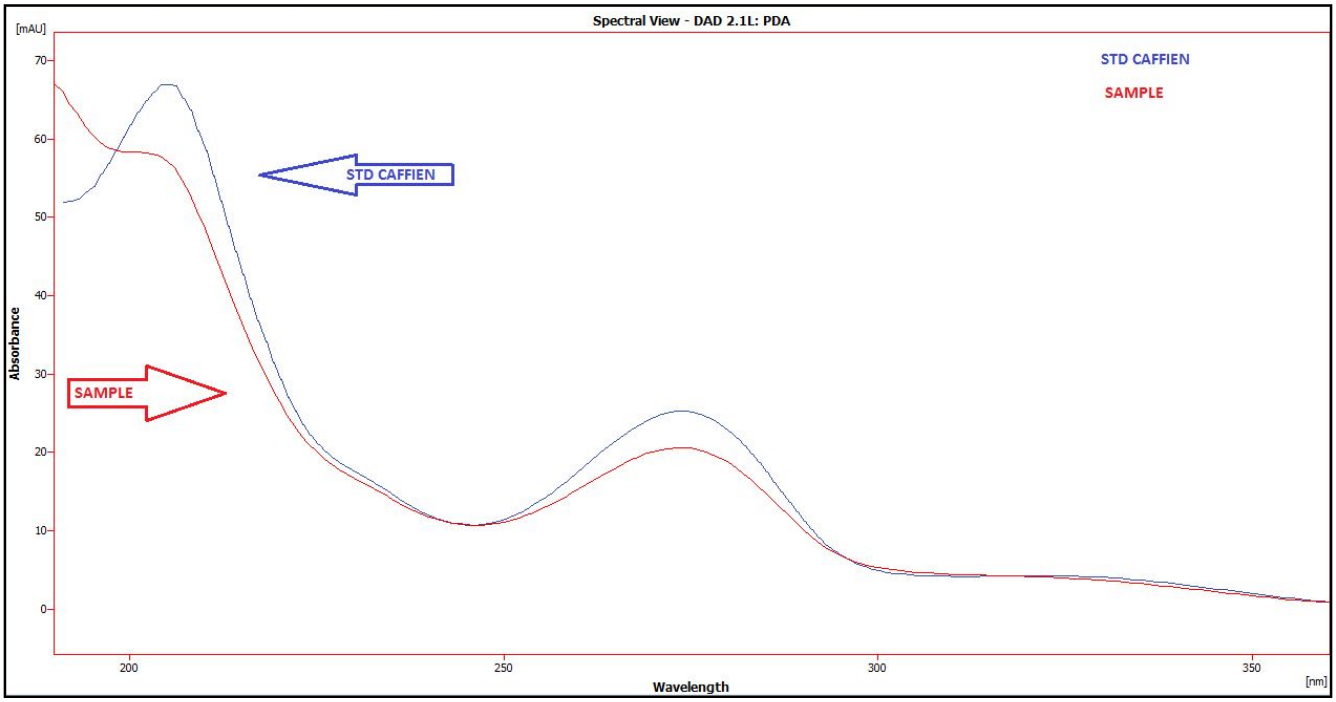



**Fig. 1:** diagram for measuring caffeine in the HPLC.

**Table 3:** The effect of water stress and some stress resistors on caffeine (µg.g$^{-1}$) in the roots of the Senna plant.

| Stress resistors(A) Mg.L$^{-1}$ | | | | | |
|---|---|---|---|---|---|
| **Average (W)** | **selenium 173(A3)** | **vitamin E 150(A2)** | **salicylic acid (A1)200** | **Distilled water (A0)** | **water stress (W)** |
| 0.77C | 0.63F | 0.24H | 1.75B | 0.48G | irrigation every two days(W0) |
| 0.97A | 0.68F | 0.26H | 2.71A | 0.23H | irrigation every 4 days(W1) |
| 0.82B | 1.11C | 1.02D | 0.79E | 0.34H | irrigation every 6 days(W2) |
| | 0.81B | 0.51C | 1.75A | 0.35D | Average(A) |

**Table 4:** The effect of water stress and some stress resistors on caffeine acid (ig .g$^{-1}$) in the leaves of Senna plant

| Stress resistors (A) Mg.L$^{-1}$ | | | | | |
|---|---|---|---|---|---|
| **Average (W)** | **selenium 173(A3)** | **vitamin E 150(A2)** | **salicylic acid (A1)200** | **Distilled water (A0)** | **water stress (W)** |
| 6.94B | 1.62GH | 3.44F | 4.85E | 17.86A | irrigation every two days(W0) |
| 3.07C | 1.03H | 2.15G | 5.09D | 4.03E | irrigation every 4 days(W1) |
| 7.14A | 4.64E | 7.06C | 5.79D | 11.08B | irrigation every 6 days(W2) |
| | 2.43D | 4.22C | 5.24B | 10.99A | Average(A) |

**Table 5:** The effect of water stress and some stress resistors on caffeine acid (µg.g$^{-1}$) in the roots of the Senna plant.

| Stress resistors (A) Mg.L$^{-1}$ | | | | | |
|---|---|---|---|---|---|
| **Average (W)** | **selenium 173(A3)** | **vitamin E 150(A2)** | **salicylic acid (A1)200** | **Distilled water (A0)** | **water stress (W)** |
| 5.57B | 3.42E | 0.60G | 16.82A | 1.42F | irrigation every two days(W0) |
| 5.16B | 1.41F | 5.29D | 10.98C | 2.98EF | irrigation every 4 days(W1) |
| 7.74A | 6.17D | 10.66C | 12.33B | 1.81 | irrigation every 6 days(W2) |
| | 3.67C | 5.51B | 13.38A | 2.07D | Average(A) |

**Characterization of the plant extract using thin layer chromatography(TLN)**

The plant extract was described using a mixture of different organic solvents with different volumetric ratios. To obtain the best results, several different systems of organic solvents were tried and the best of these systems are:

1- Chloroform: ethyl acetate. (20:80 (v / v)
2- Methanol: Ethyl acetate: distilled water. (20:60:20 v / v / v).
3- Chloroform: hexane: absolute ethanol. (1: 1: 1 v / v / v).

Activated the Silica Gel sheets (Class TLC with 0.25mm, 20 × 20cm, Albet, Germany) by placing them in the oven at 105$^{0}$C for one hour and then placing approximately (100, 150, 200, 250) lµ about 2cm from the base from the Neighbour Footings and left to dry and then conducted a light examination WV 345 UV and UV

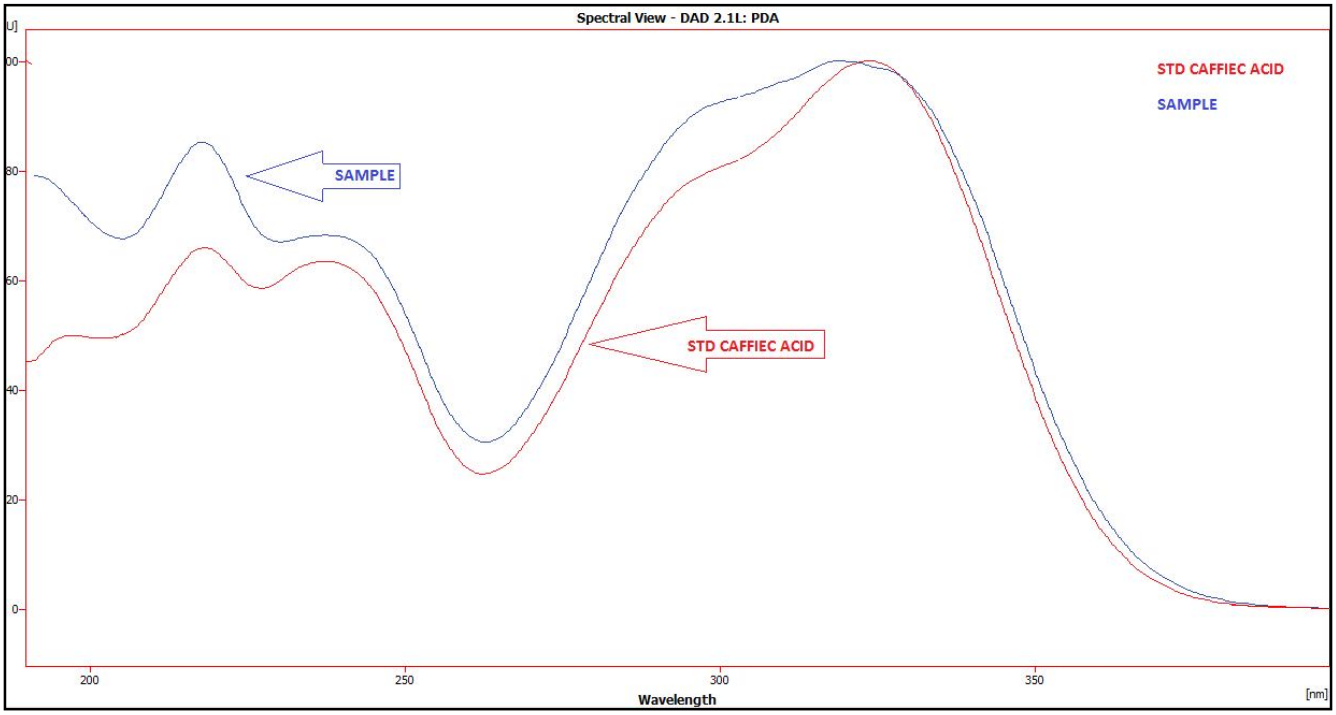



**Fig. 2:** Diagram for measuring caffeic acid in the HPLC

radiation, as the Rf Retardation Factor is defined for the constituent beams in addition to the number and colour of those beams [Vekiari *et al.,* 1993].

$$Rf = \frac{The\ distance\ that\ bundle\ was\ passed\ through}{The\ distance\ that\ phase\ was\ passed\ through}$$

**Methods of detection of some phytosanitary compounds (qualitative disclosures)**

**Detection of Alkaloid**

Mix 10 mg of vegetable extract with 50 ml of distilled acidified water with 4% HCL and put in a boiling water bath for 10 minutes, filter the resulting solution and then mix 0.5 ml of the filtrate with: The Mayer reagent indicates the appearance of a white precipitate on the positivity of the detection. Wackner Detector, The appearance of a brown precipitator indicates the positivity of detection.

**Detection of glycosides**

Mix 1ml of vegetable extract with 500 lμ of Benedict reagent and put in a boiling water bath for 10 minutes as the appearance of a red precipitate indicates a positive detection [23].

**Detection of Tannines**

Adding a solution of lead acetate 1% to 5 ml of the plant extract, as the appearance of a gel precipitate indicates a positive detection [Veerachari and Bopaiah. 2012].

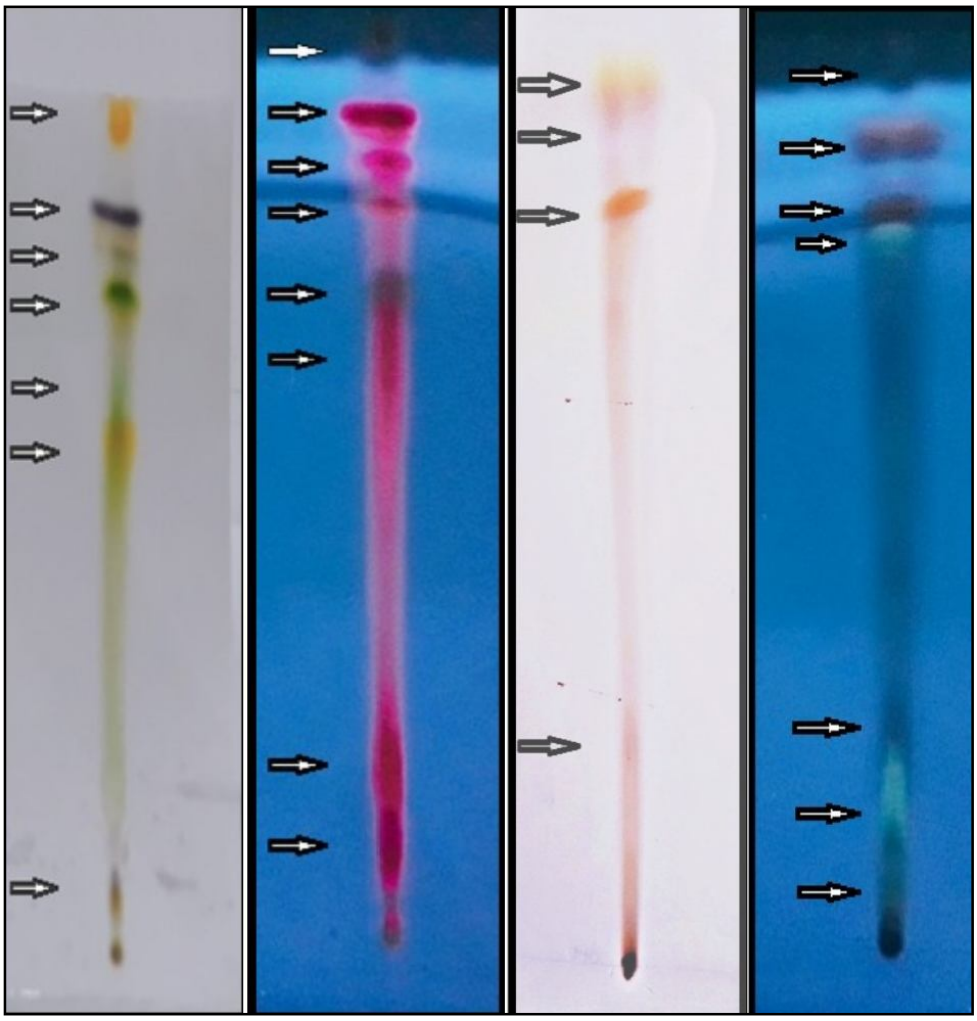
**Detection of Saponin**

Exposed by:

1. Shake a container of 5 ml of vegetable extract, as the appearance of foam indicates the positive of detection.
2. Add 3 ml of mercuric chloride 1% to 5ml of plant extract as the appearance of a white precipitate indicates positive detection [Veerachari and Bopaiah.2012].

**Detection of phenols**

A filter paper was moistened with vegetable extract and drops of ferric chloride were added, as the appearance of a bluish-green color indicates a positive detection [Indian herbal pharmacopeias. 1998].



**Detection of Flavonoids**

Two equal sizes of solutions were mixed below, as the appearance of a yellow color indicates a positive detection.

1. Mix 10 ml of plant extract with 5ml of 95% ethanol and filter the solution.

2. Mix 10ml of 50% ethanol with 50% KOH [Khasawneh *et al.,* 1980].

**Detection of coumarin: Detection of Coumarins**

The filter paper covered with NaOH was covered with a 5ml container of vegetable extract and placed in a water bath at $100^0$C for 10 minutes, then the filter paper was exposed to the UV source, the appearance of yellow color indicates the positive of detection [Harbone,1984].

**Detection of Resins**

**Table 6:** Characterization of the bundles formed on the TLC leaves of the aqueous extract of the Senna leaf using the organic solvent (chloroform: ethyl acetate) (80: 20V / V).

| Extract for the leaf | | | |
|---|---|---|---|
| Number of bundles | bundle properties | | Method of examination |
| 7 | Rf value | color | Visible |
| | 0.08 | Dark brown | light |
| | 0.48 | yellow | examination |
| | 0.63 | light green | |
| | 0.67 | dark green | |
| | 0.70 | green | |
| | 0.72 | Purple | |
| | 0.81 | orange | |
| 8 | 0.15 | dark pink | Ultraviolet |
| | 0.21 | dark pink | examination |
| | 0.27 | dark pink | |
| | 0.36 | Gray | |
| | 0.77 | light pink | |
| | 0.87 | light pink | |
| | 0.94 | dark pink | |
| | 0.96 | Dark gray | |

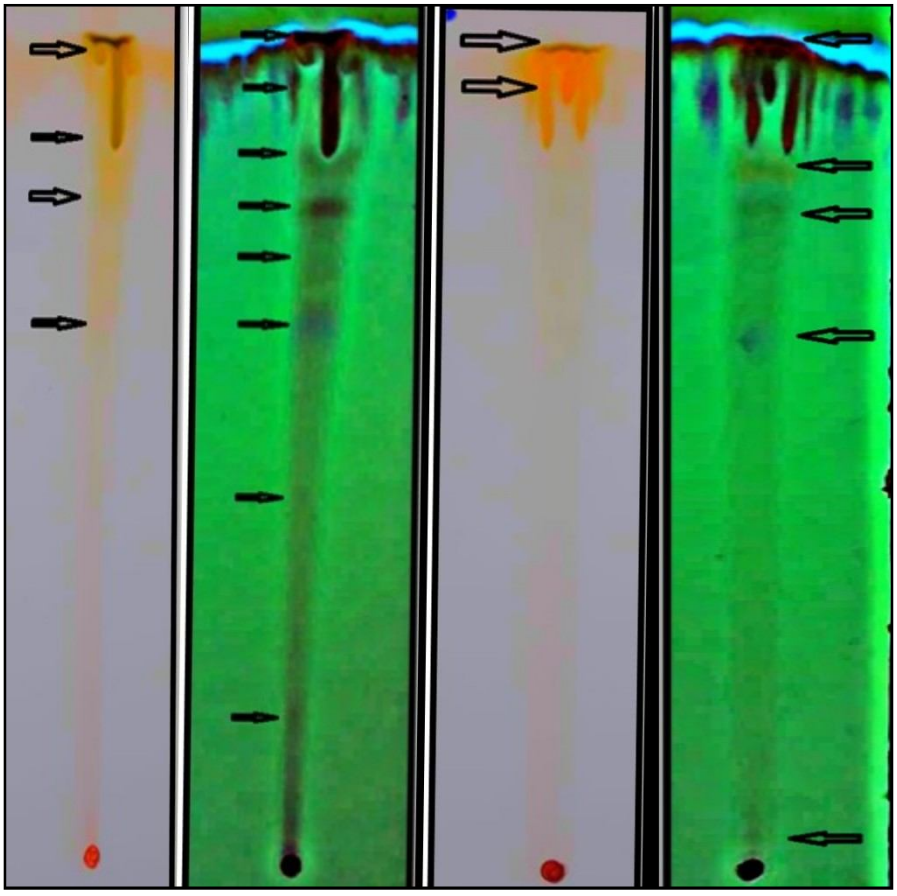


**Fig. 4:** shows the characterization of the aqueous methanol extract of the Senna plant on TLC sheets using an organic solvent (distilled water: chloroform: methanol) (60:20: 20V / V / V).

Mix 50 ml of 95% ethanol with 10 mg of vegetable extract and put in a water bath at 50$^0$C for 10 minutes, filter the solution and add to it 100ml of distilled acid with HCL 4%. The appearance of turbidity is evidence of positive detection [Veerachari and Bopaiah, 2012].

### Detection of Volatile Oil

Volatile oils were detected according to [Jaffer *et al.,* 1983]. The filter paper saturated with the vegetable extract was shown to ultraviolet light. The bright pink color indicates the positive of the detection.

### Detection of steroid and Terpenes

Mix 1mg of the extract 2 ml of chloroform and a drop of snowy acetic acid, then add a drop of $H_2SO_4$ acid. The appearance of lead color indicates that the extract contains turbines and the emergence of a blue color for the mixture later evidence of the presence of steroids [Al-Shami.1982].

## Results

### Caffeine estimating :

#### Estimating of caffeine in Senna leaves (µg.g$^{-1}$)

The results in Table 2 show that there was a significant difference between irrigation periods on the concentration of caffeine in the leaves where The irrigation every 6 days was distinguished an increase in the percentage of caffeine in the leaves amounted to 2.20 µg.g$^{-1}$ and spraying was excelled by different experiment factors in the presence of significant differences in the percentage of caffeine in the leaves where they were distinguished The control treatment with the highest caffeine content in the leaves amounted to 2.26 µg.g$^{-1}$ compared to the lowest caffeine in the leaves when sprayed with selenium and vitamin E amounted to 0.59 and 0.77 µg.g$^{-1}$, As for the water stress experience interaction factors, the W2A0 interaction treatment was excelled by giving it the highest the percentage of caffeine in the leaves amounted to 5.42 µg.g$^{-1}$, compared to the lowest percentage of caffeine in the papers at the treatment W1A0 of 0.61 µg.g$^{-1}$.

#### Estimating of caffeine in the roots of Senna (µg.g$^{-1}$)

**Table 7:** Characterization of the bundles formed on TLC root of the aqueous extract of the root of the Senna plant using an organic solvent (chloroform: ethyl acetate) (80: 20V / V).

| Extract for the root | | | |
|---|---|---|---|
| **Number of bundles** | **bundle properties** | | **Method of examination** |
| 4 | Rf value | color | Visible |
| | 0.16 | light brown | light |
| | 0.63 | orange | examination |
| | 0.66 | violate | |
| | 0.74 | Light yellow | |
| 9 | 0.15 | blue | Ultraviolet |
| | 0.21 | white | examination |
| | 0.27 | blue | |
| | 0.36 | white | |
| | 0.77 | black | |
| | 0.87 | blue | |
| | 0.94 | blue | |
| | 0.95 | white | |
| | 0.98 | blue | |

**Table 8:** Characterization of the bundles formed on the TLC leaf of the aqueous extract of the Senna leaf plant using an organic solvent (distilled water: chloroform: methanol) (60:20: 20V / V / V).

| Extract for the leaf | | | |
|---|---|---|---|
| **Number of bundles** | **bundle properties** | | **Method of examination** |
| 4 | Rf value | color | Visible |
| | 0.70 | Light yellow | light |
| | 0.82 | dark yellow | examination |
| | 0.89 | yellow | |
| | 0.94 | brouwn | |
| 8 | 0.07 | light black | Ultraviolet |
| | 0.11 | light black | examination |
| | 0.32 | blue | |
| | 0.67 | black | |
| | 0.75 | dark black | |
| | 0.80 | black | |
| | 0.84 | dark black | |
| | 0.90 | dark blac | |

**Table 9:** Characterization of the bundles formed on the TLC root of the aqueous extract of the root of the Senna plant using an organic solvent (distilled water: chloroform: methanol) (60:20: 20V / V / V).

| Extract for the root | | | |
|---|---|---|---|
| **Number of bundles** | **bundle properties** | | **Method of examination** |
| 2 | Rf value | color | Visible light |
| | 0.91 | Light orange | examination |
| | 0.97 | light brown | |
| 5 | 0.08 | light black | Ultraviolet |
| | 0.75 | blue | examination |
| | 0.83 | Gray | |
| | 0.89 | light brown | |
| | 1.00 | light black | |

The results in Table 3 show a significant difference between irrigation periods on the concentration of caffeine in the roots, as irrigation every 4 days, was excelled an increase in the percentage of caffeine in the roots amounted to 0.97 µg.g$^{-1}$ compared to the lowest percentage of caffeine when irrigation every 2 days amounted to 0.77 µg.g$^{-1}$, Spraying with different experiment factors was significantly excelled in the percentage of caffeine in the roots, where spraying with salicylic acid was excelled which gave the highest percentage of caffeine in the roots amounted to 1.75 µg.g$^{-1}$compared to the lowest percentage of caffeine in the roots when the control treatment amounted to 0.35 µg.g$^{-1}$. As for the water stress experiment factors interaction, the W1A1 interaction treatment was excelled by giving it the highest percentage of caffeine in the roots amounted to 2.71 µg.g$^{-1}$ compared to the lowest caffeine in the roots at the treatment W0A2 at 0.24 µg.g$^{-1}$.

**Estimating of caffeic acid**

**Estimating of caffeic acid in Senna leaves (µg.g$^{-1}$)**

The results in Table 4 show that there was a significant

**Table 10:** results of qualitative analysis disclosure of Senna leaves and roots

| **The material that requires to disclosed** | **The test used** | **The results of test** | **The extract test results** | |
|---|---|---|---|---|
| | | | **leaves** | **roots** |
| Alakaloids | Wagner | Brown precipitate | ++ | ++ |
| Essential oil | Uv light | Orange Color | + | + |
| Phenols compund | Ferric chloride | Yellow Color | ++ | + |
| Glycosides | Benedict | Red precipitate | ++ | + |
| Flavonoids | Ethanol (50%)+ KOH (50%)(1:1) | Yellow Color | + | + |
| Saponins | Shaken | Formation of foam | ++ | +++ |
| Terpenes | Chloroform + acetic acid + $H_2SO_4$ | Blue Color | + | + |
| Coumarins | Uv light | Bright pink Color | + | + |
| Tannines | Lead acetate 1% | White Gelatinous pellet | + | + |
| Resin | HCl4% | Turbidity | + | ++ |
| steroids | Chloroform + acetic acid + H2SO4 | gray color | - | - |

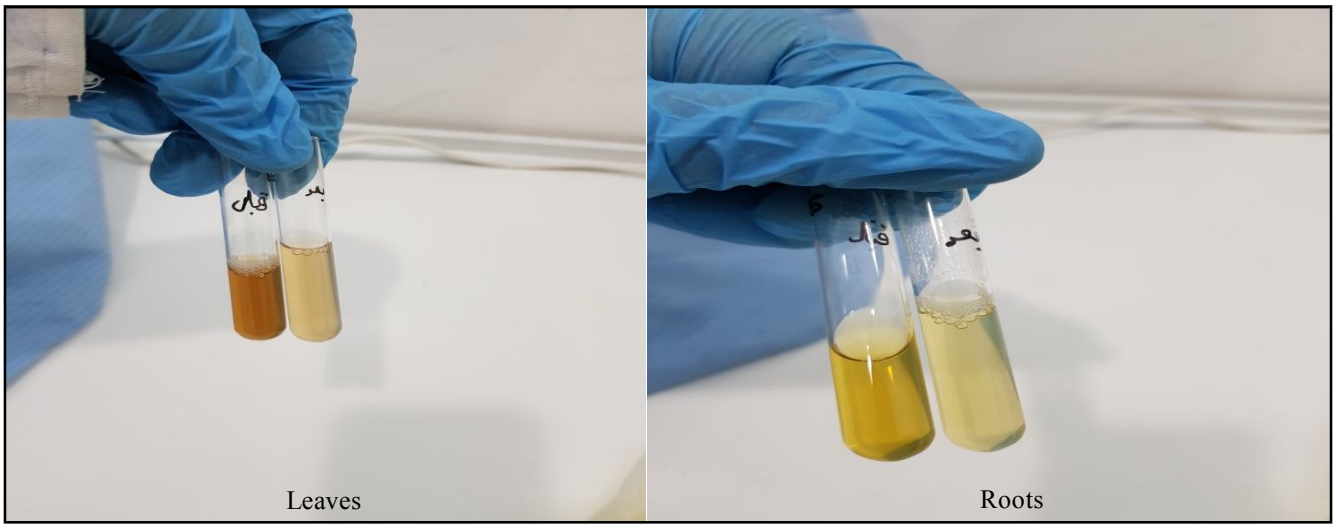



**Fig. 5:** detection of flavones

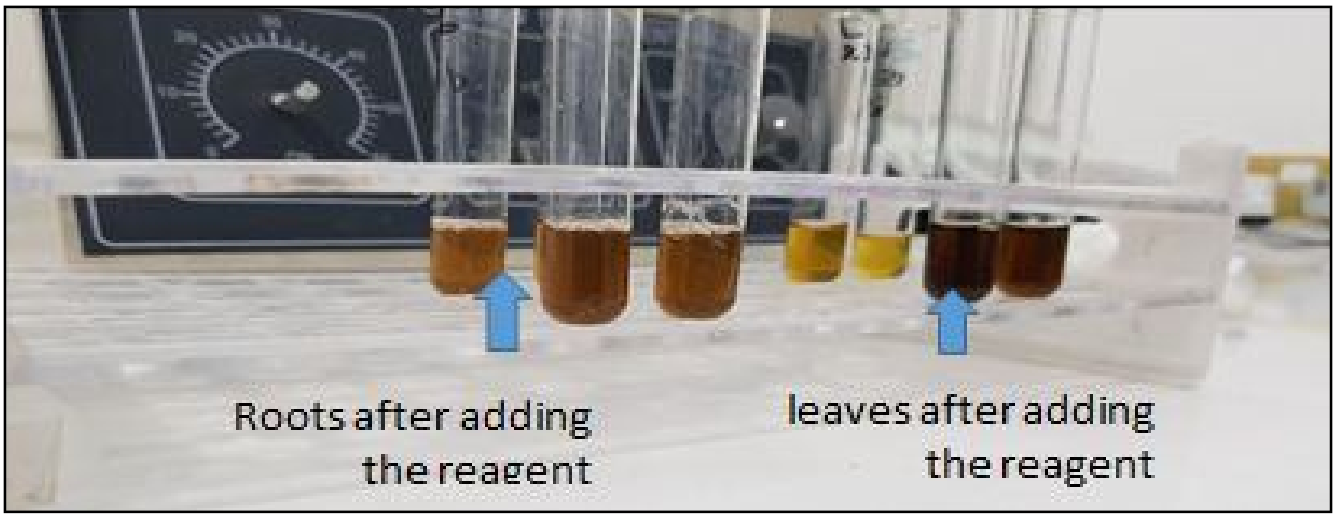



**Fig. 6:** The material that requires to disclosed

difference between irrigation periods on the concentration of caffeic acid in the Senna leaves, where irrigation every 6 days was excelled an increase in the percentage of caffeic acid in the leaves amounted to 7.14 $\mu g.g^{-1}$ respectively, Spraying with different experiment factors was excelled, in that there were significant differences for the percentage of caffeic acid in the roots, where the control treatment was excelled by the highest percentage of caffeic acid in the leaves amounted to 10.99 $\mu g.g^{-1}$. Compared to the lowest percentage of caffeine in the roots when treating selenium spray amounted to 2.43 $\mu g.g^{-1}$. while the water stress experiment factors, the interaction treatment W0A0 was excelled by giving it the highest percentage of caffeic acid in the leaves amounted to 17.86 $\mu g.g^{-1}$, compared to the lowest caffeine percentage in the leaves at the treatments W0A3 and W1A3 amounted to 1.62 and 1.03 $\mu g.g^{-1}$, respectively.

**Estimating of caffeic acid in the roots of Senna ($\mu g.g^{-1}$)**

The results in table 5 show a significant difference between irrigation periods on the concentration of caffeic acid in the roots, where irrigation every 6 days was excelled an increase in the percentage of caffeic acid in the roots amounted to 7.74 $\mu g.g^{-1}$ compared to the lowest percentage of caffeic acid when irrigation every 2 days and every 4 days amounted to 5.57 and 5 .16 $\mu g.g^{-1}$, respectively, Spraying by different experiment factors, that there were significant differences for the percentage of caffeic acid in the roots, where Spraying by salicylic acid was excelled which gave the highest percentage of caffeic acid in the roots amounted to 13.38 $\mu g.g^{-1}$. As for the water stress experiment interaction, the treatment W0A1 was excelled by giving it the highest percentage of caffeic acid in the roots amounted to 16.82 $\mu g.g^{-1}$, compared to the lowest percentage of caffeine in the roots when treatment W0A2 amounted to 0.60 $\mu g.g^{-1}$.

**Characterization of the Senna plant extract using thin layer chromatography (TLN)**

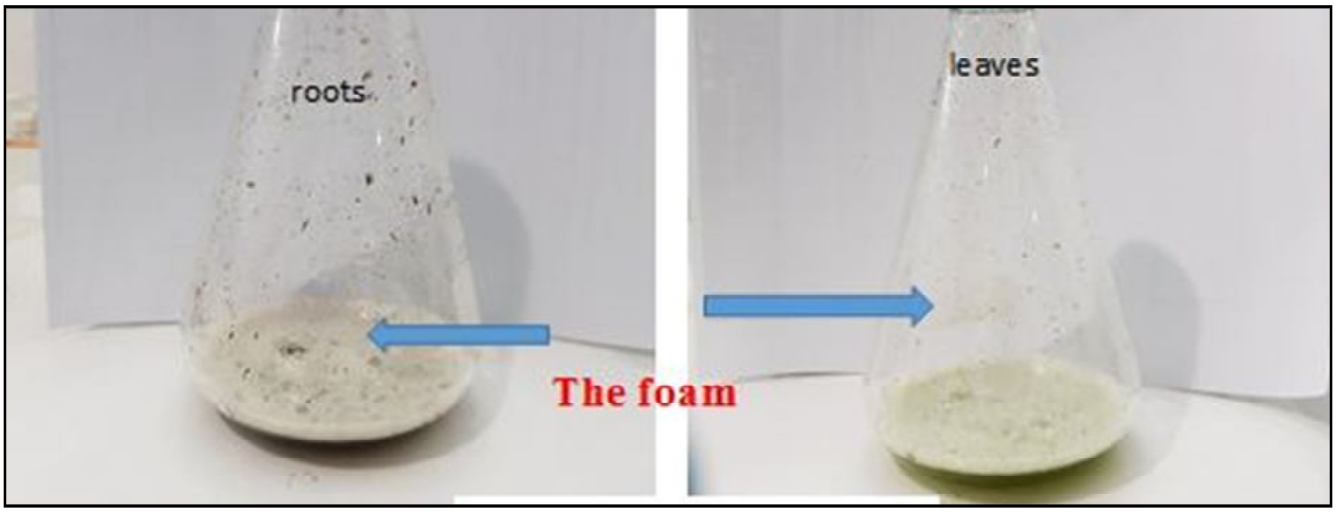



**Fig. 7:** detection of soaps

Fig. 3, 4 show the results of migrating the aqueous extract of the Senna plant on the thin layer plates when examining with visible light and ultraviolet rays. Tables (6, 7, 8, 9) show the characteristics of the beams, their number and the handicap factor Rf for each of them. Where it was observed when using the organic solvent (chloroform: ethyl acetate) (80: 20V / V), the visual light examination showed 7 paper bundles and 4 roots. The value of the handicap Rf ranged between (0.8 - 0.81) and (0.16 - 0.74), respectively.When examining the ultraviolet, 8 paper bundles and 9 root bundles appeared, the values of the handicap Rf ranged between (0.15 - 0.96) (0.15 -0.98), respectively.

As for the organic solvent (distilled water: chloroform: methanol) (60:20: 20V / V / V), visible light showed 4 paper bundles and 2 roots. The value of Rf ranged between (0.70-0.94) (0.91-0.97), respectively and 8 paper bundles And 5 for the root when examining ultraviolet radiation was the value of Rf between (0.07-0.9) (0.08-1.0), respectively

(1/3) Visual examination

(2/4) UV examination

The arrows indicate the locations of the bundle Figure (4) shows the characterization of the aqueous methanol extract of the Senna plant on TLC leaf using an organic solvent (distilled water: chloroform: methanol) (60:20: 20V / V / V)

(1/3) Visual examination

(2/4) UV examination

The arrows indicate the locations of the bundle

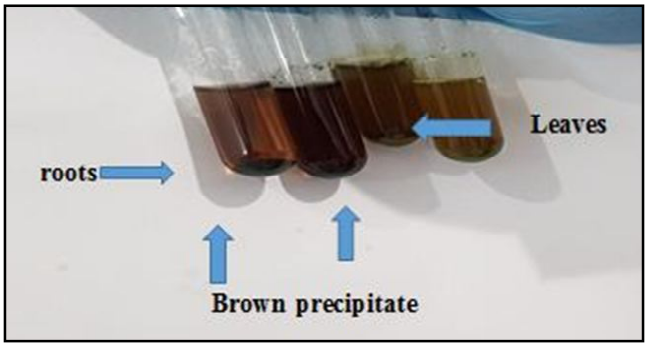



**Fig. 8:** detection of alkaloids (Wackner reagent)

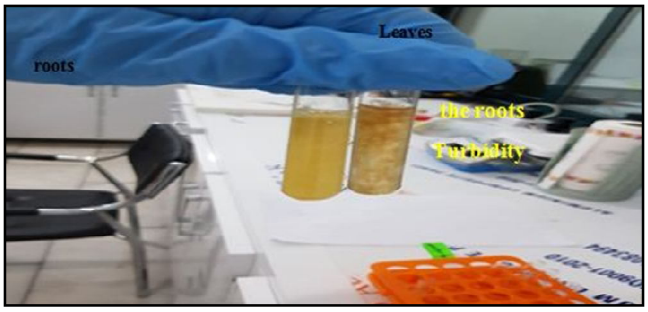



**Fig. 9:** detection of resins

**Qualitative tests related to aqueous methanol extract of Senna leaves and roots:**

After performing a set of qualitative tests on the aqueous extract of each of the leaves and roots of the Senna plant, it was found that they consist of several different chemical compounds as shown in Table 10.

## Discussion

The results of the migration of the aqueous methanolic extract of the leaves and roots of Senna using different systems of organic solvents and with different volumetric ratios show a relatively difference in the number of bundles and in the values of Rf, which indicates that the extract consists of a number of different polar chemical compounds where it was proven [Sato, 1990 ; Diogo *et al.,* 2007] Different parts of the plant contain different chemical groups that include alkaloids, anthocyanides,

phenols, proteins, phosphatins, steroids, tannins, flavonoids, anthroquinones, saponins, terpenes, resins, balsam, amino acids, glycosides carbohydrates [AL-Maisary,1999]. The Senna plant also contains the following compounds: acrosine, alloimodine, imodine, intron, epigenine, aurantectin, campstrol, kasolin, chryso-aptosine, chrysophanic acid, chrysaropine, chrysophanol, chrysoriol, rhine, iseimer, 1, 8- methyl Anthraquinone, 1, 4, 5-trihydroxy-3-methyl-7-methoxy anthraquinone, caciocidantaline A, B and C, acrosine, xanthurine, aurantisozin, cambisterol, cassolin, chrysoisericin, chrysol, galactopranosil, helmilinethrosporin, helminthenosporin, helminthenosporin, helminthenosporin, helmethineperosin, hydrophenesporin Linoceric acid, linoleic acid, acid For linolenic, sitosterol, mannitol, Manopejanorossel, Matiosenaul, Ooptossifolin, Oobtosen, oleic acid, as Cassia occidentalis extract contains total flavonoids 3.24 mg/g, carotenoids 2.9 mg / g and Alfinolex total of 6.7 micrograms [Daniyan *et al.,* 2011]. When using HPLC technology in the analysis of the aqueous extract of the leaves and roots of the Senna plant, it contains real and unreal tannins, including caffeine, caffeine acid and other compounds,Also, some qualitative disclosures on the leaves and roots of the Senna plant contain many active compounds.

## References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