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Studies on *Chenopodium murale* red leaves secondary metabolites

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Abstract: Light is a main environmental reason affecting different plants' growth, appearance, and metabolism. This work aimed to compare the green-red appearing *Chenopodium murale* plants. Acidic methanol was used to extract pigments of *C. murale* colored leaves and C-18 columns were used to fractionate the colored phytochemical compounds. UV-VIS spectrum was used to differentiate the different obtained colored fractions. This study revealed how environmental conditions could shape plants and lead them to manage their metabolites in response to the surrounding conditions..

keywords: *Chenopodium murale*; solid phase extraction; red leaves; C-18.

1. Introduction

Chenopodiaceae comprises 174 genera and approximately 2,500 species of perennial or annual herbs, shrubs, and weeds. Some of the common members of this family are *Chenopodium album* L., *Chenopodium murale* L., and *Dysphania ambrosioides* L. *C. murale* is branded as Nettle leaf goosefoot in English **[1].** Alkaloids and phenolics such as kaempferol and quercetin, are all found in *C. murale*. Sterols, essential oils, flavonoids, tannins, saponins, coumarins, and steroidal estrogen-like compounds are also present in C. murale. In preclinical experiments, several pharmacological advantages, such as hypotensive, anti-inflammatory, analgesic, diuretic, antifungal, antibacterial, phytotoxic, hepatoprotective, and renoprotective properties, were found in C. murale **[2].** This plant is popular worldwide, especially in tropical and subtropical regions. It is a common weed on roadsides and in fields. The plant is an annual herb with an upright stem that can reach 70 cm in height **[3]**.

Plant secondary metabolites (PSMs) are crucial in different types of defenses, such as plant defense against herbivory. Over 2140,000 secondary metabolites are known in the literature: secondary metabolites are categorized into five classes: alkaloids, terpenoids, steroids, polyphenols, fatty-acid-derived compounds, non-ribosomal polypeptides, and enzyme cofactors **[4].** Varied light intensity is usually coupled with temperature stress between seconds and minutes that can affect plant appearance and metabolism **[5].** One common response of plants to light stress is the accumulation of flavonoids, which play a defense function by absorbing excessive amounts of UV light and providing high antioxidant function to the cell in harsh environmental conditions **[6, 7].**

Flavonoids are categorized into six classes: anthocyanins, chalcones, flavanones, flavones, flavonols, and isoflavonoids. Flavonoids have several health-promoting effects such as antioxidative, anti-inflammatory, anti-carcinogenic, and antimutagenic. Therefore, it is an important element in pharmaceutical, nutraceutical, and cosmetics manufacturing **[8].** Flavonoids facilitate plants' ability to modify to extreme heat and cold. **[9,10].**

In this study, *Chenopodium murale* was investigated to determine the categories of secondary metabolites produced under crucial environmental conditions.

2. Materials and methods

Collection of *Chenopodium Murale*

Chenopodium Murale plant leaves were collected from Egypt's north coast near New Mansoura city (31.43734° N, 31.50410° E) in March 2023.

Extraction and fractionation of secondary metabolites from leaves.

Fresh plant red leaves were added to the extraction solution (acidic methanol volume double the plant sample weight) and kept in the dark. Then, homogenize for 5 min the solution and centrifuge at top speed at 4°C for 15 min. All supernatants were combined and dried by a rotary evaporator (SCILOGEX RE 100- Pro, USA) to remove the methanol. The extract was filtered through a 0.45 µm bacterial filter. Then, the red supernatant was loaded on a C-18 column (Ea, Phenomenex, USA). Firstly, 60 ml of deionized water was added. Then, 60 ml methanol (100%) followed by a mixture containing 30 ml methanol and 30 ml deionized water (1:1) was added **[11]**.

After that, the green supernatant was loaded on a C-18 column. 60 mL of 90% methanol was added until the majority of the yellow pigment was eluted. Then the fractions have chlorophylls were eluted by 100% methanol **[12].**

UV- VIS spectral analysis of fractions.

All the fractions were scanned using UV-VIS spectrophotometer (JENWAY 7315, UK) from 200 to 700 nm **[13].**

3. Results.

The collected normal green and stressed red leaves of the *Chenopodium murale* plant are shown in Figure 1.

Extraction and fractionation of *Chenopodium murale* leaves extracts

The *Chenopodium murale* red leaves extract has been divided into two loads in the C-18 column Figure (2A). The two loads have given the same fractions, each load has given tweeny three fractions with different colors represented in Table (1).

The *Chenopodium murale* green extract has only one load in the C-18 column Figure (2B) which gave thirteen fractions with different colors represented in Table (2). Notably, colored fractions observed in case of red leaves were not obtained here.

UV- VIS spectral analysis.

After scanning all the fractions the same fractions were collected together and finally we got eleven fractions from the red stressed leaves Table (3) Figure (3), and five fractions from the normal green leaves Table (4) Figure (4). It is apparent that red plants have produced colored compounds that were not obtained when green plants were fractionated. There are pink, purple, red, and orange fractions. The colors might be attributed to flavonoids. The pink and purple colors may be anthocyanin types. The orange is expected to be the precursor of anthocyanin which is called kaempferol. The red color is expected to be the quercetin and isoflavonoids.

Figure (1) represents the *Chenopodium murale* red stressed leaves and normal green leaves.

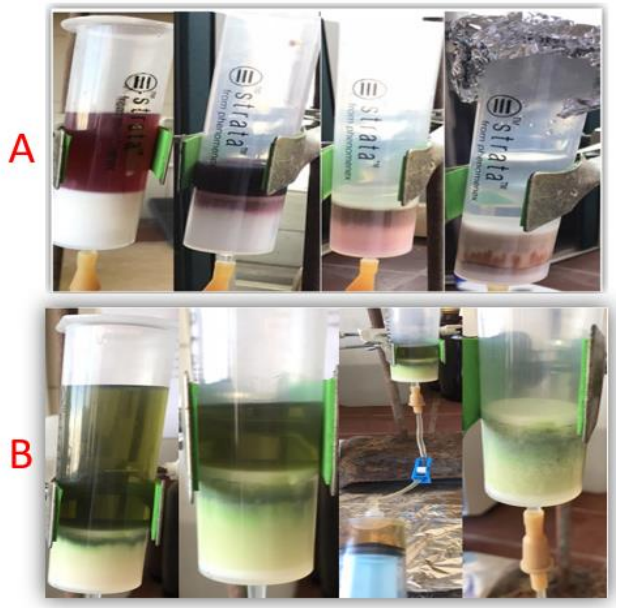


Figure (2). (A) represents the *Chenopodium murale* red extract load for separation in the

C-18 column and (B) represents the *Chenopodium murale* green extract load.

Table (1) represents the solvents, number of fractions, and color of the loads of red leaves (*Chenopodium murale*).

Number	Solvent	Color
1	Without solvent	Pink
2	Without solvent	Deep pink
3	Deionized water	Purple
4	Deionized water	Purple
5	Deionized water	Pink
6	Deionized water	No color
7	Deionized water	Faint pink
8	Deionized water	Faint pink
9	DW	Faint pink
10	DW	Faint pink
11	DW	No color
12	DW	Pink
13	Methanol	Deep Red
14	Methanol	Orange
15	Methanol	Faint orange
16	Methanol	Yellow
17	Methanol	Yellow
18	Methanol	No color
19	50 % Methanol +50 % water	Purple
20	50 % Methanol +50 % water	Pink
21	50 % Methanol +50 % water	Faint pink
22	50 % Methanol +50 % water	White
23	50 % Methanol +50 % water	No color

Table (2) represents the solvents, number of fractions, and color of the load of green leaves (*Chenopodium murale*).

Number	Solvent	Color
1	without solvent	Deep yellow
2	without solvent	Deep yellow
3	without solvent	Deep yellow
4	without solvent	Yellow
5	Methanol 90%	Yellow
6	Methanol 90%	Deep green
7	Methanol 90%	Green
8	Methanol 90%	Green
9	Methanol 90%	Faint green
10	Methanol 100%	Faint green
11	Methanol 100%	Faint green
12	Methanol 100%	Yellow
13	Methanol 100%	Yellow

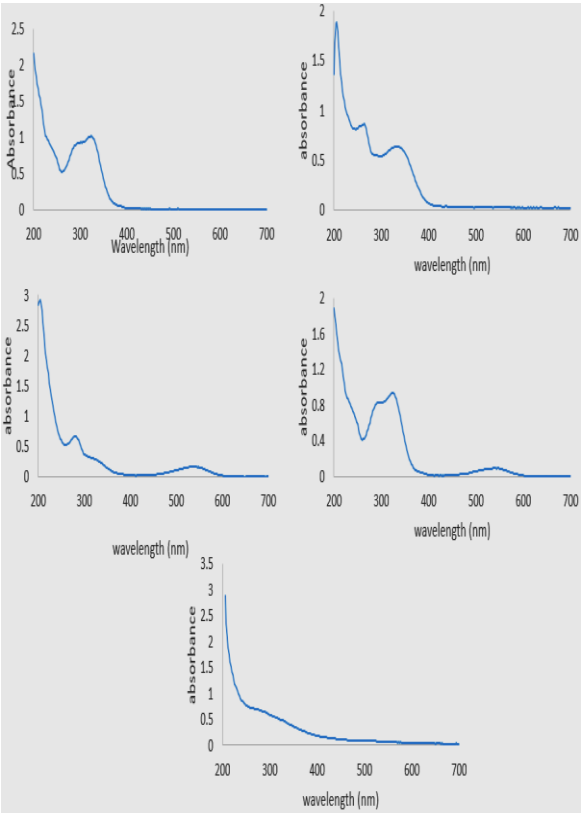
Table (3) represents the eleven fractions of red stressed leaves of *Chenopodium murale* with the fraction color and the peaks.

Number	Color	Peaks at
1	Deep pink	300- 324
2	Pink	264- 330
3	Purple	276- 540
4	Purple	290- 320
5	Pink	280- 540
6	Faint pink	296- 318- 540
7	Pink	290- 324
8	Orange	247- 397
9	Purple	268
10	Red	247- 431
11	Green	414- 660

Table (4) represents the five fractions of normal green leaves of *Chenopodium murale* with the fraction color and the peaks of each fraction.

Number	Color	Peaks at
1	Deep yellow	260-338
2	Yellow	466-664
3	Green	408-668
4	Faint green	346-468- 664
5	Yellow	412-670

Figure (3). UV spectrum of some fraction of *Chenopodium murale* red stressed leaves.



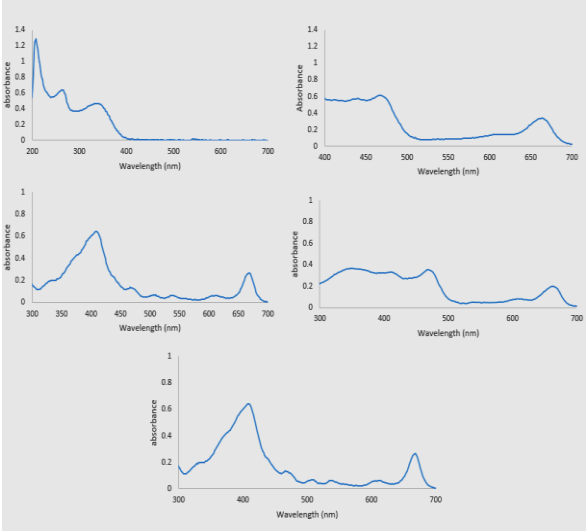


Figure (4). UV spectrum of the fraction of *Chenopodium murale* green normal leaves.

4. Discussion.

Under stress conditions, plants utilize their phenotypic plasticity to adapt to different environmental conditions **[14].** Many genes required in anthocyanin biosynthesis, transportation, and regulation were observed to be responsible for anthocyanin accumulation in Arabidopsis **[15].**

Phenol compounds are an essential component of phenylalanine-derived secondary metabolites with numerous properties. These properties make them fit for the food, pesticide, pharmaceutical, and cosmetic industries. Lighting doses may be changed to enhance plant execution and increase the amount of secondary metabolites **[17].** UV-B stress can raise the number of secondary metabolites suitable for human physical conditions. Furthermore, abiotic stress caused by UV-B exposure to *mung bean sprouts*, leads to considerable accumulation of vitamin C, phenols, and flavonoids, enhancing nutritional value. *Mung bean sprouts* contain vitamin C, phenols, and flavonoids that may be enhanced by low-dose irradiation with UV-B **[18].**

Most flavonoids are absorbed in the range of 315–400 nm **[19].** Anthocyanins have a unique UV–VIS absorption profile with a maximum in the visible range of 465-550 nm and a range of 270 nm and 280 nm **[20].**

C. murale comprises flavonoids, saponins, and terpenoids **[21].** Phytochemical investigation of *C. murale* showed flavonoids, essential oils, sterols, steroidal estrogen-like substances, alkaloids, and coumarins **[22].** The aerial regions of *C. murale* have four flavonol glycosides, three aglycones, and one coumarin. One of the glycosides was kaempferol with its 7-rhamnoside, 3-rhamnoside, 7-glucoside and 3,7-dirhamnoside. The other three glycosides were herbacetin, quercetin, and scopoletin **[23].**

Anthocyanins are phenolic compounds that make the plant leaves colored red, orange, purple, and yellow colors **[24].** Accumulation of soluble phenolics and anthocyanins has been studied as a sign of stress modification to plants **[25, 26].**

5. Conclusion

C. murale extract from red leaves contains flavonoids, especially anthocyanins, but the green leaves do not, which means these phytochemical compounds are produced due to stress conditions.

6. References

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