

Effect of ultraviolet ray and Moringa leaf extract on growth and accumulation of digoxin in *Digitalis lanata in vitro*.

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Abstract

The experiment was conducted with the aim of testing the effect of ultraviolet rays and Moringa leaf extract on growth indicators and stimulating the cardiac glycoside digoxin in the leaves of the digitalis plant, the Plant Tissue Culture Laboratory - College of Agricultural Engineering Sciences - University of Baghdad for the period from December 15, 2022, to January 1, 2023. Sterilized seeds were planted in MS medium prepared with BA at a concentration of (0.5 mg.l⁻¹) and until obtaining a sufficient number of vegetative branches with a length of (2 cm), they were exposed to ultraviolet radiation for different periods of time (0,5,10,15, 20 min). The vegetative branches were also planted in MS medium prepared with Moringa leaf extract at concentrations (0,5,10,15 g.l⁻¹) in independent experiments. The results showed that the experiment of exposure to ultraviolet radiation for a period of (15 min) was significantly superior in the average number of branches and leaves, wet and dry weight, chlorophyll and cardiac digoxin, as it recorded (44.67 branches. plant⁻¹, 4.52 leaves. plant⁻¹, 2.987 g, 0.731 g, 0.895 g. 100 g fresh weight, 28.55 µg.g⁻¹), and the results of the experiment of Moringa leaf extract showed that the concentration of 10g l⁻¹ was superior to the rest of the concentrations in the study indicators, as it reached (2.67 branch.plant⁻¹, 11.67 leaves.plant⁻¹, 1.706g, 0.832g, 0.597mg.100g⁻¹ and 30.91 µg.g⁻¹).

Keywords: *Digitalis lanata*, Moringa extract, ultraviolet ray, digoxin.

1. Introduction

Herbal medications are primarily derived from medicinal plants, either directly or through various processing methods. These plants play a vital role not only in the production of pharmaceutical drugs but also in numerous other industries, including the manufacturing of disinfectants and cosmetics [1]. Consequently, plants are now an essential component in the creation of medicines and a primary source for the treatment of diseases [2]. You may find *Digitalis lanata* L. among these plants Eastern and Western Europe, Central and Western Asia, and Northern Africa. *Digitalis* is a genus of

eukaryotic plants that is subfamily Plantaginaceae. Two dozen herbaceous or shrubby species, some of which are annuals, make up the genus *Digitalis*. Among these plant species, *lanata* stands head and shoulders above *D. purpurea* in terms of biological activity, which is two to five times higher [3,4]. The medicinal value of this plant is derived from the cardiac glycosides it contains. These glycosides control the heart rate and enhance the power of blood pouring out of the heart during contractions, particularly during myocardial relaxation [5]. In addition to treating tachycardia and atrial

fibrosis, it lowers vascular pressure. Scientists have also investigated digoxin's potential as an anti-cancer medication; the molecule is effective at destroying cancer cells; thus, it might be used to treat cardiac issues, prostate cancer, lung cancer, and kidney cancer [6,7]. It takes a lot of work and a lot of agricultural area to grow medicinal plants so that their bioactive chemicals may be extracted. Additionally, unpredictable environmental conditions can negatively impact plant growth, and the quality of the active compounds produced. Therefore, scientists primarily rely on plant tissue culture technology as an effective approach to produce pharmaceutical compounds. This technique enables the rapid and continuous generation of virus-free plants while preserving their genetic traits. Beyond its role in pharmaceutical industries, it also contributes to the study of plant growth and development. Given the significant medicinal value of Digitalis plants, optimizing their cultivation strategies is essential to enhance their growth and increase the production of their bioactive compounds [4,8]. Among the various methods employed in our approach is the utilization of ultraviolet (UV) radiation, a form of non-ionizing electromagnetic radiation with specific wavelengths used to promote plant tissue growth. Due to the high energy of photons, electron transitions between molecular orbits may occur. Ultraviolet radiation is classified based on wavelength: UVA ranges from 400 to 315 nm, followed by UVB, UVC, and finally, another UVA range between 280 and 200 nm. The effectiveness of UV radiation depends on several factors, including radiation intensity, the penetration capacity of the material, and the duration of exposure [9,10]. Exposure to ultraviolet radiation can enhance the production of secondary metabolites, improve the plant's energy storage capacity, and increase its biomass by stimulating cellular growth [11]. According to research by Jaiswal and Agrawal [12], two varieties of turmeric plants are affected by UV light, which increases the yield of aromatic oils like 1,8-cineole [13]. Aghdasi et al. [14] observed that exposure to UV radiation led to an increase in the concentrations of caffeine, chlorogenic

acid, ascorbic acid, and total phenols in lettuce callus. Extracts from plants have recently attracted a lot of attention in the realm of plant nutrition because they help plants with some of their nutritional requirements and growth-stimulating materials. This serves two purposes: first, by using only natural ingredients, it helps to preserve the environment and health. Second, it improves the nutritional efficiency and quality characteristics, which helps to stimulate growth and produce secondary metabolites. One of these intriguing extracts is moringa leaf extract. The evergreen tree *Moringa oleifera* L. grows rapidly and is a member of the Moringaceae family. Because it does well in dry climates and works well in poor soils, it is grown extensively over the globe, particularly in Asia and Africa [15]. The plant receives all the nutrients it needs and growth regulators from moringa due to its high nutrient content, which includes carbohydrates, proteins, vitamins, amino acids, oils, phenols, carotenoids, flavonoids, and cytokinins like zeatin [16,17]. This renders moringa a natural plant growth stimulant, enhancing both the quantity and quality of yield [18]. Additionally, moringa plays a crucial role in promoting cell division and expansion, thereby regulating biological and physiological processes, improving vegetative growth characteristics, and stimulating secondary metabolism. Consequently, this leads to the upregulation and increased synthesis of compounds associated with secondary metabolic pathways [19,20]. In a study conducted by Yap et al. (21) to examine the effect of moringa leaf extract at concentrations of (0, 5, and 10 g .l⁻¹) on growth indicators, yield, and active compound content in *Silybum marianum* (milk thistle), the application of moringa leaf extract resulted in the highest content of silybin (A + B). This study aims to evaluate the synergistic effects of Moringa leaf extract and UV light on the growth and development of Digitalis plants under controlled conditions, with the objective of optimizing the biosynthesis of cardiac glycosides.

2. Materials and Method

2.1 Duration

From December 15, 2022, to January 1, 2023, researchers from the University of Baghdad's College of Agricultural Engineering Sciences' Laboratory of Plant Biology, which is part of the Medicinal and Aromatic Plants Research Unit, took part in the study. The seeds came from Johnny Seeds, an American company. We followed these steps to carry out the study:

2.1.1 Seed disinfestation

After soaking the seeds for thirty minutes in a 1% sterilant that included 6% sodium hypochlorite, we rinsed them five times with distilled water to eradicate the infestation, all the disinfestation procedures were completed under a sterile laminar airflow hood [22,23].

2.1.2 Seed germination

The sterile seedlings were cultured on a robust Murashige and Skoog (MS) medium, supplemented with 30 g of sucrose and 7 g of agar. Initially, the seedlings were kept in the dark at approximately 25 °C for two days. Following this, they were exposed to a light cycle of 16 hours of light and 8 hours of darkness, in accordance [24].

2.1.3 Proliferation stage

After four weeks of germination, the seedlings were collected, and one node was selected to be cultured in a medium containing 0.5 mg/L of benzyl adenine (BA) [25,26]. Subsequently, the progeny was cultivated in a medium that retained the original components. This procedure was repeated until enough branches had developed to initiate the stimulation of secondary compounds.

2.2 Effect of ultraviolet rays on growth and increasing stimulation of some cardiac glycosides in digitalis plants.

We exposed the plants to type B UV light ten times per treatment for 0, 5, 10, 15, and 20 minutes. There were 0.5 milligrams per liter of benzyl adenine (BA) in the growth medium. A growth chamber, which maintained a constant temperature of 25 °C and provided 16 hours of light and 8 hours of darkness, kept the plants in an ideal environment [27].

2.3 Effect of Moringa leaf extract on growth and digoxin in Digitalis plants.

Before making an aqueous extract, we dried and ground the Moringa leaves into a fine powder. Next, we dissolved 100 g of the powder in 500 ml of distilled water, periodically shaking the mixture. Next, we strained the mixture, dried the resulting black powder, placed it on a glass plate, and baked it for two days at 40°C. We finished this process and stored the extract in the refrigerator at 4°C until we needed it [28].

For branch culture, MS medium was prepared with varying concentrations of Moringa leaf extract (0, 5, 10, and 15 g.l⁻¹). The cultures were maintained in a growth chamber at 25°C, with a photoperiod of 16 hours of light and 8 hours of darkness. A total of ten distinct treatments were established, and data were collected after six weeks to evaluate the study parameters.

- The count of branches . plant⁻¹.
- The Length of the branch cm.
- Number Leaf (leaf. plant⁻¹)
- wet and dry weight (g)
- Chlorophyll content estimation.

We applied Goodwin's [29] method to quantify the chlorophyll pigment content. Initially, (1 g) of vegetative growth from plant tissue cultures was collected, and 10 ml of 85% acetone . The plant tissues were ground until only colorless residues remained. The resulting mixture was then filtered into a volumetric flask using filter paper, and the volume was adjusted to 10 ml by adding more acetone. The light absorbance of the solution was measured with a spectrophotometer at wavelengths of 663 nm and 645 nm. The total chlorophyll content was subsequently calculated using the following equation :

$$\text{Total Chlorophyll Content (mg/l)} = [20.2 D (645) + 8.02 D (663) \times V] / W \times 1000$$

- High-performance liquid chromatography (HPLC) was employed for the quantitative and qualitative analysis of various cardiac glycosides. The quantification of digoxin was conducted following the method described by Fender[30](Fig.1), using the following equation:

$$\text{Sample concentration} = \frac{\text{Standard concentration} \times \text{sample area} \times \text{Dilution times}}{\text{Standard area}}$$

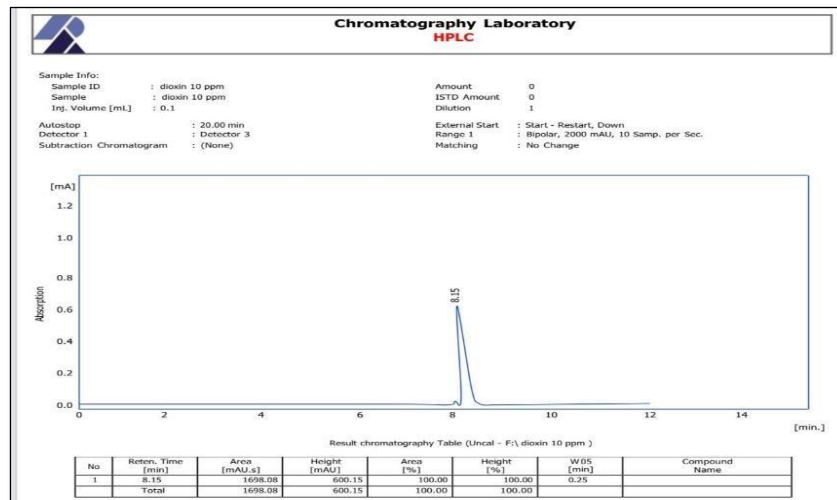


Fig 1. Standard curve of digoxin.

2.3 Statistical design

The study utilized a factorial experimental design incorporating 10 repetitions within a completely randomized design (CRD). Data analysis was

performed using GenStat software, and mean comparisons were conducted using the least significant difference (LSD) test at a significance level of 0.05 [31].

3.Results and Discussion

3.1.Effect of UV rays on the number of branches, branch length, and number of leaves of the Digitalis plant.

Table 1 shows that the quantity of leaves, the length of proliferating branches, and the number of branches exposed to UV light all have a significant impact. The average number of branches and leaves per plant significantly

increased after 15 min of UV light exposure, reaching 44.67 branches and 4.652 leaves, respectively. The control group, on the other hand, had the lowest mean values for branch count, branch length, and leaf number, averaging 7.67 branches/plant, 1.00 cm branch length, and 0.524 leaves.plant⁻¹.

3.2.Effect of UV rays on fresh and dry weight and content of chlorophyll and cardiac digoxin

Table 2 demonstrates that the period of UV light exposure significantly changed the fresh and dried weight of the leaves, the amount of chlorophyll, and the amount of digoxin.

Compared to all other exposure times, the 15-minute interval yielded the greatest values: 2.987 g, 0.731 g, 0.895 g, 100 g⁻¹ fresh weight, and 28.55 g. Fig. 2 shows that the control group had the lowest readings, with weights of 0.232 g, 0.134 g, and 0.234 g. 100 g⁻¹, and 20.08 g⁻¹.

Table 1. effect of UV on the branch number, branch length and leaf number of *D. lanata*

UV (min)	Number of branches (branch. plant ⁻¹)	Branch length (cm)	Number of leaves (leaf. plant ⁻¹)
0	7.67	1.00	0.524
5	20.00	1.67	2.065
10	3.497	33.67	4.23
15	44.67	2.67	4.652
20	38.50	5.37	3.762
LSD 0.05	1.151	0.743	0.007

Table 2. effect of UV on the fresh weight, dry weights, chlorophyll, and digoxin compound in the leaves of the *D. lanata*

UV (min)	average fresh weight(g)	average dry weight(g)	Chlorophyll (mg.100g ⁻¹)	Digoxin (µg.g ⁻¹)
0	0.232	0.134	0.234	20.08
5	1.313	0.459	0.459	23.22
10	0.695	0.679	26.73	1.502
15	2.987	0.731	0.895	28.55
20	1.631	0.532	0.723	26.20
LSD 0.05	0.001	0.004	0.0123	0.637

Tables 1. and 2. clearly show that exposing plants to 15 minutes of UV radiation treatment resulted in notable improvements in several traits. These improvements encompass the number of branches, the length of the proliferated branches, the number of leaves, as well as fresh weight, dry weight, chlorophyll content, and the concentration of the medicinal compound digoxin. Conversely, plants not exposed to radiation (UV₀) did not exhibit these enhancements. This benefit may stem from the fact that increased levels of radiation disrupt the activity of specific enzymes that impede certain

3.3.The effects of Moringa leaf extract on *Digitalis* leaf number, length, and number were investigated in this study.

Table 3 shows that different concentrations of Moringa leaf extract had a significant effect on average branch and leaf proliferation, as well as the overall number of branches and leaves

biological processes within the plant. Researchers have also found that radiation activation changes the cytoplasm's physiological properties. This makes the plant's biological responses and functional processes better stimulated. Consequently, this leads to the promotion of secondary metabolite synthesis [32,33]. These results are like what al-Mousawi [27] found. He discovered that UV radiation had a positive effect on the growth of tissue cultures *Tanacetum parthenium* plants, as evidenced by an increase in the chemical content of secondary metabolites.

produced. The treatment with a concentration of 10 g.l⁻¹ notably exceeded the other treatments, yielding 2.67 branches per plant and 11.67 leaves per plant. In contrast, the control treatment recorded the lowest averages, with only 1.00 branches per plant, 1.00 cm, and 4.67 leaves per plant.

3.4. Moringa leaf extract's impact on cardiovascular health, chlorophyll levels, and fresh and dry weight and Digoxin .

Table 4 illustrates that the amounts of Moringa plant extract had a significant impact on the percentage of chlorophyll and digoxin present in the leaves, along with their fresh and dry weights. The data presented in the table indicates that a

concentration of 10 g/L outperformed all other concentrations. In contrast, the remaining concentrations yielded lower averages, including 0.427 g, 0.198 g, 0.134 g, 100 g, and 32.08 micrograms/g, while the control treatment exhibited the lowest averages. (Photo 3.).

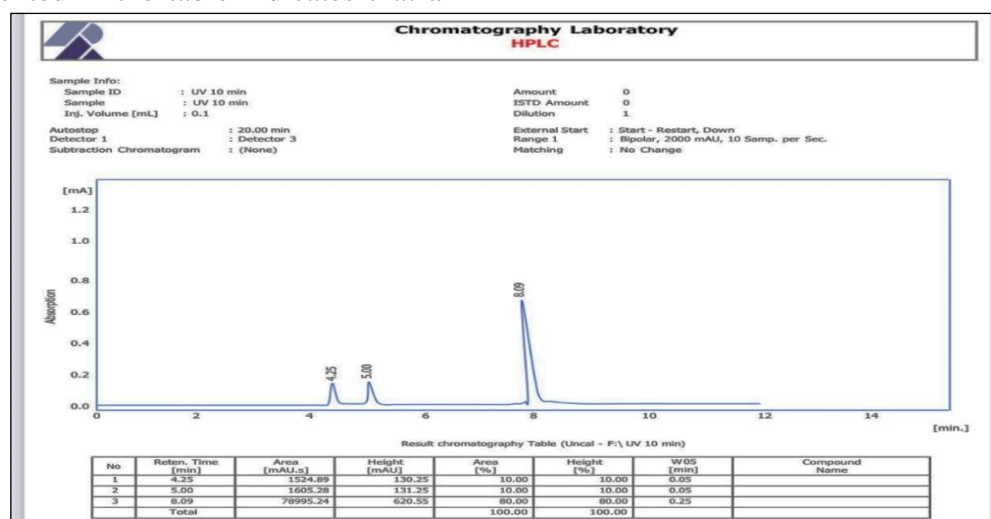


Fig 2. The best form of UV (15 min).

Table 3. Impact of Moringa leaf extract on the branch number, length of branches and number of leaves of *D.lanata*.

Moringa extract g.l ⁻¹	Number of branches (branch. plant ⁻¹)	Branch length (cm)	Number of leaves (leaf. plant ⁻¹)
0	1.00	1.00	4.67
5	1.33	2.00	8.67
10	1.667	11.67	2.67
15	1.67	2..00	8.33
LSD 0.05	0.941	0.543	1.087

Table 4. Effect of Moringa leaf extract on the fresh and dry weights, chlorophyll and digoxin compound in the leaves of *D. lanata*.

Moringa extract g.l ⁻¹	average fresh weight(g)	average dry weight(g)	Chlorophyll (mg.100g ⁻¹)	Digoxin (µg.g ⁻¹)
0	0.427	0.198	0.134	23.08
5	0.804	0.531	0.395	27.20
10	0.832	0.597	1.706	30.91
15	0.706	0.948	0.402	28.71
LSD 0.05	0.007	0.001	0.002	0.790

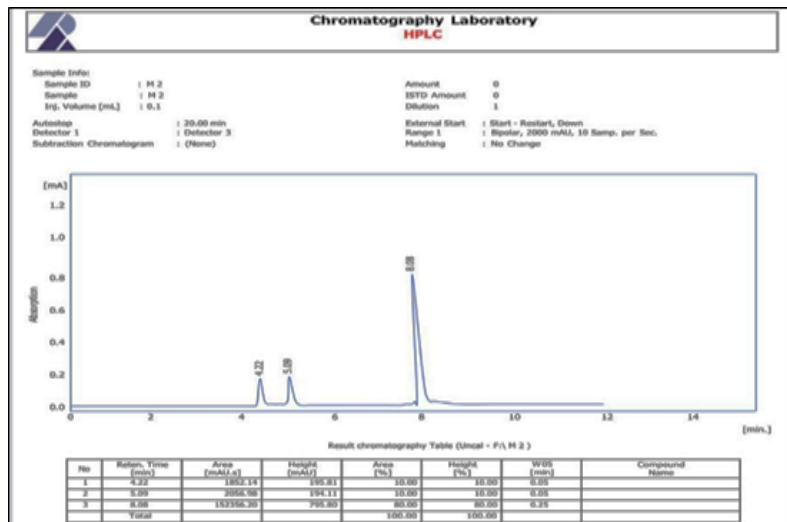


Fig 3. The best form of moringa extract (10 gm.l⁻¹).

Tables 3 and 4 show that there are a lot of nutrients and organic compounds in the Moringa leaf extract. There are many auxins, cytokinins, and gibberellins. These are important for plants at different stages of development because they control many physiological processes, The most important of these is cell elongation and division [34,35], Auxins play a crucial role in promoting plant elongation, while cytokinins regulate the transport of auxins to lateral branches, thereby enhancing branching and increasing the number of primary branches. The interaction between these hormones establishes a precise equilibrium that optimizes overall plant growth. In Moringa leaf extract, the synergistic action of these hormones, in conjunction with the plant's abundant amino acids and minerals, significantly influences plant development. Moreover, the

4. Conclusions

Based on the obtained results, it can be concluded that the treatment of the Digitalis plant with ultraviolet radiation and Moringa leaf extract

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presence of growth parameters, as well as the increased concentrations of organic acids, proteins, and amino acids. [36,37]. The majority of nitrogen metabolism in plants relies on these components for a variety of physiological functions and metabolic pathways. Therefore, they have a beneficial effect on the byproducts of carbon assimilation in the leaves, which in turn increases the accumulation of secondary metabolites and aids in the plant's development and expansion [38, 39]. These results are consistent with the findings of Farooq et al. [40], who reported that the application of Moringa leaf extract to lettuce leaves led to an increase in growth indicators and the plant's content of secondary metabolites.

significantly influenced its vegetative growth and the accumulation of the medically important compound digoxin in its leaves under in vitro conditions.

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