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Effect of Colchicine Treatment on Oxalate Content of *Talinum triangulare* Leaves

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Abstract

Excessive oxalate content in leafy vegetables like *Talinum triangulare* limits calcium absorption and increases kidney stone risk. This study investigated the effect of colchicine treatment on oxalate content in *Talinum triangulare* to enhance its nutritional quality. Stem cuttings were treated with colchicine at concentrations of 0.01%, 0.025%, and 0.1% for 12, 18, and 24 hours, then cultivated under controlled conditions. Oxalate levels were determined using titrimetric analysis. Results showed a significant reduction ($p < 0.05$) in oxalate content across all colchicine treatments compared to the control. The highest reduction (65.57%) was observed in plants treated with 0.025% colchicine for 18 and 24 hours. These findings indicate that colchicine treatment can effectively lower oxalate levels in *Talinum triangulare*, potentially improving its nutritional value. Further molecular studies are needed to unravel this plant's exact genetic mechanisms involved in oxalate biosynthesis.

Keywords*: Talinum triangulare*, colchicine, oxalate levels, nutritional value, and kidney stones.

Introduction

Talinum triangulare. (Jacq) Willd commonly known as waterleaf, is a leafy green plant belonging to the Portulacaceae family (CAROLIN, 1987). Waterleaf is also known as *Talinum fruticosum* (MANIKANDAN et al., 2023). It also has other common names such as Ceylon spinach, Surinam purslane, Philippine spinach, Java, Blatt-ginseng, and Sweetheart (USDA, NPGS 2020). It is known for its nutritional and medicinal significance, containing essential vitamins, minerals, and bioactive compounds (ADEKANMI et al., 2020). *Talinum triangulare* is primarily propagated through cuttings measuring 10–15 cm in length (FONTEM and SCHIPPERS, 2004). Its leaves and tender shoots are commonly used as thickening agents in soups and are a staple in the Southern region of Nigeria (IBEAWUCHI et al., 2007).

Despite its benefits, waterleaf contains high levels of oxalate, a compound that can impede calcium absorption and contribute to kidney stones formation when consumed excessively (MANIKANDAN et al., 2023). Excessive oxalate intake has been linked to health hazards such as hyperoxaluria, emphasizing the need to reduce oxalate levels in edible plants (DEMOULIN et al., 2022).

Colchicine, an alkaloid derived from meadow saffron *(Colchicum autumnale L.*), is widely used

as a mutagenic agent to induce genetic variations in plants, including chromosome duplication and polyploidy (ENG & HO, 2018). Studies have shown that colchicine treatment influences secondary metabolite biosynthesis by altering enzymatic activity in metabolic pathways (MADANI et al., 2021). While colchicine's effects on plant growth and secondary metabolites have been extensively documented (ÇÖMLEKÇIOĞLU and ÖZDEN, 2019; SAMADI et al., 2022). However, to the best of our knowledge no prior study has directly reported colchicine's effect on oxalate levels in plants.

This study aimed to evaluate the effect of colchicine treatment on oxalate content in *T. triangulare* leaves using various colchicine concentrations and treatment durations with the goal of reducing oxalate in the plant, which is an anti-nutrient, thereby enhancing its nutritional value. This research seeks to contribute to the development of safer and more nutritious food sources, particularly in regions where waterleaf is a staple crop.

The justification for this study lies in the significant consumption of waterleaf in Nigeria, particularly in the Southern region, where it is commonly used for thickening soups and as a staple vegetable (IBEAWUCHI et al., 2007). Lowering its oxalate content could enhance its safety and nutritional value, especially in populations prone to kidney stone formation.

Materials and Methods

Study Area and Experimental Design

This study was conducted in the northwestern region of Nigeria, specifically in the Kaduna North Local Government Area of Kaduna State. The plants used for this study were grown at the Biological Garden of the Department of Biological Sciences at the Nigerian Defence Academy in Kaduna State. Laboratory analyses were carried out at the Institute of Chemical Technology Zaria, Kaduna State. The experimental design was a randomized block design (RBD) split plot arrangement with three replicates for each treatment.

Plant Materials

Talinum triangulare plants were obtained from a commercial vegetable garden at Kawo, Kaduna State. Uniform stem cuttings, measuring 11.4 cm in length, were excised from healthy, mature mother stock. The cuttings were thoroughly washed with running tap water and surface-sterilized with a 1% sodium hypochlorite solution for 5 minutes, followed by three rinses with sterile distilled water.

The colchicine solution was prepared by dissolving the required amount of colchicine powder in distilled water. The solution was autoclaved at 121°C for 15 minutes to ensure sterility. The sterilized stem cuttings were treated with aqueous colchicine solutions at concentrations of 0.001%, 0.025%, and 0.1%. An untreated control group was included. The stem cuttings were immersed in 150ml flasks containing distinct colchicine solutions. The specimens were left undisturbed for durations of 12, 18, and 24 hours.

Post-treatment and Planting

Following the 12 hours, 18hours and 24 hours exposure to the colchicine solutions, the stem cuttings were rinsed with running tap water to remove residual colchicine and halting the effects of colchicine before planting. The treated cuttings were planted in 10 cm x 15 cm sac bags filled with a 1:1 mixture of sterile sand and leaf mold. Four treated cuttings were planted per bag in 3 replicates along with a control. The plants were maintained at the Biological Garden of the Department of Biological Sciences, Nigerian Defence Academy (NDA) Kaduna for five weeks under ambient environmental conditions (average temperature: 28 ± 3°C, relative humidity: 65 ± 10%, natural light cycle) and closely monitored before being harvested for laboratory analyses.

Screening for Changes in Oxalate Content

Mature leaf samples were randomly collected from plants treated with 0.001%, 0.025%, and 0.1% colchicine for 12 hours, 18 hours and 24 hours as well as the control group. Leaf samples were harvested at the end of the five-week growth period. Two grams of fresh leaf tissue were pulverized and homogenized in 10 ml of 6 M hydrochloric acid (HCl). The mixture was

stirred for 1 hour and filtered through Whatman No. 1 filter paper. The filtrate was adjusted to a final volume of 250 ml with distilled water. The pH of the filtrate was adjusted with concentrated ammonium hydroxide (NH_4OH) until a color change from salmon pink to pale yellow was observed. 10 ml of 5% calcium chloride ($CaCl_2$) solution was added to precipitate oxalate. The suspension was centrifuged at 2500 rpm, and the supernatant was discarded. The precipitate was dissolved in 10 ml of 20% (v/v) sulfuric acid (H_2SO_4), and the solution was diluted to 300 ml. A 125 ml aliquot was heated to 80-90°C and titrated against 0.05 M standardized potassium permanganate ($KMnO_4$) solution until a faint pink endpoint persisted for 30 seconds. The oxalate content was calculated using the titration data, as described by (KARAMAD et al., 2019). The same method was also carried out for the control plant.

Below is the overall redox reaction.

$$2MnO_4^- + 5C_2O_4^{2-} + 16H^+ \rightarrow 2Mn^{2+} + 8H_2O + 10CO_2$$

Statistical Analysis

All experiments were performed in triplicate. Data are presented as mean ± standard deviation. Statistical analysis was performed using IBM SPSS Statistics version 29. One-way analysis of variance (ANOVA) was used to determine significant differences between treatments. Mean

Results

Effect of Colchicine on Oxalate Content

Treatment of *Talinum triangulare* leaves with colchicine resulted in a significant reduction in oxalate content compared with the untreated control. (Table 1, Figure 1) displays the mean oxalate content (mg/100g) ± standard deviation (SD) of *Talinum triangulare* leaves treated with varying concentrations of colchicine (0.01%, 0.025%, and 0.1%) for 12, 18, and 24 hours, compared to an untreated control (0%). The control group exhibited the highest mean oxalate content (0.61 ± 0.09 mg/100g). All colchicine treatments resulted in a significant reduction in oxalate content compared to the control ($p < 0.05$).

The lowest oxalate content was observed in the 0.025% colchicine treatment group across all durations, with values ranging from 0.21 ± 0.06 to 0.26 ± 0.08 mg/100g. The 0.1% colchicine treatment resulted in oxalate levels between 0.30 ± 0.03 and 0.32 ± 0.04 mg/100g, while the 0.01% treatment showed oxalate contents between 0.40 ± 0.05 and 0.43 ± 0.03 mg/100g. Ploidy level of colchicine treated plants was not conducted in this study.

Percentage Reduction in Oxalate Content

Table 1 shows the percentage reduction in oxalate content relative to the control. The highest percentage reduction was observed with 0.025% colchicine, reaching 65.57% at 18 and 24 hours. The 0.1% colchicine treatment resulted in a reduction of approximately 47.54% to 50.82%, and the 0.01% treatment showed reductions between 29.51% and 34.43%. Percentage reduction in oxalate content was calculated using the formula: Mean oxalate content of control – Mean oxalate content of treated sample ÷ Mean oxalate content of control ×100.

Table 1: Percentage Reduction in Oxalate Content of *Talinum triangulare* following varying Levels of Colchicine Treatment

Concentration of colchicine (%)	**Duration of Treatment (hours) of Colchicine**	**Concentration of Oxalate(mg/100g)**	**Percentage Reduction in Oxalate (%)**
0.01	12	0.43± 0.03[b]	29.51
	18	0.40 ± 0.05[b]	34.43
	24	0.41 ± 0.02[b]	32.79
0.025	12	0.26 ± 0.08[d]	57.38
	18	0.21 ± 0.06[d]	65.57
	24	0.21 ± 0.07[d]	65.57
0.1	12	0.32 ± 0.04[c]	47.54
	18	0.30 ± 0.03[c]	50.82
	24	0.31 ± 0.01[c]	49.18
0	0	0.61 ± 0.09[a]	

Values are means of triplicate measurements ± standard deviation. Mean values that differ significantly at 95% confidence level were assigned different letters. Significant differences were observed across columns

Colchicine concentration of 0 represents the control (non-colchicine treated) The highest mean value was assigned letter "a"

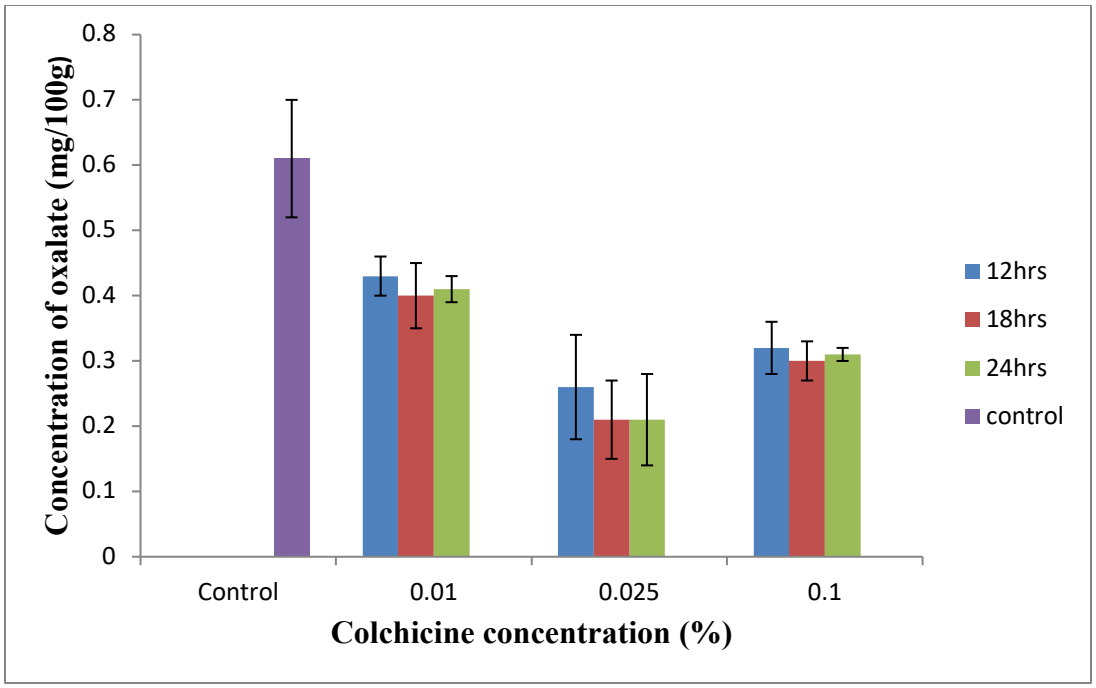


Fig 1. Effect of Varying Colchicine Concentrations and Treatment Durations on Oxalate Content of *Talinum triangulare*

Discussion

This study shows that there was a significant reduction in oxalate content of *Talinum triangulare* leaves after treatment with colchicine. This highlights the potential of colchicine treatment in regulating bioactive compounds like oxalates in plants. This finding is consistent with (MADANI et al., (2021) who reported that colchicine a widely used antimitotic inhibitor can alter plant metabolism by modifying physiological and biochemical traits including secondary metabolite production.

This research revealed a dose and time-dependent effect of colchicine treatment on oxalate levels of leaves of *T. triangulare.* The 0.025% colchicine treatment with 65.57% reduction in oxalate levels was the most effective in reducing oxalate content, particularly at 18- and 24-hours post-treatment compared with the control and other treatments. 0.1% had 50.82% reduction at 18 hours while 0.01% had 34.43% as its highest reduction at 18 hours. This aligns with the findings of SING et al. (2020) which showed a reduction in some macronutrients in sweet orange (*Citrus sinensis*) treated with different doses of colchicine. Different studies have highlighted that pre-treatment of plants with adequate concentration of colchicine and treatment duration could result in development of improved plants in morphology, physiology and anatomy (MANGENA and MUSHADA, 2023).

The reduction in oxalate content in *Talinum triangulare* has significant implications for its nutritional value. Oxalates, acting as anti-nutrients, can impede the bioavailability of essential minerals like calcium (RAJAN and NAYAGAM, 2024). Consequently, the colchicine-induced decrease in oxalate could enhance the nutritional quality of *Talinum triangulare*, making it safer for human consumption without the mineral-binding effects associated with high oxalate levels.

This study is the first to document the effect of colchicine treatment on oxalate content in *Talinum triangulare.* However, previous research

has explored alternative methods of reducing oxalate levels in other plant species. For instance, studies have shown that blanching Taro with NaCl or EDTA can significantly reduce oxalate concentrations (DAHAL and SWAMYLINGAPPA, 2006). Similarly, NaCl treatment of purple yam has decreased its oxalate levels (ROFI'ANA et al*.,* 2018). These findings, along with the results of this research, highlight the potential of both chemical and physical treatments to regulate oxalate content in plants. Although ploidy level was not examined in this study, the observed reduction may be linked to colchicine's influence on plant metabolic pathways. The long-term stability and heritability of this effect remain unclear. It is recommended that future studies assess ploidy status, confirm trait persistence across generations, and evaluate any unintended physiological changes. This will help determine the safety and practical application of colchicine in enhancing the nutritional quality of *Talinum triangulare*. Further investigation to unravel the specific pathways involved in oxalate metabolism in *Talinum triangulare* should be explored*.*

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