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DOI: <https://dx.doi.org/10.4314/acsj.v33i2.9>



## IMPACT OF PHARMACEUTICAL WASTEWATER ON PHYTOCHEMICAL CONSTITUTION OF COMMONLY CONSUMED VEGETABLES IN NIGERIA

### IMPACT DES EAUX USÉES PHARMACEUTIQUES SUR LA CONSTITUTION PHYTOCHIMIQUE DES LÉGUMES COURAMMENT CONSOMMÉS AU NIGÉRIA

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(Received 2 February 2025; accepted 25 March 2025)

## ABSTRACT

The manufacture of pharmaceutical medicines is a desired industrialisation process, though the production of wastewater during its operations and its release into surrounding water systems may pose environmental pollution challenges. The objectives of this study was to examine the effect pharmaceutical industrial processing wastewater on the phytochemical constitution of commonly consumed vegetables in Nigeria. A study was conducted at the University of Ilorin Botanical Garden, involving two commonly consumed vegetables, namely *Amaranthus viridis* (African spinach) and *Corchorus olitorus* (jute mallow) cultivated in soils treated with different concentrations of pharmaceutical wastewater (20, 40, 60, 80 and 100%). Phytochemical analyses included the test to determine the presence and quantities of alkaloids, saponins, tannins, glycosides and terpenoids. Results revealed that the alkaloid content was marginally present in spinach leaves from soil treated with 100% wastewater, but moderately present in *Amaranthus viridis* from other treatments. The study revealed that the alkaloid content in *Amaranthus viridis* ranged from 4.22 to 9.8 mg 100 g<sup>-1</sup>; while glycoside content in *Corchorus olitorius* leaves varied between 0.50 to 2.72 mg 100 g<sup>-1</sup>. On the other hand, low pharmaceutical wastewater concentrations enhanced phytochemicals, with high concentrations causing heavy metal stress, resulting in reduced phytochemical yields. Phytochemical yield is the amount of bioactive plant compounds (phytochemicals) extracted from a plant sample, and it is usually expressed as a percentage of the weight of the initial plant material.

**Key Words:** *Amaranthus viridis*, contamination, *Corchorus olitorus*

## RÉSUMÉ

La fabrication de médicaments pharmaceutiques est un processus d'industrialisation souhaité, bien que la production d'eaux usées pendant ses opérations et son rejet dans les systèmes d'eau environnants puissent poser des problèmes de pollution environnementale. Les objectifs de cette étude étaient d'examiner l'effet des eaux usées de traitement industriel pharmaceutique sur la constitution phytochimique des légumes couramment consommés au Nigéria. Une étude a été menée au Jardin botanique de l'Université d'Ilorin, portant sur deux légumes couramment consommés, à savoir *Amaranthus viridis* (épinard africain) et *Corchorus olitorius* (mauve de jute) cultivés dans des sols traités avec différentes concentrations d'eaux usées pharmaceutiques (20, 40, 60, 80 et 100 %). Les analyses phytochimiques comprenaient le test pour déterminer la présence et les quantités d'alcaloïdes, de saponines, de tanins, de glycosides et de terpénoïdes. Les résultats ont révélé que la teneur en alcaloïdes était marginalement présente dans les feuilles d'épinards provenant de sols traités avec 100 % d'eaux usées, mais modérément présente dans *Amaranthus viridis* provenant d'autres traitements. L'étude a révélé que la teneur en alcaloïdes d'*Amaranthus viridis* variait de 4,22 à 9,8 mg 100 g<sup>-1</sup> ; tandis que celle en glycosides des feuilles de *Corchorus olitorius* variait de 0,50 à 2,72 mg 100 g<sup>-1</sup>. En revanche, de faibles concentrations dans les eaux usées pharmaceutiques augmentaient les composés phytochimiques, tandis que des concentrations élevées provoquaient un stress dû aux métaux lourds, entraînant une diminution du rendement phytochimique. Le rendement phytochimique correspond à la quantité de composés végétaux bioactifs (composés phytochimiques) extraite d'un échantillon végétal et est généralement exprimé en pourcentage du poids de la matière végétale initiale.

*Mots Clés :* *Amaranthus viridis*, contamination, *Corchorus olitorius*

## INTRODUCTION

The manufacture of pharmaceutical medicines is a desired industrialisation process, though the production of wastewater during its operations and its release into surrounding water systems, poses environmental pollution challenges. Consuming contaminated crops can cause excessive concentrations of contaminants from the wastewater, such as heavy metals, organic pollutants, and excess nutrients, to build up in the soil, thus hindering plant growth and causing illnesses in humans (Maukeeb *et al.*, 2022).

Heavy metals from contaminated soil tend to accumulate in leafy vegetables, such as *A. viridis* and *C. olitorius*, due to their vast surface area and root systems, which enable plants to absorb the metals from the soil (Ahmed *et al.*, 2022). The microbial population of the soil is also altered by industrial pollutants, thus reducing the quantity of helpful bacteria and fungi that are essential to plant health (Gaikwad *et al.*, 2023). Heavy metals in pharmaceutical

wastewater can hinder the development of chloroplasts and other organelles, causing abnormal plant growth and suboptimal nutrient utilisation (Rai *et al.*, 2019).

*Amaranthus viridis* (commonly known as African Spinach), and *Corchorus olitorius* (jute mallow) are two vegetables that are widely consumed as foods in many parts of the world. There are a number of health benefits derived from eating these vegetables. The leaves are a storehouse of essential phytonutrients and antioxidants (Aderibigbe *et al.*, 2020). They are rich in fiber and water contents which makes them very good for the digestive tract. Consumption of these vegetables also help to reduce cholesterol levels; and this is beneficial to those suffering from hypertension and cardiovascular diseases (Aderibigbe *et al.*, 2020).

An increasing volume of industrial wastewater is discharged into the environment, often without adequate treatment. Phytochemicals are plant based bioactive compounds produced by plants for their

protection against environmental threats (Kumar *et al.*, 2023) like predator insects, pollution and diseases. Examples of phytochemicals include alkaloids, steroids, tannins, phenols, saponins, and flavonoids.

Phytochemicals are also beneficial to human health in several ways, one of which is that some of them are known to help maintain a balanced immune system (Paudel *et al.*, 2023; Muscolo *et al.*, 2024). Some work as antimicrobial agents (Khameneh *et al.*, 2021) and others help to reduce ongoing inflammation associated with inflammatory diseases (Gonfa *et al.*, 2023).

Pharmaceutical wastewater contains a variety of pollutants and chemicals that can impact aquatic ecosystems and the therapeutic properties exhibited by plants because of their phytochemical content (Kayode-Afolayan *et al.*, 2022). The objective of this study was to examine the effect pharmaceutical industrial processing wastewater on the phytochemical constitution of commonly consumed vegetables in Nigeria.

## MATERIALS AND METHODS

**Collection of seed and identification of plants.** This study involved two commonly consumed vegetables in Nigeria; namely, *Amaranthus viridis* L. (African spinach) and *Corchorus olitorius* L. (Jute mallow). Seeds of both crops were purchased from Ministry of Agriculture, Ilorin. Samples of the plants that germinated from the purchased seeds, were collected and taken to the Department of Plant Biology, University of Ilorin Herbarium for identification. The Herbarium voucher numbers were UILH/003/1776/2022 for *Amaranthus viridis* and UILH/002/1774/2022 for *Corchorus olitorius*.

Collection of pharmaceutical industry wastewater and preparation of different concentrations of wastewater samples from a pharmaceutical industry in Nigeria, were collected in plastic containers from its outlet at the point of wastewater release before it

exits into the treatment plant. The wastewater samples were considered as 100% (wastewater). Different dilutions (concentrations) (20, 40, 60, 80, 100% and control) of the wastewater were prepared using borehole water. The borehole water had been previously analysed and results indicated that the heavy metal content was low and would not interfere with the treatments. Distilled water could not be used because a large quantity of water was required for irrigation. The preparations were used as treatments to irrigate the potted experimental plants, every other day at 100 ml per 5 kg pot for the first week after planting; and 200 ml for the second week after planting and thereafter. The control was irrigated with water, 20% dilution consisted of 20 ml of wastewater + 80 ml of water, 40% dilution was prepared with 40 ml of wastewater + 60 ml of water, 60% dilution consisted of 60 ml of wastewater + 40 ml of water, 80% dilution consisted of 80 ml of wastewater + 20 ml of water and 100% was prepared with undiluted wastewater.

**Preparation of experimental pots.** Eight seeds of *A. viridis* and *C. olitorius* were planted in plastic pots, containing 5 kg of loamy soil, three replicates. The seeds were sown after two weeks of treating the soil with the different concentrations of wastewater from the pharmaceutical industry.

The experiment was laid out in a Completely Randomised Design. The control replicates were irrigated daily with borehole water; while the other replicates were irrigated with different concentrations of the wastewater and borehole water on alternate days. Hand weeding was carried out weekly. Twelve weeks were allotted for the planting season.

**Phytochemical analysis.** Sample of leaves of *A. viridis* and *C. olitorius*, at 6 weeks after planting, were collected from healthy, and fully expanded leaves in the middle portion of the

plants, thoroughly rinsed with distilled water to remove any debris; and air dried for 14 days at room temperature (27 °C). Each sample was then ground into a fine powder, using a Kenwood blender. The sample was then packaged in air tight containers to prevent moisture absorption and contamination. They were labeled for easy identification, and kept in a clean dry place until when required for use. The samples were analysed for alkaloids, tannins, saponins, flavonoids and terpenoids using chromatographic and mass spectrometric techniques. The analytical values of each sample were obtained in triplicates.

### Qualitative phytochemical analysis

**Alkaloids.** The plant aqueous extract was prepared from leaves of *A. viridis* and *C. olitorius*. A total of 3 cm<sup>3</sup> of the plant aqueous extract was combined in a test tube, with 1 cm<sup>3</sup> of 1% HCl. After being heated for 20 minutes, the mixture was cooled and filtered. The test was then conducted using the filtrate. One milliliters of the aqueous extract received 2 drops of Meyer's reagent. Alkaloids were detected in the extract by a creamy or yellow precipitate, depending on the nature of the alkaloid. (Shaikh and Patil, 2020).

**Tannins and saponins.** A total of 2 ml of 5% ferric chloride was added into 1 ml of plant extract in a test tube, and left standing for three minutes. The appearance of dark blue/green/black colour was rated using colorimetric analysis to indicate the presence of tannins (Mussa *et al.*, 2024).

For determination of saponins, a total of 2 cm<sup>3</sup> of the extract in a test-tube was vigorously shaken with distilled water for 2 minutes and then warmed. Frothing which persisted on warming indicated the presence of saponins, while frothing failing to occur meant absence of saponins (El Aziz *et al.*, 2019).

**Flavonoids.** Equal volume of detanned water extracts of the plant was added to 5 cm<sup>3</sup> of sodium hydroxide. A yellow solution indicated

the presence of flavonoids, while other colours apart from yellow indicated the presence of other substances which are not flavonoids. (Muhammad and Abubakar, 2016).

**Terpenoids.** The presence of terpenoids was determined by mixing 0.5 ml of the plant extract with 2 ml of chloroform and concentrated sulphuric acid that had a molarity of 18M. The formation of a reddish-brown colour at the interface indicated the presence of terpenoids and colour was rated using colorimetric analysis. Absence of the reddish brown colour indicated absence of terpenoids (Mussa *et al.*, 2024).

### Quantitative phytochemical analysis

**Alkaloids.** A total of 2 g of finely ground plant sample was transferred into 100 ml beaker and 20 ml of 80% absolute alcohol was added to give a smooth paste. The mixture was transferred to a 250 ml flask and more alcohol was added to make up to the mark; and about 1 g of magnesium oxide was then added. The mixture was digested in a boiling water bath for 1 hr and 30 minutes under a refluxed air condenser with occasional shaking. The mixture was filtered while hot through a Buchner funnel. The residue was poured back into the flask and re-digested for another 30 minutes, with 50 ml alcohol; after which the alcohol was evaporated using a rotary evaporator. Distilled water was added to replace the lost alcohol.

When all alcohol had evaporated, 3 drops of 10% HCl was added. The whole solution was later transferred into 250 ml volumetric flask; 5 ml of zinc acetate solution; and 5 ml of potassium ferric cyanide solution were thoroughly mixed together to give a homogenous mixture. The flask was allowed to stand for a few minutes, filtered through a dry filter paper and 10 ml of the filtrate was transferred into a separating funnel.

The alkaloids present were extracted vigorously by shaking with five successive portions of chloroform. The residue obtained

was dissolved in 10 ml of hot distilled water and transferred into a Kjeldahl tube with the addition of 0.2 g of selenium for digestion to a clear colourless solution. The clear colourless solution was used to determine Nitrogen content, using Kjeldahl distillation apparatus. The distillate was back titrated with 0.01N HCl and the titre value obtained was used to calculate the % Nitrogen using Equation 1:

$$\% \text{ Nitrogen} = \frac{\text{TV} \times \text{AM N} \times 100}{\text{Weight of sample (mg)}}$$

..... Equation 1

Where:

TV = titre value; AM N = atomic mass of nitrogen of HCl

% Alkaloid = % Nitrogen x 3.26 (Ameen *et al.*, 2021)

**Flavonoids.** For flavonoids determination, 10 g of the plant sample was extracted with 100 ml of 80% aqueous methanol at room temperature. This was done by immersing the plant material in the aqueous methanol in an airtight container and allowing the extraction to sit for eight hours. The extract was stirred using a magnetic stirrer and then filtered through Whatman filter paper No. 42 (125 mm). The filtrate was transferred into a crucible and evaporated to dryness over a water bath; to a constant weight (Odeyemi *et al.*, 2023). The concentration of flavonoids was computed using the Equation 2:

$$\text{Flavonoids \%} = \frac{(\text{weight of crucible} + \text{residue}) - (\text{weight of crucible}) \times 100}{\text{Weight of sample analysed}}$$

..... Equation 2

**Saponin.** One gramme of dried sample that had been passed through 125 mm sieve, was transferred into a 250 ml beaker, and 100 ml of isobutyl alcohol added. The filtrate was

transferred into a 100 ml beaker containing 20 ml of 40% saturated solution of  $\text{MgCO}_3$ . About 1 ml of the colourless filtrate was pipetted into a 50 ml volumetric flask and 2 ml of 5%  $\text{FeCl}_3$  solution was added and made up to the marked level with distilled water. This was then allowed to stand for 30 minutes for a blood red colour to develop. Percentage saponin was calculated using Equation 3 (Ameen *et al.*, 2021):

$$\% \text{ Saponin} = \frac{\text{AS} \times \text{AG} \times \text{DF}}{\text{Weight of sample} \times 10,000}$$

..... Equation 3

Where:

AS = Absorbance of sample; AG = Average gradient; DF = Dilution factor and Wt of sample = Weight of sample

**Tannins.** Twenty milliliters of 50% methanol was added to 0.2 g of sample in the 50 ml beaker. The mixture was covered with parafilm and placed in a water bath at 77- 80 ° C for an hour. After cooling, the extract was filtered using a double layered Whatman No. 41 filter paper and transferred into a 100 ml volumetric flask. Twenty milliliters of distilled water, 2.5 ml Folin-Dennis reagent and 10 ml of 17%  $\text{Na}_2\text{CO}_3$  were added and mixed properly. The mixture was made up to volume with distilled water and allowed to stand for 20 minutes. A bluish-green colour develops at the end of range. The concentration of tannins was detected through the evaluation of bluish-green colour that developed, using of TGP 210 Buck atomic absorption spectrophotometer (AAS) (Ameen *et al.*, 2021)

**Data analysis.** Data collected were subjected to descriptive statistics using the Statistical Package of Social Sciences (SPSS) version 16 and Origin 7.0 and Significance Difference (LSD) at 0.05% level of significance.

## RESULTS

***Amaranthus viridis*.** Table 1 presents the findings of a qualitative phytochemical examination of *Amaranthus viridis* plant leaves in response to varying concentrations of the pharmaceutical industry wastewater. Compared to the control, the different wastewater concentrations had a significant effect ( $P < 0.05$ ) on the qualitative phytochemical analysis of *A. viridis* leaves. Alkaloids were found in all treatments in moderate amounts, with the exception of 100%, where they were found in very trace amounts. Saponin was slightly present in all treatments, except in 60% in which it was

moderately present. In the case of tannins, glycosides and terpenoids, they were slightly present in all treatments; except in the 40 and 60% wastewater treatment in which they were recorded to be moderately present.

Results of data pertaining to quantitative phytochemical analysis of *Amaranthus viridis* leaf, as affected by different concentrations of wastewater from pharmaceutical industry are presented in Table 2. The results show that the concentrations of pharmaceutical industrial wastewater significantly ( $P < 0.05$ ) affected the quantitative phytochemical analysis of *A. viridis* leaf at different treatments, compared to the control. Alkaloids, saponin, tannins, glycosides, terpenoids increased significantly

TABLE 1. Qualitative phytochemical analysis (mg 100 g<sup>-1</sup>) of *Amaranthus viridis* plant leaf as affected by different concentrations of wastewater from pharmaceutical industry

| Treatment       | Alkaloids | Saponin | Tannins | Glycosides | Terpenoids |
|-----------------|-----------|---------|---------|------------|------------|
| Control         | ++        | +       | +       | +          | +          |
| 20% wastewater  | ++        | +       | +       | +          | +          |
| 40% wastewater  | +++       | +       | ++      | ++         | ++         |
| 60% wastewater  | +++       | ++      | ++      | ++         | ++         |
| 80% wastewater  | ++        | +       | +       | +          | +          |
| 100% wastewater | +         | +       | +       | +          | +          |

Legend: + = slightly present, ++ = moderately present, +++ = highly present

TABLE 2. Quantitative phytochemical analysis (mg 100 g<sup>-1</sup>) of *Amaranthus viridis* plant leaf as affected by different concentrations of wastewater from pharmaceutical

| Treatment             | Alkaloids               | Saponin                 | Tannins                | Glycosides              | Terpenoids              |
|-----------------------|-------------------------|-------------------------|------------------------|-------------------------|-------------------------|
| Control               | 5.48±0.38 <sup>d</sup>  | 0.68±0.11 <sup>ab</sup> | 2.22±0.13 <sup>c</sup> | 3.11±0.15 <sup>c</sup>  | 0.30±0.04 <sup>bc</sup> |
| 20% wastewater        | 7.55±0.05 <sup>c</sup>  | 0.83±0.22 <sup>ab</sup> | 3.50±0.20 <sup>b</sup> | 3.83±0.23 <sup>c</sup>  | 0.58±0.03 <sup>b</sup>  |
| 40% wastewater        | 8.12±0.20 <sup>b</sup>  | 1.10±0.05 <sup>a</sup>  | 4.93±0.27 <sup>a</sup> | 4.60±0.17 <sup>b</sup>  | 0.73±0.05 <sup>ab</sup> |
| 60% wastewater        | 9.80±0.26 <sup>a</sup>  | 1.58±0.43 <sup>a</sup>  | 4.97±0.09 <sup>a</sup> | 5.69±0.06 <sup>b</sup>  | 1.12±0.22 <sup>a</sup>  |
| 80% wastewater        | 4.86±0.09 <sup>d</sup>  | 0.65±0.16 <sup>b</sup>  | 2.26±0.20 <sup>c</sup> | 2.80±0.15 <sup>a</sup>  | 0.28±0.09 <sup>c</sup>  |
| 100% wastewater       | 4.22±0.41 <sup>de</sup> | 0.58±0.08 <sup>b</sup>  | 2.16±0.18 <sup>c</sup> | 2.73±0.09 <sup>cd</sup> | 0.25±0.04 <sup>c</sup>  |
| Mean                  | 6.67                    | 0.91                    | 3.34                   | 3.80                    | 0.54                    |
| LSD <sub>(0.05)</sub> | 1.63                    | 0.51                    | 1.25                   | 2.08                    | 0.54                    |

LSD = Least Significant Difference, Means followed by the same letter(s) are not significantly different at 5 percent probability level

in plants with wastewater, with the highest value observed in *A. viridis* leaf in 60% treatments. The least values were recorded in the 80 and 100% treatments.

***Corchorus olitorius*.** The results of data pertaining to qualitative phytochemical analysis of *Corchorus olitorius* plant leaf as affected by different concentrations of wastewater from pharmaceutical industry is presented in Table 3. It was evident from the result that the various concentrations of wastewater significantly ( $P<0.05$ ) affected the qualitative phytochemical analysis of *C. olitorius* at

different treatments, compared to the control. Alkaloids were slightly present in all treatments, with the exception of 60 and 80%, in which they were moderately present. On the other hand, saponins were abundant in all treatments. Tannins, glycosides, and terpenoids were slightly present in all treatments; with the exception of 60 and 80%, where they were found to be moderately present. Results of data pertaining to quantitative phytochemical analysis (in mg 100 g<sup>-1</sup>) of *C. olitorius* plant leaf, as affected by different concentrations of wastewater from pharmaceutical industry, are presented in Table 4. It was clear that the

TABLE 3. Qualitative phytochemical analysis (mg 100 g<sup>-1</sup>) of *Corchorus olitorius* plant leaf as affected by different concentrations of wastewater from pharmaceutical industry

| Treatment       | Alkaloids | Saponin | Tannins | Glycosides | Terpenoids |
|-----------------|-----------|---------|---------|------------|------------|
| Control         | +         | +++     | +       | +          | +          |
| 20% wastewater  | +         | +++     | +       | +          | +          |
| 40% wastewater  | +         | +++     | +       | +          | +          |
| 60% wastewater  | ++        | +++     | ++      | ++         | ++         |
| 80% wastewater  | ++        | +++     | ++      | ++         | ++         |
| 100% wastewater | +         | +++     | +       | +          | +          |

Legend: + = slightly present, ++ = moderately present, +++ = highly present, — = absent

TABLE 4. Quantitative phytochemical analysis (mg 100 g<sup>-1</sup>) of *Corchorus olitorius* plant leaf as affected by different concentrations of wastewater from pharmaceutical

| Treatment             | Alkaloids              | Saponin                 | Tannins                 | Glycosides              | Terpenoids             |
|-----------------------|------------------------|-------------------------|-------------------------|-------------------------|------------------------|
| Control               | 1.48±0.28 <sup>c</sup> | 4.10±0.11 <sup>c</sup>  | 1.22±0.24 <sup>c</sup>  | 0.53±0.15 <sup>cd</sup> | 0.58±0.22 <sup>a</sup> |
| 20% wastewater        | 1.55±0.05 <sup>c</sup> | 6.10±0.12 <sup>d</sup>  | 2.50±0.20 <sup>b</sup>  | 0.91±0.23 <sup>c</sup>  | 0.69±0.10 <sup>a</sup> |
| 40% wastewater        | 2.12±0.20 <sup>b</sup> | 7.60±0.05 <sup>c</sup>  | 3.93±0.30 <sup>ab</sup> | 1.60±0.17 <sup>b</sup>  | 0.78±0.16 <sup>a</sup> |
| 60% wastewater        | 2.80±0.16 <sup>a</sup> | 9.68±0.09 <sup>b</sup>  | 4.21±0.10 <sup>a</sup>  | 1.69±0.06 <sup>b</sup>  | 0.91±0.21 <sup>a</sup> |
| 80% wastewater        | 2.86±0.09 <sup>a</sup> | 10.45±0.60 <sup>a</sup> | 4.26±0.22 <sup>a</sup>  | 2.72±0.15 <sup>a</sup>  | 0.98±0.18 <sup>a</sup> |
| 100% wastewater       | 1.22±0.11 <sup>c</sup> | 4.08±0.38 <sup>c</sup>  | 1.16±0.13 <sup>c</sup>  | 0.50±0.17 <sup>cd</sup> | 0.52±0.20 <sup>a</sup> |
| Mean                  | 2.01                   | 7.05                    | 2.88                    | 1.33                    | 0.75                   |
| LSD <sub>(0.05)</sub> | 0.48                   | 1.21                    | 0.54                    | 0.47                    | Ns                     |

LSD = Least Significant Difference; Means followed by the same letter(s) are not significantly different at 5 percent probability level; and ns = no significant difference

various concentrations of wastewater significantly ( $P < 0.05$ ) affected the quantitative phytochemical analysis of *C. olitorius* plant leaf compared to the control. In plants treated with pharmaceutical industrial wastewater, the levels of alkaloids, saponin, tannins, glycosides, and terpenoids increased dramatically. The highest value was found in the plant in the 80% treatment. The 100% wastewater treatment and control groups had the lowest values.

## DISCUSSION

Results from the qualitative phytochemical analysis showed that the alkaloid content varied from moderately to highly present in *Amaranthus viridis* from soil treated with different concentrations of pharmaceutical wastewater (Table 1). Saponin content varied from marginally to moderately present. However, in *Corchorus olitorius* alkaloid content varied from being slightly present to moderately present; while saponin content was highly present in all treatments and the control.

The tannin, glycoside and terpenoids contents were recorded to vary from slightly to moderately present (Table 1). It was observed that results from qualitative phytochemical analysis showed a reduction in content of all the phytochemicals in spinach grown in soils treated with the highest concentrations 80 and 100% wastewater and 100% wastewater in *C. olitorius*.

Results from quantitative phytochemical analysis indicated that the tannin content in *A. viridis* from soil contaminated with different concentrations of pharmaceutical wastewater varied between 2.16 - 4.97 mg 100 g<sup>-1</sup> (Table 3); while the saponin content ranged from 0.58 - 1.58 mg 100 g<sup>-1</sup>. Results from this study also showed that the glycoside content in *A. viridis* ranged from 2.73 - 5.69 mg 100 g<sup>-1</sup> (Table 3), while the alkaloid content was between 4.22 and 9.80 mg 100 g<sup>-1</sup>. In *C. olitorius* alkaloid content was between 1.22 - 2.86 mg 100 g<sup>-1</sup>, quite lower than in the *A. viridis*. On the other

hand, saponin content ranged from 4.08 - 10.45 mg 100 g<sup>-1</sup> (Table 4).

The results from the qualitative and quantitative phytochemical analysis indicated that the phytochemical content increased as concentration of pharmaceutical wastewater increased from 20, 40 and 60%. However, at the highest wastewater concentrations (80% and 100% in *A. viridis*), (and 100% in *C. olitorius*), the phytochemical content reduced and had the lowest values. The quality and quantity of phytochemicals were recorded to have significantly reduced in vegetables from soils contaminated with the highest concentration of pharmaceutical wastewater, thereby also resulting in a reduction in the attendant benefits derived from the phytochemicals. This could also be attributed to the fact that the plants increased their rate of producing phytochemicals in an attempt to strengthen their immune system to resist the effects of environmental changes caused by stress caused by the contents of pharmaceutical wastewater (Adele *et al* 2024).

The term “soapy plants” refers to plants that, when disturbed in aqueous solutions, produce foam and saponins, which are also known to have antibacterial qualities, are responsible for these plants’ capacity to foam (Kunatsa and Katere, 2021). Many plants contain the lathering components known as saponins, but *C. olitorius* has a notably high concentration of these compounds, which explains why its saponin content is larger than that of *A. viridis* (Table 4).

Some plant families inherently generate a higher concentration of phytochemicals compared to others (Pawase *et al.*, 2024). *Amaranthus*, belongs to the Amaranthaceae family (Adegbola *et al.*, 2020) and *Amaranthus viridis* and other leafy green plants in the Amaranthaceae family are excellent providers of nutritional fiber, vitamins, minerals and phytochemicals such as alkaloids, tannins, glycosides, and terpenoids (Fouad *et al.*, 2024). These phytochemicals

are additionally found in *C. olitorius*, a member of the Malvaceae family (Atalar *et al.*, 2023); although in smaller amounts than in *A. viridis* and this should explain why *A. viridis* has higher levels of alkaloids, tannins, glycosides, and terpenoids than *C. olitorius* (Table 2).

Stressful environmental factors, such as the presence of pharmaceutical wastewater in the soil, can encourage plants to produce more phytochemicals, which is likely why plants from soils treated with 20, 40, 60 and 80% contain more phytochemicals (Tables 2 and 4). Although a diet high in phytochemical content can help prevent chronic diseases like diabetes, heart disease, and cancer (Aune *et al.*, 2017), it is important to take into account potential drawbacks like potential toxicity at high doses and potential interactions with medications depending on the specific phytochemicals involved (Oluyori *et al.*, 2020). Nevertheless, an increase in phytochemical production in plants could have significant positive implications for human health by potentially leading to a higher intake of antioxidants, anti-inflammatory compounds, and other bioactive substances.

Benefits include decreased disease risk (because of their anti-inflammatory, anti-cancer, and antioxidant qualities, a higher intake of phytochemicals may reduce the risk of chronic diseases), enhanced immune function and improved detoxification as some phytochemicals can support the body's detoxification processes by activating enzymes involved in the breakdown of toxins (Kumar *et al.*, 2023) and the possibility of developing new medications since researching phytochemicals may result in the creation of novel medications due to their bioactive characteristics (Kumar *et al.*, 2023). However, some phytochemicals are generally thought to be safe in regular dietary levels, yet in excessive concentrations, they can become toxic (Oluyori *et al.*, 2020), which is a possible concern.

A decrease in phytochemical production in plants would likely result in a reduced intake

of beneficial compounds for humans, potentially increasing the risk of chronic diseases like cancer, cardiovascular diseases, and inflammatory conditions. Phytochemicals also act as antioxidants and anti-inflammatory agents and can help regulate various bodily functions. Because phytochemicals are often a plant's natural defense mechanism against herbivores and pathogens (Adele *et al.* 2024), a decrease in phytochemical production in plants can have a significant impact on the environment (Corona-España *et al.*, 2024). This is mainly because it can upset the delicate balance of plant-herbivore interactions, which could result in increased herbivory (Fernandez-Conradi *et al.*, 2022), resulting in altered ecosystem dynamics, and potential impacts on biodiversity. Additionally, as phytochemicals are often a plant's natural defense mechanism against herbivores and pathogens, a decrease in phytochemical production may impede the nutritional and medicinal benefits that humans derive from plant-based foods (Frances and Nwakuche, 2024).

Certain phytochemicals help draw pollinators and a reduction in their output may have an effect on pollination rates and plant reproduction (Bernklau *et al.*, 2019) and decreased medical value as a decrease in these molecules could lessen the therapeutic potential of many plants with high phytochemical content, which are employed for medicinal purposes (Frances *et al.*, 2023). Therefore, plant defense mechanisms, interactions with herbivores and pollinators, soil ecology, and possibly the dynamics of the food web can all be significantly impacted by an increase in phytochemical production.

Some phytochemicals can offer protection against stressors like pests and UV radiation (Saleem *et al.*, 2020); but excessive production may change plant communities and have adverse effects on specific organisms in the ecosystem. Because pharmaceutical processes are complex, pharmaceutical wastewater has certain characteristics like high concentration

and poor biodegradability, which makes advanced treatment of pharmaceutical wastewater essential and there are numerous types of advanced treatment that can successfully improve the quality of pharmaceutical wastewater (Guo *et al.*, 2017). The present study recommends the use of pharmaceutical wastewater for irrigation after it has undergone advanced treatment.

### CONCLUSION

Pharmaceutical wastewater, when used for irrigation of popularly consumed vegetables in Nigeria, causes considerable alteration in the phytochemical contents of *A. viridis* and *C. olitorius* which can affect its nutritive safety of humans and the environment. *Amaranthus viridis* was more affected than *C. olitorius*, because phytochemical content significantly reduced in plants from 80 and 100% treatment in *A. viridis* but only in *C. olitorius* plants from 100% treatments. Communities located near pharmaceutical industries frequently use pharmaceutical wastewater for domestic tasks like farming. Even though increased phytochemical production can give plants advantages in terms of defense and adaptation, it is important to take into account the possible ecological effects on other organisms and the overall ecosystem balance that may arise from the alteration of phytochemical levels in plants when vegetables are irrigated with pharmaceutical wastewater. In view of this, advanced treatment of pharmaceutical wastewater is necessary by pharmaceutical industries.

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