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THE EFFECTS OF FOLIAR APPLICATION OF SOME SELECTED PHYTOHORMONES ON THE PROXIMATE, PHYTOCHEMICAL, AND MINERAL COMPOSITIONS OF ROSELLE (*HIBISCUS SABDARIFFA* L.) LEAVES

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ABSTRACT

Recent research has shown that agricultural crops can be improved through the use of phytohormones. In this study, we tested the effects of coconut water (CW), Gibberellic Acid (GA_3), Indole Acetic Acid (IAA), 6-Benzylaminopurine (BAP), and Naphthalene Acetic Acid (NAA) through foliar spray, on the seedlings of *Hibiscus sabdariffa* L. (Roselle) to understand the best treatment to improve nutritional quality. Roselle seedlings were raised in 30cm × 19cm polythene bags and arranged using a completely randomized design in a screened house for 4 months. The phytohormones were applied as treatments on seedlings both singly and combined in the range of 10-20 (%) for CW and 50-200 (mg/L) for the other treatments. Proximate, vitamin C and mineral elements were determined using standard analytical procedures, while phytochemical screening was carried out on plant extracts. The best nutritional composition was achieved using 200mg/L GA_3 and 20% CW with crude protein, fibre, vitamin C, and flavonoid contents of 4.17, 1.36, 46.7, and 3.72 mg/100g respectively. Treatments with 20% CW also recorded the highest calcium and magnesium contents of 193.20 and 89.20 mg/100 g respectively. Generally, the single hormone treatments outperformed the combined hormone treatments. The exceptional performance of CW proved that CW has the potential to improve the nutritional content of agricultural crops and can also serve as a cheap and accessible bio-stimulant for agricultural crops such as *H. sabdariffa*.

Keywords: foliar spray, phytohormones, phytochemical, roselle, proximate

INTRODUCTION

Roselle (*Hibiscus sabdariffa* L.) belongs to the family Malvaceae, and it is mostly cultivated in the tropical and sub-tropical regions (Borrás-Linares *et al*., 2015; Stella *et al*., 2023). The growth requirement for *H. sabdariffa* L. ranges from 4 to 8 months, with minimum night-time temperature of 20 ^{o}C, as well as 13 hours of sunlight and a monthly rainfall ranging from 130-250 mm in the early months to prevent early flowering (Da-Costa-Rocha, 2014; Paraiso *et al*., 2020). *H. sabdariffa* L. are mostly cultivated for their unique calyces, which are very rich in anthocyanins, flavonoids and many other secondary metabolites. These anthocyanins are composed of polyphenols responsible for the colour in the calyx of roselle (Buljeta *et al*., 2022; Stella *et al*., 2023) and are mostly used in culinary and pharmaceutical industries (USDA, 2007; Eslaminejad and Zakaria, 2011). The use of plants in pharmaceutical and nutraceutical industries has always been a trend and this is largely due to the vital minerals and phytochemicals inherent in plants, which are of high pharmacological importance (Anyasor *et al*., 2011; Khan *et al*., 2023).

In some regions in China, *H. sabdariffa* is cultivated mainly for its medicinal properties while in some part of Africa, specifically in the West, the leaves and seeds of *H. sabdariffa* are cultivated mainly for use in meals (Da-Costa-Rocha, 2014; Boukerche *et al*., 2023). For example, in Lubumbashi area of Congo, the leaves of *H. sabdariffa* is the economic important part of the plant as it is consumed as vegetable (Shakalenga *et al*., 2021). Also, in Nigeria, the calyces are processed and consumed as beverages or tea, while the seeds are processed as seasoning, popularly called "Dadawa" in the Northern part of Nigeria (Borrás-Linares *et al*., 2015; Daudu *et al*., 2015; Hinojosa-Gómez *et al*., 2018). In recent years, the need for the consumption of vegetables has gathered followership due to the high increase in obesity and cancer-causing processed foods. However, while several studies have been carried out on the calyces of *H. sabdariffa*, the same cannot be said for the leaves. The studies on the leaves are rarely or limitedly available despite their continuous use as vegetable in meals or as medicines. In lieu of this, this study was carried out to assess the physico-chemical properties of *H. sabdariffa* leaves as influenced by the foliar spraying of some selected phytohormones.

MATERIALS AND METHODS

Study Area

This research was carried out at the Botanical Garden of Federal University of Agriculture, Abeokuta, Ogun State, Nigeria (FUNAAB).

Seed collection and viability test.

Seeds of Roselle (*Hibiscus sabdariffa* L.) were obtained from local farmers at Suleja market, Abuja and the viability test of seeds were carried out using the simple floating method of the International Seed Testing Association (ISTA, 1976). The viability of the seeds used in the experiment were very high and ranged from 80 to 100 %.

Initial germination test

Routine germination tests were carried out using randomly selected seeds of *H. sabdariffa* L. They were spread on a moist filter paper and gently placed in Petri dishes. The germination was monitored and calculated using the formula (Total number of seeds germinated/ initial total number of seeds multiplied by 100).

Collection and preparation of soil

The soil sample used for raising the seedlings is a sandy-loamy soil, and it was collected at 20 cm depth at the Botanical Garden, FUNAAB, after which it was sieved to

remove pebbles and stones, and then dispensed into dark polythene bags for planting.

Soil pH determination

Twenty milliliters (20 ml) of distilled water were added to 20 g of sieved air-dried soil in a 50 ml beaker. It was allowed to stand for thirty minutes and stirred occasionally with a glass rod. The pH meter (HANNA Instruments) was standardized by dipping its electrode into buffers of pH 4 and pH 9 (standards), after which it was dipped into a partly settled suspension and the reading on the pH meter (6.54) was recorded.

Experimental site and seedling raising

The seedlings of *H. sabdariffa* L. was raised from treated seeds using perforated black polythene bag of 37cm by 31cm, in the screened house at the Botanical Garden, FUNAAB. Seedlings were thinned to three per polythene bag after emergence (Plate 1). This study was carried out using Complete Randomized Design (CRD).

Treatments used for the experiment

The foliage of the seedlings of *Hibiscus sabdariffa* L. were sprayed separately with Gibberellic acid (GA_3), 6-Benzylaminopurine (BAP), coconut water (CW), Naphthalene Acetic Acid (NAA) and Caffeic acid (an inhibitor), while distilled water treatment served as the control. There were both single and combined hormones treatments. For the single hormone treatments, concentrations of 10, 15, 20 (% CW), and 50, 100, and 200 (mg/L) for GA_3, IAA, NAA, BAP, Caffeic acid were used. While for the combined treatments, 50mg/L GA_3 + 10% CW, 100mg/L GA_3 + 15% CW, 200 mg/L GA_3 + 20% CW, 50mg/L GA_3 + 50mg/L IAA, 100mg/L GA_3 + 100 mg/L IAA, 200mg/L GA_3 + 200mg/L IAA, 50mg/L GA_3+ 50mg/L NAA, 100mg/L GA_3+ 100 mg/L NAA, 200mg/L GA_3+ 200mg/L NAA, while distilled water was used as the control.

Plate 1: Experimental site (Screenhouse at the botanical gardens, Federal University of Agriculture, Abeokuta).

Qualitative and Quantitative Analysis of Phytochemicals in *Hibiscus sabdariffa* L. Plants

The leaf extracts were screened qualitatively and quantitatively for their alkaloid, saponin, flavonoid, tannin, terpene, and phenolic contents.

Qualitative phytochemical screening

The leaves of *Hibiscus sabdariffa* was prepared by cutting the leaves into smaller bits and air dried for three weeks, after which they were pulverized into powder, using electric blender. Ten grams of each powder were dissolved in 70ml of absolute ethanol and acetone separately and allowed to stand for 72 hours, after which filtration was carried out. The filtrates were subjected to alkaloid, flavonoids, tannins, terpenes and phenolic tests according to the methods described by Ciulci (1994), while saponins was tested according to the frothing method described by Usman and Ismail (2023).

Quantitative phytochemical screening

Quantitative analysis of alkaloids

The determination of alkaloids was carried out according to the methods of Sofowora (2008). The % N was converted to Percentage % Total Alkaloid by multiplying by a factor of 3.26.

Therefore, % Total Alkaloid = %N × 3.26.

Quantitative analysis of saponin

The saponin determination was carried out according to the method described by Usman and Ismail (2023). The

absorbance of the sample and the standard saponin solutions were read after the colour development in a Jenway V6300 spectrophotometer at a wavelength of 380 nm.

% Saponin was calculated using the formular:

$$\% \text{ Saponin} = \frac{\text{Absorbance of sample} \times \text{Average gradient factor} \times \text{Dilution factor}}{\text{Wt.sample} \times 10{,}000}$$

Quantitative analysis of flavonoid

The determination of flavonoids was carried out according to the method described by Sofowora (2008), and the absorbance of magenta red coloration of sample and standard solutions were read on a digital Jenway V6300 spectrophotometer at a wavelength of 520nm. The percentage flavonoid was calculated using the formular:

$$\% \text{ Flavonoids} = \frac{\text{Absorbance of sample} \times \text{Average gradient factor} \times \text{Dilution factor}}{\text{Wt.sample} \times 10{,}000}$$

Quantitative analysis of tannin

Tannin contents was determined according to the method described by Ciulci (1994). Percentage (%) Tannin was calculated using the formular:

$$\% \text{ Tannin} = \frac{\text{Absorbance of sample} \times \text{Average gradient factor} \times \text{Dilution factor}}{\text{Wt.sample} \times 10{,}000}$$

Quantitative analysis of terpene

Terpene was determined according to the method of Sofowora (2008).

The percentage Terpene was calculated using the formular below:

$$\% \text{ Terpene} = \frac{\text{Absorbance of sample} \times \text{Average gradient factor} \times \text{Dilution factor}}{\text{Wt.sample} \times 10{,}000}$$

Quantitative Analysis of Phenol

The determination of phenol was carried out according to the method described by Robert (2010). Percentage yield of phenol was calculated by the formular:

$$\% \text{ Yield Residue} = \frac{\text{weight of residue} \times 100}{\text{Total weight of mixture of ether and residue}}$$

Determination of crude protein

The determination of crude protein content was carried out using micro-kjeldal method as described by A.O.A.C. (2015).

Determination of crude fibre

The crude fibre was determined according to the method of A.O.A.C. (2005). The percentage crude fiber was calculated by using the following formula:

$$\text{Percentage Crude Fiber} = \frac{A - B \times 100}{\text{Weight of Sample}}$$

Where: A = weight of Crucible and residue

B = weight of crucible and ash

Determination of chlorophyll content

The chlorophyll contents were determined using the method of Witham *et al*. (1971) and A.O.A.C. (2015). The amount of chlorophyll present was calculated and expressed as (mg) of chlorophyll per gram of leaf tissue using the following equation;

$$\text{Mg Total Chlorophyll Tissue} = \frac{\text{O.D.652} \times 1000}{34.5} \times \frac{\text{v}}{1000 \times \text{w}}$$

Where,

O. D. = Optical Density reading of the chlorophyll extract at 652 nm.

V = The final value of the 80 % acetone-chlorophyll extract (100 ml).

W = The fresh weight in grams of the leaf tissue extracted (1g).

Determination of Vitamin C content

The determination of vitamin C was carried out using the indophenol method described by A. O. A. C. (2005).

Determination of minerals elements

The determination of the mineral elements started with the digestion of the Roselle leaves with Perchloric acid according to the method described by A.O.A.C. (2015). Then, the calcium, magnesium and sodium were determined by Atomic Absorption Spectrometer, while phosphorus and potassium were determined using colorimeter and flame photometer respectively, according to the methods described by A. O. A. C. (2015).

Statistical Analysis

The data was subjected to Analysis of Variance (ANOVA) and means were separated using Duncan Multiple Range Test (DMRT) at $P<0.05$.

RESULTS

Qualitative Analysis of Some Phytochemicals in *Hibiscus sabdariffa* Plants

Alkaloids and tannins were present in all the treatments (both single and combined). Others such as saponins, terpenes, flavonoids and phenols were also found present across the treatments, but some were recorded to be present in trace quantities while no activity were recorded in some cases (Table 1 and 2).

Effects of single and combined plants hormone treatments on the phytochemical contents of *H. Sabdariffa* seedlings at 10 W.A.P.

The treatments considered for this analysis were 15 single hormone treatments, 9 combined hormone treatments, 3 treatments for negative control and 1 treatment for positive control and were measured in (mg/100 g) (Table 3 and 4). For Alkaloids treatments with 20 % CW and 200 mg/L IAA showed highest values of 2.43 ± 0.024 and 2.44 ± 0.023 respectively. The lowest value was obtained in 50 mg/L caffeic acid treatment (Table 4). For Saponin, treatment with 200mg/L NAA had the highest value of 3.26 ± 0.001 (Table 3), while distilled water treatment showed the lowest value (Table 4). Flavonoids were shown to be highest in treatments with 20 % CW, 200 mg/L NAA, and 100 mg/L BAP with 3.57 ± 0.001, 3.32 ± 0.002, and 3.72 ± 0.002 respectively and with no significant difference. Tannin was highest in 50 mg/L NAA, followed by treatments with 100 mg/L BAP and 100 mg/L NAA, while the lowest value was recorded in 50 mg/L Caffeic acid. As for terpenes, the highest value of 2.68 ± 0.025 was shown in 50 mg/L NAA, while the lowest value of 1.23 ± 0.013 was recorded in 100 Mg/L GA_3 + 100 Mg/L IAA (Table 2b). Phenol has its highest value of 1.75 ± 0.018recorded in 50 mg/L NAA and the lowest value of 0.13 ± 0.013 in 15 % CW.

Effects of single and combined plant hormone treatments on the crude protein, crude fibre, vitamin C, chlorophyll A and B of *H. sabdariffa* plants at 10 W.A.P.

In the single plant hormone treatments, highest crude protein content of 4.68 mg/100g was obtained in 200 mg/L IAA treatment, while the lowest crude protein of 3.44 mg/100g was recorded in both 100 mg/L GA_3 and 15% CW treatments (Table 5 and 6). There was no significant difference in the crude fibre contents among the treatments ($p> 0.05$), However, there were significant differences across the treatments ($p< 0.05$) as regards vitamin C contents, with the highest value of 54.6mg/100g obtained in 200mg/L NAA

treatment, and the lowest value of 38.5mg/100g in 200mg/L BAP treatment (Table 5). Chlorophyll A content was highest in 50 mg/L NAA treatment, while the lowest content was obtained in 50 mg/L GA_3 treatment.

Table 1: Phytochemical screening of some phytochemicals in *H. Sabdariffa* leaves using single hormone treatments

Single Hormones Treatments	Alkaloids	Saponins	Flavonoids	Tannin	Terpenes	Phenol
10% CW	++	++	++	++	+	+
15% CW	+++	++	++	++	+	++
20% CW	++	++	++	+	-	+
50 mg/L GA_3	++	++	++	++	-	++
100 mg/L GA_3	++	-	++	++	++	+
200mg/L GA_3	+	++	+	++	++	++
50 mg/L IAA	++	++	++	+	+	++
100 mg/L IAA	++	++	++	++	+	++
200mg/L IAA	++	++	++	++	++	++
50 mg/L BAP	++	-	+	++	++	++
100 mg/L BAP	+++	++	++	++	++	++
200mg/L BAP	++	-	++	++	-	++
50 mg/L NAA	++	++	++	+	++	++
100 mg/L NAA	++	++	+	++	+	+
200mg/L NAA	+	++	++	++	++	++

***Key:** (+++) = Highly present; (++) = moderately present; (+) = slightly present; (−) = Absent

Table 2: Phytochemical screening of some phytochemicals in *H. Sabdariffa* seedlings using combined hormone treatments

Combined hormones treatments	Alkaloids	Saponins	Flavonoids	Tannin	Terpenes	Phenol
50 mg/L GA_3+ 10% CW	++	-	++	++	+	++
100 mg/L GA_3+ 15% CW	++	++	++	++	-	++
200mg/L GA_3+ 20% CW	++	++	++	++	++	++
50 mg/L GA_3+ 50 mg/L IAA	++	-	++	++	++	++
100 mg/L GA_3+ 100 mg/L IAA	++	++	++	+	-	++
200mg/L GA_3+ 200mg/L IAA	++	+	++	++	-	++
50 mg/L GA_3+ 50 mg/L NAA	++	++	++	++	+	++
100 mg/L GA_3+ 100 mg/L NAA	++	++	+	++	++	++
200mg/L GA_3+ 200mg/L NAA	++	++	-	++	++	++
50 mg/L Caffeic acid	++	++	++	++	-	-
100 mg/L Caffeic acid	++	++	++	++	++	++
200mg/L Caffeic acid	++	++	++	++	+	++
Distilled water	++	++	++	++	++	++

***Key:** (+++) = Highly present; (++) = moderately present; (+) = slightly present; (−) = Absent

Table 3: Mean value of some phytochemical contents ± S.E. of *H. sabdariffa* given single hormone treatments at 10 WAP

Single Hormone Treatments	Alkaloids	Saponin	Flavonoids	Tannins	Terpenes	Phenol
10% CW	1.85 ± 0.015^{ef}	2.40 ± 0.006^{efg}	2.91 ± 0.001^{de}	0.19 ± 0.001^{e}	1.46 ± 0.013^{gh}	0.14 ± 0.021^{h}
15% CW	1.80 ± 0.008^{ef}	2.32 ± 0.001^{fgh}	3.33 ± 0.001^{c}	0.15 ± 0.011^{f}	1.52 ± 0.033^{fgh}	0.13 ± 0.013^{i}
20% CW	2.43 ± 0.024^{a}	2.00 ± 0.001^{ij}	3.57 ± 0.001^{bc}	0.14 ± 0.001^{f}	1.31 ± 0.005^{i}	0.15 ± 0.012^{h}
50 mg/L GA_3	1.88 ± 0.002^{ef}	2.36 ± 0.003^{fgh}	2.60 ± 0.005^{ef}	0.22 ± 0.012^{cd}	2.14 ± 0.002^{de}	1.11 ± 0.032^{e}
100 mg/L GA_3	1.79 ± 0.011^{ef}	2.40 ± 0.004^{efg}	3.16 ± 0.002^{cd}	0.22 ± 0.003^{cd}	2.12 ± 0.004^{de}	1.07 ± 0.013^{ef}
200mg/L GA_3	2.17 ± 0.021^{d}	2.28 ± 0.002^{fgh}	2.77 ± 0.088^{ef}	0.17 ± 0.003^{e}	1.86 ± 0.012^{f}	1.12 ± 0.043^{e}
50 mg/L IAA	2.40 ± 0.008^{ab}	2.51 ± 0.003^{def}	3.26 ± 0.001^{c}	0.22 ± 0.002^{cd}	1.61 ± 0.031^{fg}	1.11 ± 0.022^{e}
100 mg/L IAA	2.36 ± 0.008^{abc}	2.66 ± 0.001^{de}	2.72 ± 0.021^{ef}	0.25 ± 0.005^{c}	1.34 ± 0.022^{h}	1.11 ± 0.013^{e}

200mg/L IAA	2.44 ± 0.023^{a}	2.42 ± 0.001^{efg}	3.41 ± 0.001^{bc}	0.17 ± 0.015^{e}	1.59 ± 0.012^{fg}	1.21 ± 0.001^{d}
50 mg/L BAP	2.11 ± 0.026^{de}	2.71 ± 0.002^{cd}	3.48 ± 0.001^{bc}	0.21 ± 0.023^{cd}	2.17 ± 0.017^{d}	1.47 ± 0.014^{c}
100 mg/L BAP	2.37 ± 0.083^{abc}	2.32 ± 0.001^{cde}	3.72 ± 0.002^{a}	0.32 ± 0.002^{a}	2.07 ± 0.015^{ef}	1.68 ± 0.002^{b}
200mg/L BAP	1.94 ± 0.015^{def}	2.42 ± 0.067^{efg}	2.51 ± 0.002^{f}	0.19 ± 0.041^{e}	1.68 ± 0.019^{fg}	1.39 ± 0.011^{cd}
50 mg/L NAA	1.81 ± 0.024^{ef}	2.67 ± 0.001^{de}	2.71 ± 0.001^{bcd}	0.33 ± 0.061^{a}	2.68 ± 0.025^{a}	1.75 ± 0.018^{a}
100 mg/L NAA	2.26 ± 0.011^{cd}	3.12 ± 0.001^{b}	3.64 ± 0.001^{ab}	0.32 ± 0.031^{a}	2.59 ± 0.022^{ab}	1.52 ± 0.031^{bc}
200mg/L NAA	2.36 ± 0.008^{abc}	3.26 ± 0.001^{ab}	3.32 ± 0.002^{c}	0.29 ± 0.021^{ab}	1.88 ± 0.042^{f}	1.42 ± 0.010^{c}

*Means followed by the same letters on the same column are not significantly different according to Duncan Multiple Range Test at 5% probability

*GA_3 = Gibberellic Acid ; NAA = Naphthalene Acetic acid; IAA = Indole Acetic Acid; and BAP = 6-Benzyl- amino purine.

Table 4: Mean value of some phytochemical contents ± S.E. of *H. sabdariffa* given single hormone treatments at 10 WAP

Combined hormone treatments	Alkaloids	Saponin	Flavonoids	Tannins	Terpenes	Phenol
50 Mg/L GA_3 + 10% CW	2.41 ± 0.006^{a}	3.05 ± 0.332^{bc}	2.59 ± 0.001^{de}	0.26 ± 0.011^{c}	2.27 ± 0.014^{bc}	1.06 ± 0.019^{cd}
100 Mg/L GA_3 + 15% CW	2.32 ± 0.066^{ab}	1.98 ± 0.001^{def}	2.24 ± 0.002^{ef}	0.34 ± 0.013^{ab}	2.34 ± 0.016^{bc}	1.14 ± 0.014^{c}
200mg/L GA_3 + 20% CW	2.11 ± 0.040^{c}	3.21 ± 0.001 ^{Ab}	3.36 ± 0.001^{b}	0.25 ± 0.016^{c}	1.57 ± 0.010^{de}	1.12 ± 0.013^{c}
50 Mg/L GA_3 + 50 Mg/L IAA	2.03 ± 0.013^{d}	2.45 ± 0.002^{cd}	2.74 ± 0.001^{d}	0.22 ± 0.012^{cd}	1.46 ± 0.011^{fg}	1.31 ± 0.011^{a}
100 Mg/L GA_3 + 100 Mg/L IAA	2.15 ± 0.006^{bc}	2.61 ± 0.005^{cd}	2.52 ± 0.001^{de}	0.20 ± 0.021^{cd}	1.23 ± 0.013^{g}	1.24 ± 0.001^{bc}
200mg/L GA_3 + 200mg/L IAA	2.31 ± 0.006^{ab}	2.58 ± 0.002^{cd}	3.17 ± 0.001^{bc}	0.25 ± 0.023^{c}	1.61 ± 0.001^{ef}	1.24 ± 0.021^{bc}
50 Mg/L GA_3 + 50 Mg/L NAA	2.26 ± 0.017^{bc}	2.35 ± 0.001^{cd}	2.57 ± 0.001^{de}	0.37 ± 0.012^{a}	2.47 ± 0.002^{ab}	0.27 ± 0.010^{e}
100 Mg/L GA_3 + 100 Mg/L NAA	2.06 ± 0.006^{d}	2.11 ± 0.001^{de}	2.72 ± 0.332^{d}	0.36 ± 0.011^{ab}	1.97 ± 0.002^{de}	0.27 ± 0.029^{e}
200mg/L GA_3 + 200mg/L NAA	1.99 ± 0.004^{d}	3.21 ± 0.001^{ab}	3.51 ± 0.002^{a}	0.35 ± 0.001^{ab}	2.66 ± 0.033^{a}	0.32 ± 0.031^{e}
50 Mg/L Caffeic acid	1.77 ± 0.035^{e}	2.71 ± 0.001^{c}	3.17 ± 0.001^{bc}	0.11 ± 0.006^{e}	2.16 ± 0.011^{cd}	0.98 ± 0.015^{de}
100 Mg/L Caffeic acid	1.80 ± 0.006^{de}	2.86 ± 0.002^{c}	2.94 ± 0.001^{cd}	0.12 ± 0.004^{e}	2.07 ± 0.021^{d}	1.10 ± 0.014^{cd}
200mg/L Caffeic acid	1.85 ± 0.008^{de}	3.30 ± 0.001^{a}	2.24 ± 0.002^{ef}	0.21 ± 0.009^{cd}	2.15 ± 0.012^{cd}	1.27 ± 0.015^{b}
Distilled Water	2.32 ± 0.013^{ab}	1.96 ± 0.001^{def}	2.17 ± 0.001^{f}	0.25 ± 0.002^{c}	2.41 ± 0.015^{ab}	0.19 ± 0.022^{f}

*Means followed by the same letters on the same column are not significantly different according to Duncan Multiple Range Test at 5% probability

*GA_3 = Gibberellic Acid ; NAA = Naphthalene Acetic acid; IAA = Indole Acetic Acid; and BAP = 6-Benzyl- amino purine.

Table 5: Mean crude protein, crude fibre, vitamin C, chlorophyll A and B ± S.E. of *H. sabdariffa* given single hormone treatments at 10 weeks after planting (WAP)

Single hormone treatments	Crude protein (mg/100g)	Crude fibre (mg/100g)	Vitamin c (mg/100g)	Chlorophyll A (mg/100g)	Chlorophyll B (mg/100g)
10% CW	3.55 ± 0.029^{cd}	1.48 ± 0.020^{bc}	51.7 ± 0.799^{ijkl}	41.0 ± 0.535 ^{hij}	37.8 ± 0.320^{c}
15% CW	3.44 ± 0.015^{f}	1.41 ± 0.007^{cd}	54.2 ± 0.099^{ab}	43.1 ± 0.176^{cd}	38.5 ± 0.361^{bc}
20% CW	4.22 ± 0.092^{bc}	1.37 ± 0.007^{cde}	50.9 ± 0.366^{ab}	42.8 ± 0.307^{cd}	40.7 ± 0.328^{b}
50 mg/L GA_3	3.60 ± 0.003^{cd}	1.59 ± 0.010^{ab}	39.8 ± 0.167^{ef}	28.4 ± 0.300^{h}	23.6 ± 0.578^{h}
100 mg/L GA_3	3.44 ± 0.020^{f}	1.40 ± 0.023^{cd}	42.6 ± 0.233^{cde}	34.2 ± 0.173^{efg}	30.9 ± 0.451^{de}
200mg/L GA_3	4.17 ± 0.041^{bc}	1.36 ± 0.012^{cde}	46.7 ± 0.200^{bc}	33.1 ± 0.351^{efg}	30.2 ± 0.361^{de}
50 mg/L IAA	4.60 ± 0.015^{ab}	1.40 ± 0.003^{cd}	43.6 ± 0.320^{cde}	36.1 ± 0.270^{ef}	30.9 ± 0.116^{de}
100 mg/L IAA	4.52 ± 0.015^{ab}	1.28 ± 0.012^{f}	50.6 ± 0.047^{ab}	35.8 ± 0.569^{ef}	33.6 ± 0.538^{de}
200mg/L IAA	4.68 ± 0.044^{a}	1.56 ± 0.007^{ab}	52.5 ± 0.247^{ab}	33.2 ± 0.310^{efg}	29.2 ± 0.246^{def}
50 mg/L BAP	4.05 ± 0.050^{bc}	1.46 ± 0.012^{bc}	50.5 ± 0.100^{ab}	37.9 ± 0.407^{e}	36.0 ± 0.184^{cd}
100 mg/L BAP	4.36 ± 0.021^{bc}	1.35 ± 0.012^{cde}	49.4 ± 0.207^{bc}	40.2 ± 1.059^{cde}	39.7 ± 0.580^{bc}
200mg/L BAP	3.71 ± 0.028^{cd}	1.59 ± 0.010^{ab}	38.5 ± 0.217^{f}	43.2 ± 0.764^{cd}	40.8 ± 0.447^{b}
50 mg/L NAA	3.46 ± 0.045^{ef}	1.28 ± 0.012^{f}	49.7 ± 0.587^{bc}	50.1 ± 0.820^{a}	42.8 ± 0.153^{a}
100 mg/L NAA	4.33 ± 0.021^{bc}	1.33 ± 0.007^{de}	51.4 ± 0.220^{ab}	47.5 ± 0.42^{b}	40.4 ± 0.219^{b}
200mg/L NAA	4.52 ± 0.015^{ab}	1.42 ± 0.012^{cd}	54.6 ± 0.053^{a}	44.3 ± 0.314^{c}	37.9 ± 0.347^{c}

*Means followed by the same letters on the same column are not significantly different according to Duncan Multiple Range Test at 5% probability

*GA_3 = Gibberellic Acid ; NAA = Naphthalene Acetic acid; IAA = Indole Acetic Acid; and BAP = 6-Benzyl- amino purine.

Table 6: Mean crude protein, crude fibre, vitamin C, chlorophyll A and B ± S.E. of *H. sabdariffa* given combined hormone treatments at 10 weeks after planting (WAP)

Combined hormone Treatments	Crude protein (mg/100g)	Crude fibre (mg/100g)	Vitamin c (mg/100g)	Chlorophyll A (mg/100g)	Chlorophyll B (mg/100g)
50 mg/L GA_3 + 10% CW	4.62 ± 0.012^{a}	1.61 ± 0.007^{a}	50.7 ± 0.033^{ab}	29.9 ± 0.167^{ef}	26.1 ± 0.374^{e}
100 mg/L GA_3 + 15% CW	4.56 ± 0.023^{ab}	1.52 ± 0.015^{b}	48.6 ± 0.047^{bc}	39.9 ± 0.440^{bc}	33.1 ± 0.404^{bc}
200mg/L GA_3 + 20% CW	4.05 ± 0.077^{h}	1.47 ± 0.006^{bc}	52.1 ± 0.020^{ab}	42.1 ± 0.319^{b}	38.5 ± 0.238^{b}
50 mg/L GA_3 + 50 mg/L IAA	3.89 ± 0.024^{bc}	1.36 ± 0.006^{d}	47.9 ± 0.333^{bc}	37.9 ± 0.517^{bc}	35.5 ± 0.665^{bc}
100 mg/L GA_3 + 100 mg/L IAA	4.12 ± 0.012^{bc}	1.41 ± 0.007^{c}	52.7 ± 0.366^{ab}	41.4 ± 0.354^{bc}	38.9 ± 0.808^{b}
200mg/L GA_3 + 200mg/L IAA	4.42 ± 0.012^{ab}	1.61 ± 0.007^{a}	52.2 ± 0.120^{ab}	49.6 ± 0.751^{a}	43.7 ± 0.463^{a}
50 mg/L GA_3 + 50 mg/L NAA	4.33 ± 0.033^{ab}	1.52 ± 0.017^{b}	53.9 ± 0.167^{ab}	34.3 ± 0.272^{cd}	28.1 ± 0.300^{bcd}
100 mg/L GA_3 + 100 mg/L NAA	3.94 ± 0.011^{bc}	1.45 ± 0.017^{bc}	56.5 ± 0.100^{a}	34.8 ± 0.058^{cd}	28.7 ± 0.294^{bcd}
200mg/L GA_3 + 200mg/L NAA	3.81 ± 0.007^{bc}	1.36 ± 0.005^{d}	49.6 ± 0.313^{bc}	41.3 ± 0.291^{bc}	39.4 ± 0.435^{b}
50 mg/L Caffeic acid	3.45 ± 0.029^{d}	1.35 ± 0.018^{d}	45.8 ± 1.414^{d}	29.6 ± 0.412^{f}	27.0 ± 0.410^{bcd}
100 mg/L Caffeic acid	3.46 ± 0.012^{d}	1.40 ± 0.003^{c}	48.0 ± 0.233^{bc}	31.8 ± 0.305^{cd}	28.1 ± 0.577^{bcd}
200mg/L Caffeic acid	3.55 ± 0.015^{cd}	1.25 ± 0.009^{e}	48.6 ± 0.047^{bc}	30.9 ± 0.050^{cd}	29.0 ± 0.503^{bcd}
Distilled water	4.44 ± 0.023^{ab}	1.61 ± 0.007^{a}	48.5 ± 0.033^{bc}	44.4 ± 0.199^{b}	38.0 ± 0.272^{b}

*Means followed by the same letters on the same column are not significantly different according to Duncan Multiple Range Test at 5% probability

*GA_3 = Gibberellic Acid ; NAA = Naphthalene Acetic acid; IAA = Indole Acetic Acid; and BAP = 6-Benzyl- amino purine.

The highest chlorophyll B content was obtained in 50 mg/L NAA treatment, while the lowest chlorophyll B content was obtained in 50 mg/L GA_3 treatment. In the combined plant hormones, crude protein content was greatest in in 10% CW + 50 mg/L GA_3 treatment (Table 6). The highest value of crude fibre was obtained in 10% CW + 50 mg/L GA_3, 200mg/L GA_3 + 200mg/L NAA and distilled water treatments, with a mean value of 1.61mg/L, while the lowest crude fibre was obtained in 200mg/L Caffeic acid with a value of 1.25mg/100g. Vitamin C content was highest in 100 mg/L GA_3 + 100 mg/L NAA treatment, while the lowest vitamin C content was obtained in 50 mg/L Caffeic acid treatment (Table 6). The highest chlorophyll A content with a value of 49.6mg/100g was obtained in 200mg/L GA_3 + 200mg/L IAA treatment, while the lowest mean value of 29.6 mg/100 g was obtained in 50 mg/L Caffeic acid treatment (Table 6). The highest chlorophyll B content was obtained in 200mg/L GA_3 + 200mg/L IAA treatment, while the lowest chlorophyll B content was obtained in 10% CW + 50 mg/L GA_3 treatment (Table 6).

Effects of plant hormone treatments on the mineral contents of *H. sabdariffa* plants at 10 W.A.P. based on promotional effect.

The treatments considered for this analysis were 20 % CW, 200 mg/L GA_3, 200 mg/L IAA, 200 mg/L NAA, and 100 mg/L BAP, while 200 mg/L Caffeic acid and Distilled water served as the negative and positive control respectively. The aforementioned treatments were selected based on their promotional effects on the leaves of *H. sabdariffa* as observed in previous results. For sodium, the highest mean value of 81.48 mg/g was obtained in 200 mg/L NAA treatment, while the lowest mean value of 61.6 mg/g was obtained in 200 mg/L Caffeic acid treatment (Table 4). The highest potassium value was obtained in 200 mg/L IAA treatments, while the lowest value was obtained in 100 mg/L BAP (Table 4). Calcium had its highest mean value of 193.20 mg/g recorded in 20 % CW treatment, followed by 100 mg/L BAP treatment with a value of 189.69 mg/g, while the lowest mean value of 164.11mg/g was obtained in 200 mg/L Caffeic acid treatment. There was no significant difference in Ca contents of 200 mg/L GA_3 and distilled water treatments ($p > 0.05$), as they both showed values of 171.53mg/g and 174.20mg/g respectively (Table 10). Similarly, to Ca contents, there was no significant difference in Mg contents of 20 % CW and100 mg/L BAP treatments. Again, the lowest contents of Ca was obtained in 200mg/L Caffeic acid treatment (Table 4). Highest phosphorus was obtained in 100 mg/L BAP treatment, but there was no significant difference in 20 % CW, 200 mg/L NAA and distilled water treatments. However, the lowest phosphorus was obtained in 200 mg/L IAA treatment (Table 4).

Table 4: Effects of plant hormone treatments on the mineral contents of *H. sabdariffa* plants at 10 W.A.P. based on promotional effect.

Hormones Treatment	Sodium	Potassium	Calcium	Magnesium	Phosphorus
20 % CW	73.69 ± 2.273^{b}	217.70 ± 14.555^{ab}	193.20 ± 2.28^{a}	89.20 ± 0.493^{a}	98.58 ± 1.039^{b}
200 mg/L GA_3	70.81 ± 0.007^{b}	211.77 ± 0.088^{b}	171.53 ± 0.273^{e}	74.43 ± 0.318^{d}	96.77 ± 0.0361^{c}
200 mg/L IAA	74.93 ± 0.038^{b}	229.15 ± 0.829^{a}	178.45 ± 0.578^{d}	84.43 ± 0.368^{bc}	95.49 ± 0.319^{c}
200 mg/L NAA	81.48 ± 0.061^{a}	198.98 ± 0.313^{bc}	184.37 ± 0.649^{c}	83.45 ± 0.227^{c}	98.17 ± 0.264^{b}
100 mg/L BAP	72.03 ± 3.357^{b}	188.75 ± 0.135^{d}	189.69 ± 0.047^{b}	88.10 ± 0.699^{a}	102.36 ± 0.380^{a}
200 mg/L Caffeic acid	61.6 ± 0.340^{c}	198.70 ± 0.208^{bc}	164.11 ± 0.397^{f}	62.77 ± 0.017^{e}	90.83 ± 0.208^{d}
Distilled water	80.78 ± 0.330^{a}	217.65 ± 0.830^{ab}	174.20 ± 0.728^{e}	85.52 ± 0.839^{b}	99.49 ± 0.059^{b}

*Means followed by the same letters on the same column are not significantly different according to Duncan Multiple Range Test at 5% probability
*GA_3 = Gibberellic Acid ; NAA = Naphthalene Acetic acid; IAA = Indole Acetic Acid; and BAP = 6-Benzyl- amino purine.

DISCUSSION

The result of this experiment showed that foliar application of coconut water (CW) and some other phytohormones can help boost the performance of vegetables, thus, increasing the phytochemical contents and mineral contents, and ensuring a safe cultivation of the plant. According to Iqbal *et al.* (2011), the phytochemical constituents of plants make them valuable for therapeutic, insecticidals, antibacterial, antifungal, anti-constipative, spasmolytic, anti-plasmodial and antioxidant uses (Riaza and Chopra, 2018; Hapsari *et al*., 2021).

The qualitative phytochemical analysis carried out on the leaves of *H. sabdariffa* showed the presence of alkaloids, saponins, flavonoids, terpenes, tannins, and phenols in all the samples, although in varying proportions. The presence of this consortium of phytochemicals will not only help the stability of flavonoid in the leaves but also suggest the potential of the leaves to possess antioxidant and anti-inflammatory capability (Zhen *et al*., 2015; Riaza and Chopra, 2018).

In order to have an idea of the varying proportions of these phytochemicals, a quantitative analysis was further carried out on the samples and the results revealed that the highest quantity of flavonoids was recorded in the single treatments of 20 % CW, 100 mg/L NAA, and 100 mg/L BAP. Similar trend can be seen in alkaloids, saponin, terpenes, tannin, and phenols. This result further supports the claims by Fadimu *et al.* (2012) and Keshinro *et al.* (2017) who suggested that coconut water and coconut milk contain Kinetin-like compound which helps in stimulating plant growth and increases the nutritional contents of plants.

The study has also shown that the leaves of *H. sabdariffa* is quite rich in protein, fibre, and minerals such as sodium, magnesium, potassium and calcium which are all essential for the proper growth and development of humans. For example, the current calcium requirement by a male adult between 19 to 65 years of age is about 1000mg according to FAO and WHO (2001), and the calcium content of the leaves of *H. sabdariffa* treated with 20 % CW was 193.20 mg per 100 g. Therefore, an adult will comfortably have a daily dose of his required calcium by consuming 500 g of the aforementioned. This can be further corroborated by the work of Shakalenga *et al*. (2021) which confirmed that *H. sabdariffa* cultivated in Lubumbashi, Congo are highly rich in essential minerals and flavonoids. Overall, the single treatment of 20 % CW has the best nutritional status been highest in most of the phytochemicals, minerals, protein, fibre, chlorophyll and vitamin c.

CONCLUSION

Roselle (*Hibiscus sabdariffa* Linn.) plants given single hormone treatments had better growth and nutritional contents than the combined hormone treatments; twenty percent CW and 200 mg/L GA_3 were the most effective for roselle cultivation.

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