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Antioxidant activity of phenolic compounds in *Hibiscus sabdariffa* from Congo

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Hibiscus sabdariffa **is a plant of the Malvaceae family, commonly found in tropical and subtropical regions of Africa, Asia, and the Americas. Its leaves, seeds, and calyxes are utilized in both human and animal nutrition. The objective of this study was to evaluate the antioxidant activity of phenolic compounds in leaves and calyxes harvested from** ***H. sabdariffa*** **plants that were fertilized with chicken manure in Congo. The total polyphenol, flavonoid, and anthocyanin contents of each extract were determined using a UV-visible spectrophotometer. Whereas, the antioxidant activities were assessed using the 2, 2-diphenyl-1-picrylhydrazyl free radical scavenging assay. The highest contents of total polyphenols, flavonoids and anthocyanins observed in leaf extracts were 364, 91 and 134.6 mg EC3G/g MS, respectively. While the calyx extracts, showed for the same compounds from** ***H. sabdariffa*** **plants fertilized with chicken droppings, were 362, 322, and 162 mg EC3G/g MS, respectively. The IC50 mean values from leaves and calyxes were 0.64 and 1.01 mg/ml, respectively. This demonstrated that the leaves and fruits of** ***H. sabdariffa*** **cultivars from Congo are rich in phenolic compounds and can be used as a source of natural antioxidants which are essential in human and other mammalian foods.**

Key words: Calyxes, chicken manures, leaves, polyphenols, flavonoids, anthocyanins, *Hibiscus sabdariffa*.

INTRODUCTION

Hibiscus sabdariffa is a plant cultivated in the tropical and subtropical regions of Africa, Asia, and America. It belongs to the Malvaceae family, and its leaves, calyces, and seeds are utilized for their various properties (Eva et al., 2022; Riaz and Chopra, 2018). The leaves are included in the diets of African consumers, while the calyxes are used as ingredients in sauces, seasonings, and refreshing drinks. The seeds find applications in cattle feed and oil extraction and the plant are listed in the African Pharmacopoeia. For human consumption, these plant organs are rich in nutrients, including phenolic compounds. They are also abundant in antioxidants with high biological value (Eva et al., 2022; Riaz and Chopra, 2018; Valenzuela and Viuda-Martos, 2021; Teixeira et al.,

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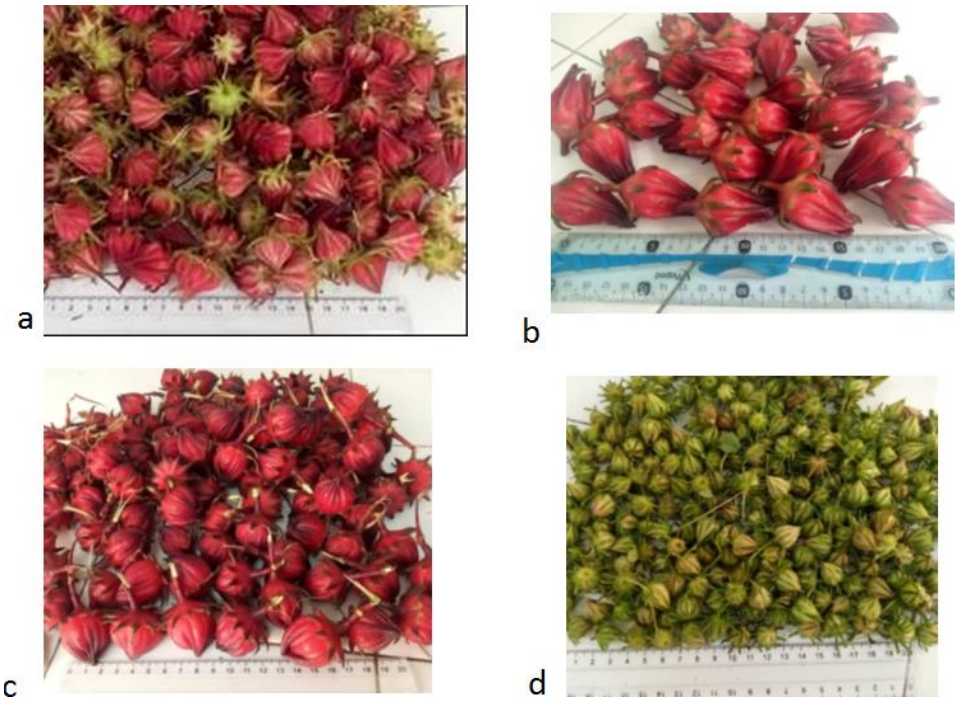

Figure 1. Fruit of different *H.sabdariffa* cultivars: (a) Poutou-poutou (C1); (b) Ngoma (C2); (c) Mbamou (C3); (d) Woolo (C4).

2022), which are essential for eliminating free radicals. This crucial role has been confirmed in antioxidant-rich foods, which can help in preventing cancer, cardio-vascular diseases, and neurodegenerative disorders (Blazovics et al., 2003). Unfortunately, in the Republic of Congo, the nutritional benefits offered by *H. sabdariffa* leaves, calyxes, and seeds are not well-known among consumers. To make *H. sabdariffa* organs more accessible, farmers often resort to the use of synthetic fertilizers, including chicken manure. These fertilizers are used in peri-urban farm, to boost up production on overexploited lands.

Furthermore, the abusive use of chemical fertilizers, accompanied by numerous harmful effects on the environment has been discussed (Roussos et al., 2019). Besides these side effects, synthetic fertilizers are expensive and can strain the budgets of small-scale farmers. The use of organic manures presents an alternative to synthetic fertilizers, particularly animal manures, which are available at a lower commercial cost. These organic manures can enhance the production of plant secondary metabolites, including essential phenolic compounds (Ramos-Agüero and Terry-Alfonso, 2014; Taokaenchan et al., 2020). To date, no study has reported the impact of organic manures on the secondary metabolite content of *H. sabdariffa* organs in Congo, and consequently, their antioxidant activity. This study aims to provide data on the antioxidant activity of phenolic compounds in the leaves and calyxes harvested from *H. sabdariffa* plants fertilized with chicken manure in Congo.

MATERIALS AND METHODS

Plant

The plant materials used for this study included seeds, leaves, and fruit calyces from four different *H. sabdariffa* cultivars collected from four distinct agroecological areas in Congo, namely Bouenza, Pool, Brazzaville, and Plateaux (Figures 1 and 2). The seeds of these cultivars vary in size and coat color. In the local dialect, plants from these ecotypes are referred to as "Poutou-Poutou," "Ngoma," "Mbamou," and "Woolo." These ecotypes also differ in botanical types: stem color, calyx, leaf shape, and the type of production (calyx, leaves and seeds). In this study, the local cultivars of *H. sabdariffa*, namely Poutou-Poutou, Ngoma, Mbamou, and Woolo, are denoted as C1, C2, C3, and C4, respectively.

Extraction method

The leaves and calyxes of four *H. sabdariffa* cultivars (C1, C2, C3 and C4) were taken from plants fertilized with 50 and 100 g of chicken manures per pocket (15 cm wide and 20 cm deep) (Mpika et al., 2022). *H. sabdariffa* leaves and calyxes were dried in the laboratory in darkness at room temperature. 25 g of dried leaves or calyxes were ground in a porcelain mortar and reduced to a homogeneous powder. The powder was then sieved with 500 µm mesh. The powder obtained was thereafter diluted in 250 ml distilled water for 24 h under magnetic stirring, and then filtered

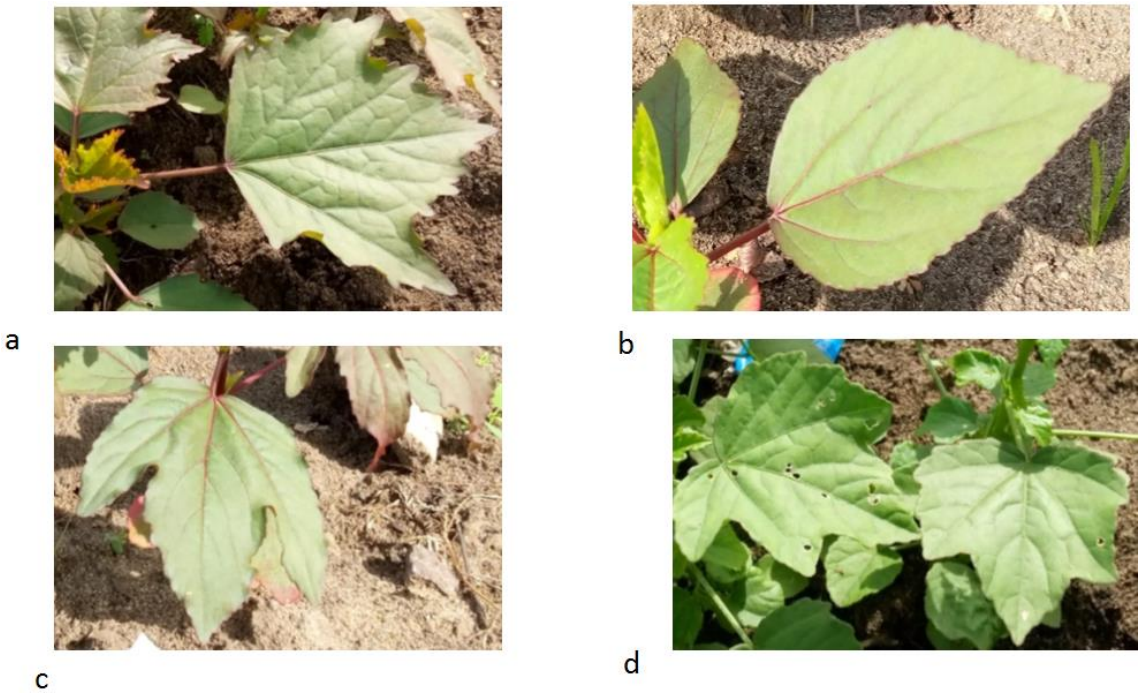

Figure 2. *H. sabdariffa* cultivars leaves morphologies: (a) Cultivar 1 (C1); (b) cultivar 2 (C2); (c) cultivar 3 (C3); (d) cultivar 4 (C4).

through absorbent cotton and Wattman paper. The filtrate obtained was evaporated at 55°C in an oven. The concentrated powdered macerate was stored in brown bottles for phenolic compound assays and antioxidant activity evaluation.

Determination of total polyphenols

The total polyphenols (TPP) was determined using the Folin-Ciocalteu reagent method in the spectrophotometer (Singleton et al., 1999), based on the oxidation of phenolic compounds by the Folin-Ciocalteu reagent (Daels-rakotoarison, 1999). A total of 0.1 g of solid extract was diluted in 10 ml of extraction solvent and the resulting solution was diluted on one-quarter (¼) with the same extraction solvent. The total phenolic compounds were determined using 0.2 ml of the suspension and placed in a test tube. The solution was then mixed with 1.8 ml distilled water, followed by 1.8 ml Folin-Ciocalteu reagent (1 N). After gently mixing, 0.2 ml of Na_2CO_3 solution (at 20%) was added to the mixture.

The mixture was incubated at room temperature for 40 min in a dark environment. The absorbance was measured with a spectrophotometer at 725 nm in ethanol solution used as a blank reaction. Total polyphenol content was obtained (in mg) of gallic acid equivalent per gram of dry matter (EAG/g MS).

Determination of total flavonoids

Two types of reagents were used for the determination of total flavonoids, (i) the sodium nitrite ($NaNO_2$, 5%) and (ii) the aluminium chloride ($AlCl_3$, 10%) in solution. The principle of the assay was based on the oxidation of flavonoids by these reagents, leading to the formation of a brownish complex which absorbs a wave length of 510 nm. The comparison between the optical density (OD) observed and that obtained by a catechin standard of known concentration, the total flavonoid content was then assessed. The total flavonoids was obtained by colorimetry and described by Subhasree et al. (2009). Briefly, 0.1 g of solid extract was diluted in 10 ml of each extracting solvent and 0.2 ml of the extract was mixed with 4 ml of distilled water. Successively after, 0.3 ml of sodium nitrite solution (5% $NaNO_2$) and 0.3 ml of aluminium trichloride (10% $AlCl_3$) were added to the mixture, 5 and 6 min, respectively. 6 min after, 2 ml of sodium hydroxide (1 N NaOH) and 2.5 ml of distilled water were added to this mixture. Counting after 30 min of incubation, the absorbance of the mixture was measured with an UV-visible spectrophotometer at 510 nm. The results were expressed in mg of catechin equivalent per gram of dry matter (EQ/g MS). A calibration plot was established with catechin standard solutions prepared at different concentrations.

Anthocyanin determination

The determination of anthocyanin was conducted using a spectrophotometer, following the previously described method in Ribereau-Goyon and Stonestre (1965). This method is based on the transformation of colorless derivatives through the action of bisulfite ions. The variation in absorbance was measured at 520 nm after adding bisulfite ions in equal proportion to the anthocyanin content. In a test tube, 1 ml of the extract from the extraction medium (stock solution) was mixed with 1 ml of 1% acidified ethanol. Following this, a volume of 20 ml of diluted 2% HCL was added to the solution.

In cuvette A, 5 ml of the mixture was combined with 2 ml of distilled water, and in cuvette B, 5 ml of the mixture was combined with 2 ml of bisulfite. The mixtures in each cuvette were then

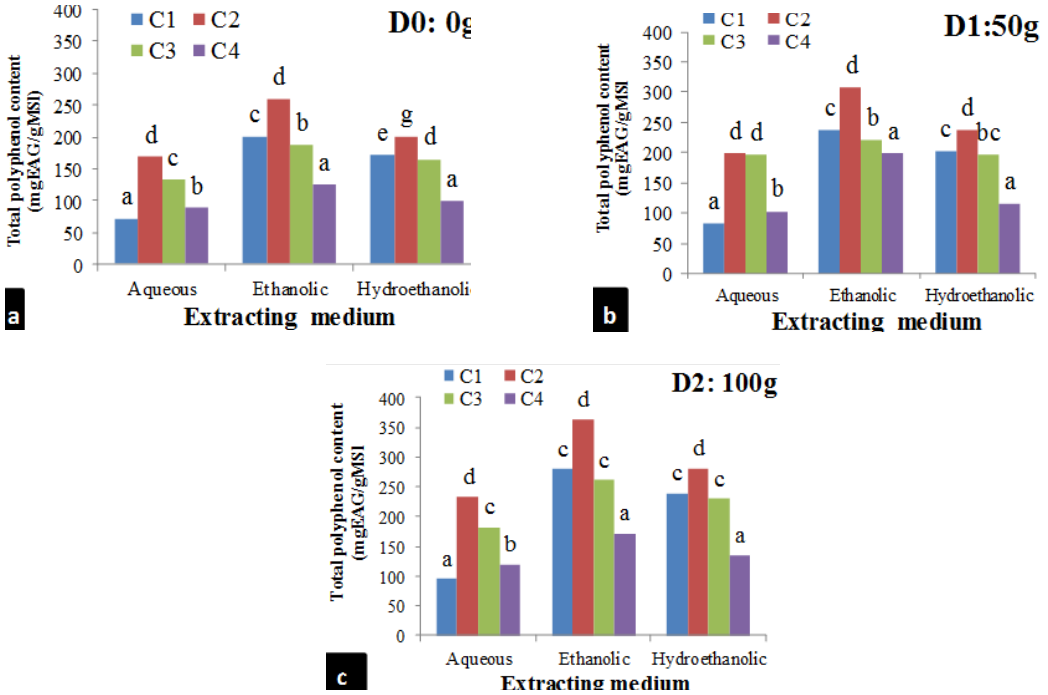

Figure 3. Total polyphenol content in leaves of *H. sabdariffa* cultivars fertilized with chicken manures: (a) 0 g (unfertilized control); (b) 50 g/pocket; (c) 100 g/pocket. C1 = cultivar 1; C2 = cultivar 2; C3= cultivar 3; C4 = cultivar.

incubated at room temperature for 20 min in a dark environment. After incubation, the absorbance was measured at 520 nm. The change in absorbance was obtained and expressed as the difference between the OD of cell A and that of cell B (ODA-DOB). Such that the anthocyanins concentration [anthocyanins] mg/l = (DOA-DOB) × λ, where λ= 875 (slope of calibration line).

Assessment of antioxidant activity

The assessment of antioxidant activity was done following the protocol described by Adjila and Azzoug (2021). A total of 1250 µl of a methanolic solution of DPPH (0.04 mg/ml) was added to 50 µl of each extract at 2, 4, 6 and 8 mg/ml or 50 µl of the solvent (blanking solution). After shaking, incubation was carried out for 30 min at room temperature in the dark.

The absorbance was then measured at λ = 517 nm. Quercetin was used as a positive control (dissolved in distilled water) and each solution was repeated four times. The percentage inhibition of the DPPH free radical was calculated according to the following formula:

$$I\% = [(A\ control - A\ sample) / A\ control] \times 100$$

where I%: Percentage of DPPH free radical inhibition, Acontrol: Absorbance of the blank solution, and Asample: Absorbance of the sample.

Statistical analysis of data

Data analysis for both field and laboratory data were performed using computer-based statistical tools, specifically the SPSS 22.0 software. The selection of statistical methods was based on the adopted sampling methods. These methods encompassed two-factor analysis of variance (ANOVA) following Cohen (1992). Post hoc comparisons of means were carried out using the Student Newman and Keuls test and the Bonferroni test at a 5% significance level.

RESULTS

Variation in total polyphenol content of *H. sabdariffa* leaves

After fertilization with 100 g of chicken manure, the total polyphenol contents of 172.2, 61.4, 282, and 364.4 mg EAG/g MS were recorded for each cultivar C4, C3, C1 and C2, respectively (Figure 3).

Higher values were obtained than those observed in control plants and in those that were fertilized with 50 g of chicken manure. For all the solvents used for extraction, high levels of total polyphenols were observed in ethanolic media. The aqueous solvent contained 96.2, 120.7, 183.9, and 234.5 mg EAG/g MS for cultivars C1, C4, C3, and C2 (Figure 3).

Analysis of variance revealed significant differences in total polyphenol content among the studied cultivars. A significant "chicken manure dose" effect ($P < 0.001$) was

Table 1. Classification of total polyphenol content (mgEAG/gMS) in leaves of *Hibiscus sabdariffa* cultivars fertilized with chicken manures according to extraction medium.

Organ	Treatment	Extraction medium		
		Aqueous	Ethanoic	Hydroethanol
Leave	C1D0	72.12[a]	199.67[e]	171.51[e]
	C4D0	88.33[b]	124.83[a]	98.53[a]
	C1D1	83.33[b]	237.33[g]	203.33[h]
	C1D2	96.17[c]	279.5i	238.83[j]
	C4D1	103.36[c]	147.33[b]	115.33[b]
	C4D2	119.5[d]	172.33[c]	134.17[c]
	C3D0	132.5[e]	186.67[d]	165.17[d]
	C3D1	156.33[f]	221.67[f]	195.67[f]
	C2D0	167.83[g]	258.83[h]	200.33[g]
	C3D2	183.23[h]	260.67[h]	229.83[i]
	C2D1	198.83[i]	308.33[j]	238.17[j]
	C2D2	233.67[j]	363.83[k]	280.5[k]

C1 = Cultivar 1; C2 = cultivar 2; C3 = cultivar 3; C4 = cultivar 4. Numbers with different letters in the column are significantly different at the $p<0.05$ threshold.

also observed (Table 1).

Variation in total polyphenol content of *H. sabdariffa* fruits

Figure 4 illustrates that the total polyphenol content of *H. sabdariffa* fruits varies according to the dosage of manure applied. The highest levels were found in cultivars fertilized with 100 g of chicken manure per bunch. The total polyphenol levels were recorded as 110.2, 175.2, 248.3, and 324.4 mg EAG/g MS for cultivars C2, C4, C3, and C1, respectively. These values surpass those observed in control plants and plants treated with 50 g of chicken manure. Nevertheless, the highest levels of total polyphenols were observed in extracts obtained from ethanolic and hydroethanolic solvents. In aqueous medium, the highest content, 362.1 mg EAG/g MS, was observed in cultivar C2, surpassing the levels of 349.5 and 215 mg EAG/g MS recorded for C4, C3, and C1, respectively. When it comes to the calyx, the lowest levels were recorded in cultivar C1 across all extraction media. The doses of chicken manure strongly promoted the accumulation of total polyphenols ($P < 0.05$) in all *H. sabdariffa* cultivars. Statistical analysis revealed a significant "chicken manure dose" effect on the accumulation of total polyphenols (Table 2).

Variation in total flavonoid content of *H. sabdariffa* leaves

The levels of flavonoids varied based on the plants grown in soil enriched with chicken manure (Figure 5). A total of 67 mg EQ/g MS in the medium was recorded for cultivar C2, which is higher than the values recorded for the leaves of cultivars C3, C4, and C1, with respective values of 61, 48.7, and 39 mg EQ/g MS. When 50 g of chicken manure was used, flavonoid contents reached 77, 62, 61, and 39 mg EQ/g MS in the leaves of cultivars C2, C3, C4, and C1, respectively, in the ethanolic medium. However, with the hydroethanolic medium, a content of 40.3 mg EQ/g MS was obtained for C4, which was higher than the 39.7 mg EQ/g MS recorded for cultivar C1 (Figure 5b). These values were higher than those of C2 and C3. Moreover, when 100 g of chicken manure was added per pocket, flavonoid contents reached 91, 73, 69, and 57 mg EQ/g MS in the leaves of cultivars C2, C3, C2, and C1. These values were higher than those in the plants fertilized with 50 g of chicken manure and the control plants (Figure 5c). Analysis of variance revealed a significant "chicken manure dose" effect ($P < 0.05$) on the flavonoid content (Table 3). This allowed for the discrimination of six homogeneous groups (a, b, c, cd, ed, f), with the strongest effect observed in C4 cultivar leaves after 100 g of chicken manure (group d).

Variation in total fruit flavonoid content.

Concerning calyx levels, the total flavonoid content varied according to the extraction medium in relation to the dose of chicken manures applied (Figure 6). In the aqueous medium, in cultivar C3, we recorded 270 mg EQ/g MS for 50 g of chicken manure; this content is higher compared

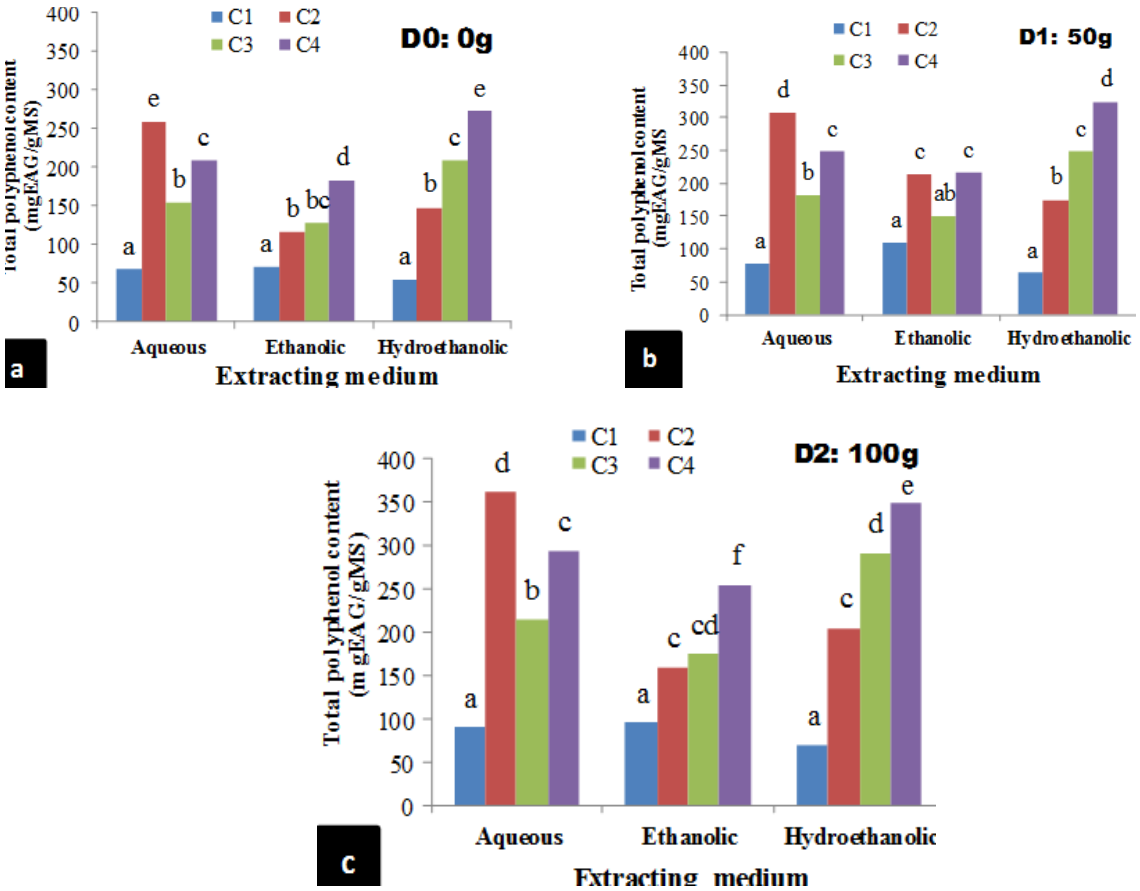

Figure 4. Total polyphenol levels in fruits of *H. sabdariffa* cultivars fertilized with chicken manures: (a) 0 g (unfertilized control); (b) 50 g/pocket; (c) 100 g/pocket.

Table 2. Classification of total polyphenol content (mgEAG/gMS) in fruits of the four *Hibiscus sabdariffa* cultivars after chicken manures, according to extraction medium.

Organ	Treatment	Extraction medium		
		Aqueous	Ethanoic	Hydroethanol
Fruit (calyx)	C1D0	67.83^{a}	71.67^{a}	54.67^{a}
	C1D1	78.33^{a}	83.23^{ab}	62.51^{a}
	C1D2	90.17^{b}	95.67^{b}	71.33^{a}
	C3D0	154.5^{c}	127.56^{ef}	208.33^{f}
	C3D0	154.5^{c}	127.56^{ef}	208.33^{f}
	C3D1	182.67^{d}	149.67^{d}	247.83^{d}
	C4D0	209.33^{d}	182.67^{c}	271.50^{e}
	C2D0	257.50^{e}	116.17^{b}	148.47^{b}
	C4D1	248.83^{f}	216.67^{e}	323.83^{g}
	C3D2	214.33^{f}	175.17^{e}	291.83^{f}
	C2D1	306.83^{g}	136.67^{e}	175.11^{c}
	C4D2	293.17^{g}	254.67^{f}	349.47^{h}
	C2D2	362.17^{h}	159.67^{e}	205.17^{e}

C1 = cultivar 1; C2 = cultivar 2; C3 = cultivar 3 ; C4 = cultivar. Figures in in each collum in bold with letters are significant ($p<0.05$).

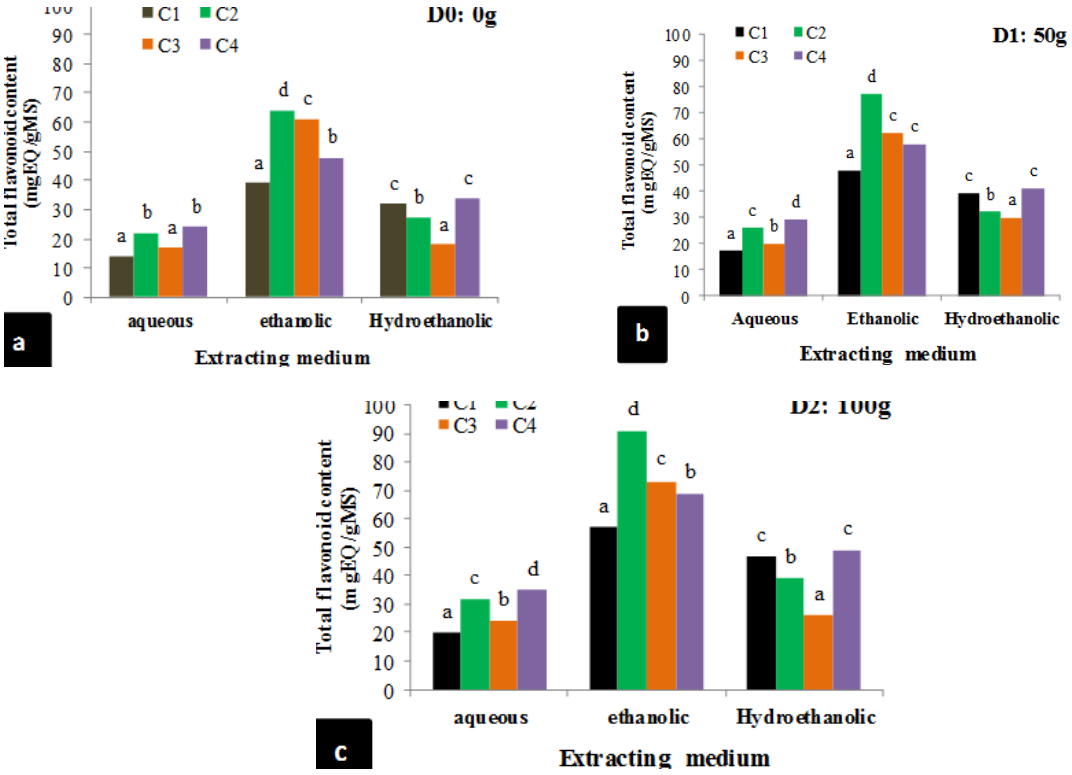

Figure 5. Total flavonoid content of leaves of *H. sabdariffa* cultivars fertilized with chicken manures: (a) 0 g (unfertilized control); (b) 50 g/pocket; (c) 100 g/pocket.

Table 3. Classification of total flavonoid content (mg EQ/g MS) in the leaves of four *Hibiscus sabdariffa* cultivars fertilized with chicken manures, according to extraction medium.

Organ	Treatment	Extraction medium		
		Aqueous	Ethanoic	Hydroethanol
Leave	C1D0	13.67^{a}	39.33^{d}	32.33^{d}
	C1D1	16.67^{a}	47.50^{e}	39.11^{e}
	C3D0	16.50^{a}	61.40^{f}	17.67^{b}
	C1D2	19.83^{a}	56.67^{ef}	46.50^{e}
	C3D2	21.83^{b}	73.33^{g}	25.83^{d}
	C2D0	23.50^{c}	63.50^{f}	26.67^{cd}
	C3D1	20.12^{cd}	61.50^{f}	21.50^{d}
	C4D0	23.67^{d}	47.83^{e}	33.67^{d}
	C2D1	25.67^{d}	76.67^{g}	32.17^{d}
	C4D1	28.83^{d}	57.67^{f}	40.67^{e}
	C2D2	31.83^{de}	91.33^{i}	38.50^{e}
	C4D2	34.50^{de}	68.67^{g}	48.5^{ef}

C1 = Cultivar 1; C2 = cultivar 2; C3 = cultivar 3; C4 = cultivar. Numbers with different letters in the column are significantly different at the p<0.05 threshold.

to the 224.1 and 207.2 mg EQ/g MS obtained in the same cultivar using ethanolic and hydroethanolic media. At 100 g of chicken manure per pocket, the highest contents of total flavonoids, 322, 266, and 247 mg EQ/g MS were obtained for calyxes in cultivar C3 in aqueous, ethanolic, and hydroethanolic media. These levels surpassed the 183, 200, and 156 mg EQ/g MS obtained in fruits from cultivars C2, C1, and C4 (Figure 6c). In the

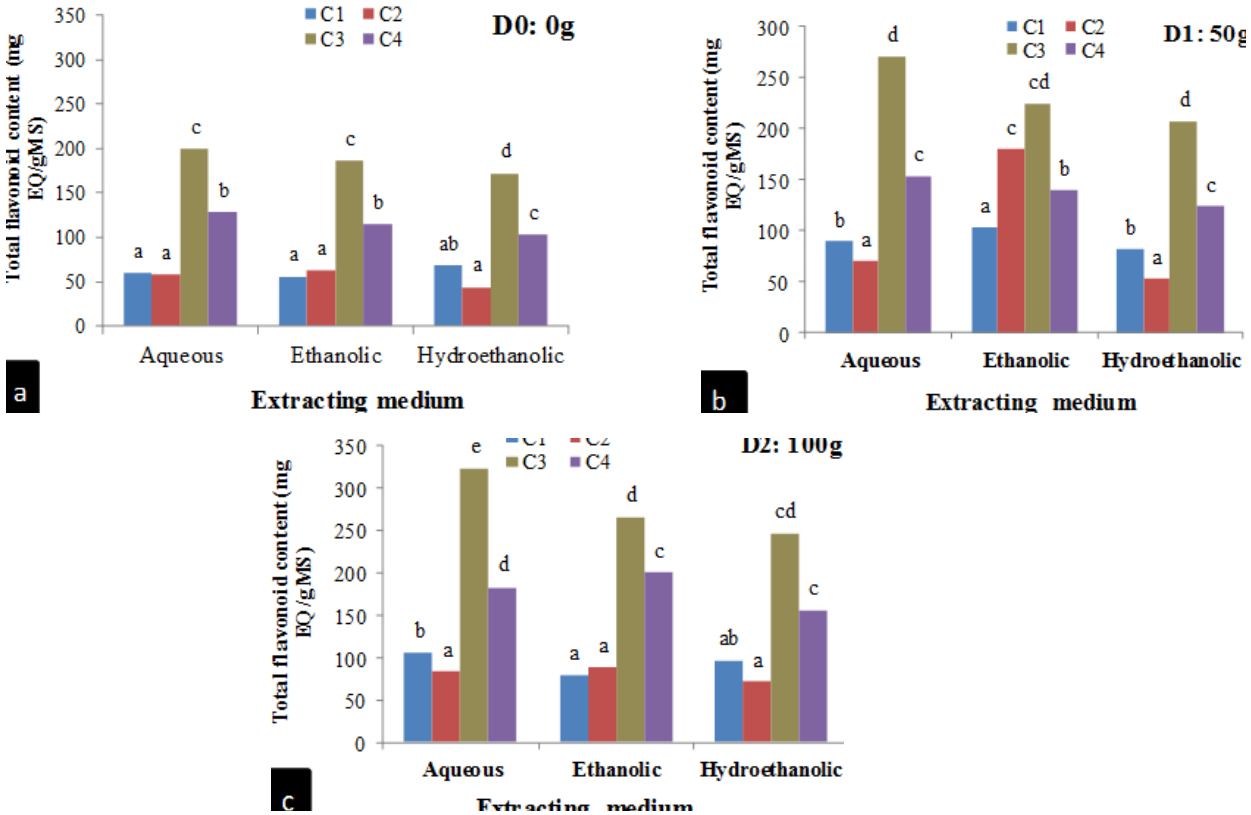

Figure 6. Total flavonoid content in fruits of four *H. sabdariffa* cultivars: (a) control plants; (b) plants fertilized with 50 g of manures; (c) plants fertilized with 100 g of manures.

aqueous extract, the flavonoid content varied among the tested cultivars. Statistical analysis revealed a significant "chicken manure dose" effect ($P < 0.05$) on the flavonoid content of the fruits from the cultivars. Analysis of variance (ANOVA) allowed us to categorize them into six homogeneous groups (a, b, c, d, e, and f) (Table 4). The most representative group was observed in plants treated with 50 and 100 g of chicken manure per pocket.

Variation in total anthocyanin content of *H. sabdariffa* leaves

The anthocyanin content of all cultivars was low in both aqueous and hydroethanol media and a high anthocyanin content of 94 mg EC3G/g MS was observed in the leaves of C4 cultivars extracted in ethanolic medium. This is higher than the 54 mg EC3G/g MS recorded in cultivars C1 and C2 as well as in cultivar C3 (27 mg EC3G/g MS) (Figure 7a). This is evident that anthocyanin levels increase with the dose of chicken manures applied. In ethanolic medium, anthocyanin contents of 113.6 and 134.2 mg EC3G/g MS were recorded in plants leaves fertilized with 50 and 100 g of chicken manures (Figure 7b and c). By increasing the fertilization dose to 100 g, these levels rose to 113, 69, 65 and 34 mg EC3G/g MS for cultivars C4, C2, C1 and C3. Levels are relatively low in aqueous and hydroethanolic media, with an average of 27.9 and 51 mg EC3G/g MS, respectively. Statistical analysis revealed significant differences in anthocyanin content (Table 5) and "chicken manures dose" effect ($P < 0.05$). It enabled us to discriminate between 6 homogeneous groups (a, b, bc, c, cd and d) at leaf level, the most representative of which is from the C4 cultivar in ethanolic medium, for plants fertilized with 50 and 100 g of chicken manures.

Variation in total anthocyanin content of *H. sabdariffa* fruits

Figure 8 illustrates the evolution of anthocyanin levels in the fruit (calyx) of *H. sabdariffa* cultivars. These levels vary according to the dose of chicken manures fed to the plants. Plants fertilized with 50 g of chicken manures per planting unit had levels of 137.2, 117.3 and 103 mg EC3G/g MS for cultivars C2, C1, C3 and C4. These values are higher than the 77, 37 and 24 mg EC3G/g MS

Table 4. Classification of total flavonoid content (mg EQ/g MS) in fruits of four *Hibiscus sabdariffa* cultivars fertilized with chicken manures, according to extraction medium.

Organ	Treatment	Extraction medium		
		aqueous	Ethanoic	Hydroethanol
Calyx	C1D0	73.5^{b}	55.5^{b}	67.67^{c}
	C2D0	57.67^{b}	61.5^{b}	44.17^{b}
	C2D1	69.5^{b}	74.17^{c}	53.33^{bc}
	C2D2	82.83^{b}	88.33^{ef}	63.67^{b}
	C1D1	88.67^{c}	66.83^{b}	81.67^{c}
	C1D2	105.67^{c}	79.67^{d}	97.33^{c}
	C4D0	127.5^{d}	115.33^{d}	102.5^{d}
	C4D1	153.83^{d}	139.17^{e}	123.5^{d}
	C4D2	183.17^{e}	165.67^{ef}	155.5^{e}
	C3D0	224.00^{f}	185.50^{f}	171.83^{e}
	C3D1	270.00^{g}	223.50^{f}	207.17^{f}
	C3D2	321.67^{g}	266.00^{f}	246.67^{g}

C1 = Cultivar 1; C2 = cultivar 2; C3 = cultivar 3; C4 = cultivar. Numbers with different letters in the column are significantly different ($p<0.05$).

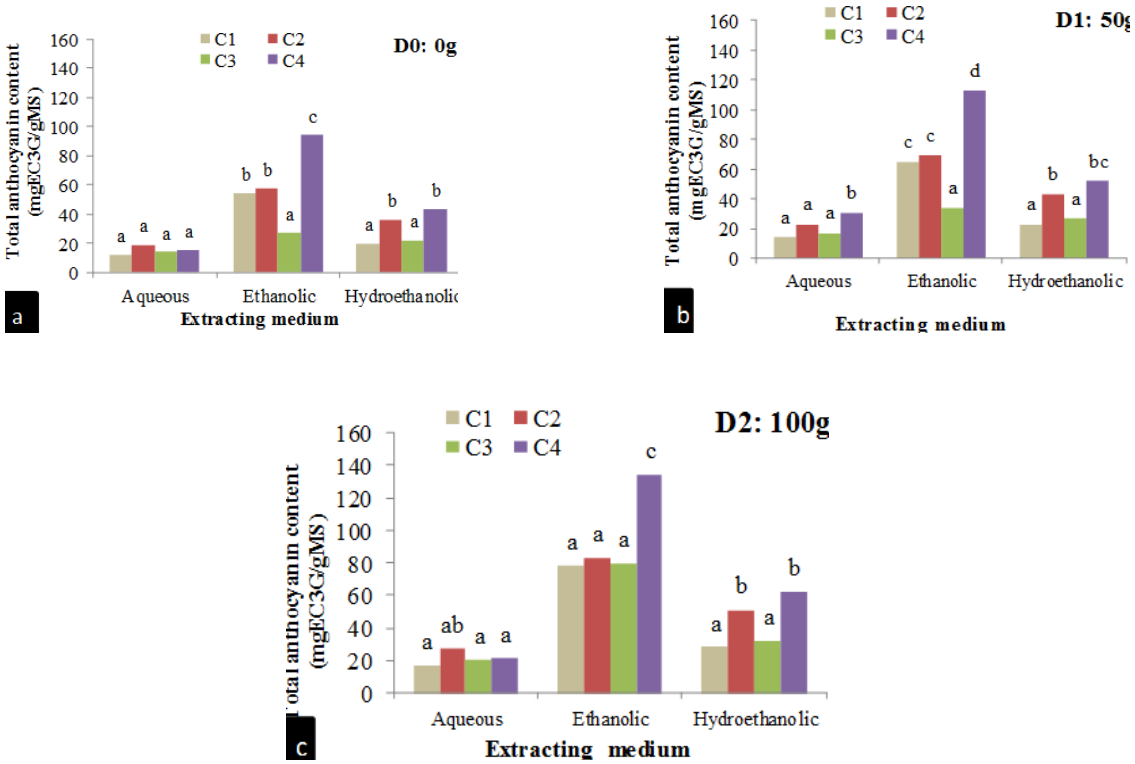

Figure 7. Total anthocyanin levels in leaves of four *H. sabdariffa* cultivars fertilized with chicken manures: (a) control plants; (b) plants fertilized with 50 g manures; (c) plants fertilized with100 g manures.

found in fruits from C4, C2 and C1 cultivars. In hydroethanolic medium, anthocyanin levels were comparable to those obtained in ethanolic medium, with an average of 106.5 mg EC3G/g MS for cultivars C4 and

Table 5. Classification of total anthocyanin content (mg EC3G/g MS) in the leaves of four *Hibiscus sabdariffa* cultivars according to extraction medium.

Organ	Treatment	Extraction medium		
		Aqueous	Ethanoic	Hydroethanol
Leave	C1D0	11.96[a]	54.25[c]	19.54[a]
	C1D1	14.29[a]	65.33[d]	23.33[a]
	V1D2	16.92[a]	77.58[e]	27.71[b]
	V2D0	19.25[a]	57.46[c]	35.58[b]
	V2D1	23.04[a]	69.42[e]	42.88[c]
	V4D2	21.01[a]	134.46[h]	62.13[d]
	V3D0	14.29[a]	27.42[ab]	22.46[ab]
	V3D1	16.92[a]	33.54[c]	26.83[b]
	V3D2	20.42[a]	40.25[f]	31.79[b]
	V4D0	14.58[a]	94.21[f]	43.17c
	V4D1	17.79[a]	113.17[g]	52.21[d]
	V2D2	27.42[ab]	82.83f	51.04[d]

C1 = Cultivar 1; C2 = cultivar 2; C3 = cultivar 3; C4 = cultivar. Numbers with different letters in the column are significantly different at the p<0.05 threshold.

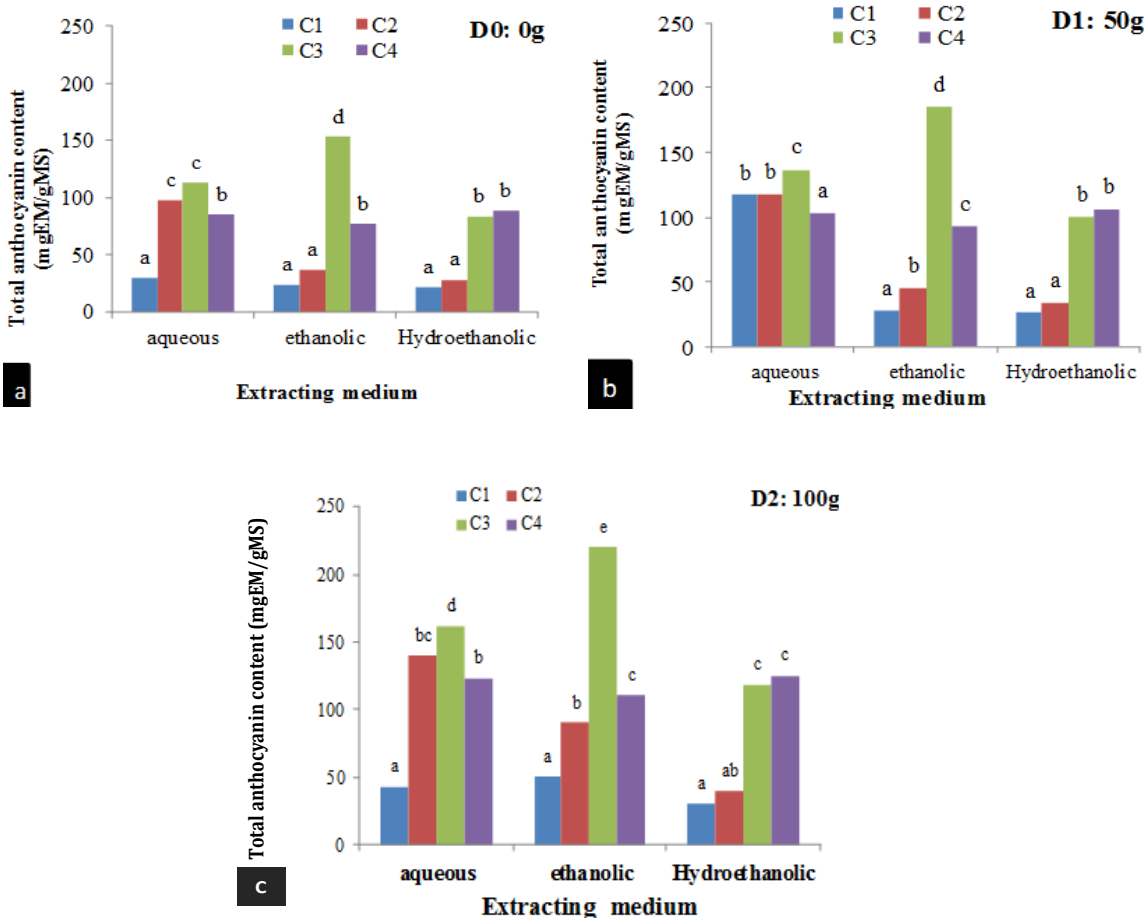

Figure 8. Total anthocyanin levels in fruits of four *H. sabdariffa* cultivars after chicken manures: (a) control plants; (b) plants fertilized with 50 g of manures; (c) plants fertilized with 100 g of manures.

Table 6. Classification of total anthocyanin content (mg EC3G/g MS) in fruits of four *Hibiscus sabdariffa* cultivars according to extraction medium.

Organ	Treatment	Extraction medium		
		Aqueous	Ethanoic	Hydroethanol
Calyx	C1D0	30.33[a]	23.63[a]	21.88[a]
	C1D1	36.46[a]	28.29[a]	25.96[a]
	C1D2	43.46[b]	33.25[b]	30.92[b]
	C4D0	85.46[b]	77.29[b]	87.79[b]
	C4D1	102.96[c]	93.04[c]	105.7[c]
	C2D0	98.29[d]	37.33[a]	28[a]
	C2D1	118.1[e]	45.21[b]	33.83[a]
	C2D2	140.29[ef]	53.67[d]	40.25[b]
	C3D0	113.17[d]	153.13[e]	83.42[b]
	C3D1	136.5[ef]	184.63[f]	100.33[c]
	C3D2	162.46[f]	219.92[g]	119[e]
	C4D2	122.5[e]	110.83[e]	125.42[e]

C1 = Cultivar 1; C2 = cultivar 2; C3 = cultivar 3; C4 = cultivar. Numbers with different letters in the column are significantly different at the $p<0.05$ threshold.

C3 (Figure 8b). With 100 g of chicken manures, contents varied, reaching 126, 140, 123 and 43 mg EC3G/g MS, respectively with cultivars C3, C2, C1 and C4 in aqueous medium. In ethanolic and hydroethanolic media, these values ranged from 125 to 90 mg EC3G/g MS (Figure 8c). Statistical analyses revealed a significant "chicken manures dose" effect on fruit anthocyanin content (Table 6). It discriminated 8 statistically different homogeneous groups (a, ab, bc, cd, de, ef, fg and g). The strongest effect was recorded for plants amended with 50 and 100 g of chicken manures.

Inhibition rate of *H. sabdariffa* fruit extract

The free radical scavenging capacity varied according to the cultivar and the dose of chicken manure applied to the plants. Plants fertilized with 100 g of chicken manure showed high antioxidant activity, resulting in very low IC50 values.

At the leaf level, the 50% inhibition concentrations (IC50) were 0.64, 2.45, 3.25, and 4.27 mg/ml, respectively, for cultivars C3, C4, C2, and C1 (Figure 9). These leaf IC50 values are comparable to the 1.01, 2.81, 2.83, and 3.17 mg/ml recorded by cultivars C2, C3, C4, and C1 in fruit. By reducing the dose of chicken manure to 50 g per pocket, the 50% inhibition concentration (IC50) increased to 4.12 and 5.14 mg/ml at the leaf and fruit levels, respectively. Unfertilized plants exhibited the highest IC50 values, regardless of the cultivar and organ tested. The percentage of free radical inhibition increased proportionally with the dose of chicken manure applied to the plants. Statistical analysis revealed a significant chicken manure dose effect ($P < 0.05$) at the 5% threshold and identified three homogeneous groups (a, b, cd) according to the Student Newman and Keul test (Figure 9).

DISCUSSION

The phenolic compound content of *H. sabdariffa* leaves and fruits was measured, along with their antioxidant activity, in relation to the dose of chicken manure. The results demonstrate that the levels vary depending on the dose of chicken manure and the specific organ under consideration. All tested cultivars exhibited elevated levels of total polyphenols, total flavonoids, and total anthocyanins. This high concentration of compounds is believed to be associated with their accumulation in the chloroplast and vacuole during the growth of *H. sabdariffa* plants (Satish and Manju, 2018). Fertilization with chicken manure enhanced the total polyphenol contents in all cultivars. High polyphenol contents were observed in the leaves and fruits of plants amended with 50 and 100 g of chicken manure per pocket.This trend was previously observed and reported in improving polyphenol content in the *Ocimum citriodorum* cultivar after the application of chicken manure (Javanmardi and Ghorbani, 2012; Benchikh et al., 2016). The enhancement in phenolic compound content is linked to the richness of the droppings in mineral elements (Brglez Mojzer et al., 2016; Guo et al., 2022). The biosynthesis of secondary metabolites, including phenolic compounds, is closely connected to the nutritional quality of the plant. Mineral elements, including nitrogen, enter the metabolic pathway

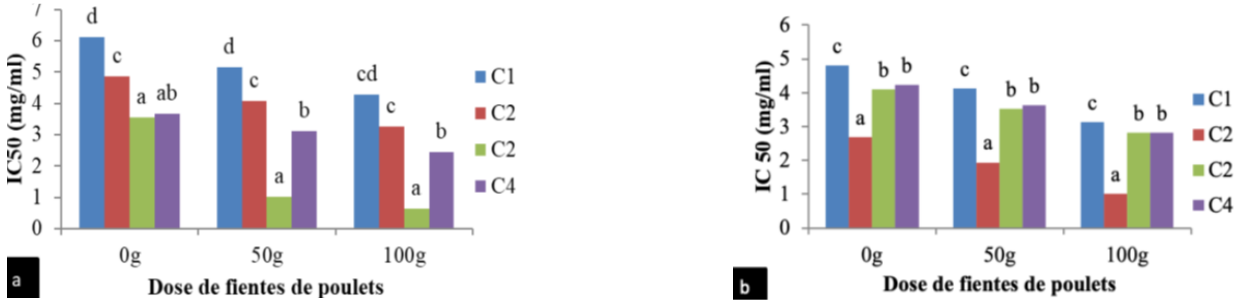

Figure 9. 50% inhibition concentration of leaf (a) and fruit (b) extracts from four *Hibiscus sabdariffa* cultivars dosed with chicken manures.

for phenolic compound synthesis from the shikimate pathway through the modification of aromatic amino acids (Heimler and Romani, 2017; Satish and Manju, 2018). The high concentration of flavonoids in the fruit (calyx) is believed to be attributed to their roles in tissue coloration. Indeed, flavonoids are responsible for the attractive colors of flower petals, bracts, and fruits (Hegnauer and Grayer-Arkmeijer, 1993; Fadel et al., 2011). However, all cultivars exhibited a high flavonoid content in both aqueous and hydroethanol media. This observation aligns with previous findings in *H. sabdariffa* calyx (Chinedu et al., 2011). Additionally, the high content of flavonoids in aqueous media may be attributed to their chemical nature, as they form glycosides (complexes with a sugar moiety). This hydroxyl group makes flavonoids hydrophilic and water-soluble (Heim et al., 2002; Kumar and Pandey, 2013). In contrast, the high levels of anthocyanins in aqueous media can be explained by their solubility in this solvent (Eun-Ji et al., 2021). Similar to flavonoids, the elevated levels of anthocyanins in the fruit are associated with their role in organ coloration (Chloe, 2010; Maria et al., 2020). In terms of antioxidant activity, the results demonstrated that the leaves and fruit (calyx) of plants fertilized with chicken manures exhibited higher activity than those of unfertilized plants. This activity increased with the dose applied to the plants. The high antioxidant activity in *H. sabdariffa* leaves and fruit is believed to be linked to their high content of total flavonoids, total polyphenols, and anthocyanins (Alejandro et al., 2016). When chicken manure decomposes, it releases a substantial quantity of mineral elements into the soil, which play a role in the biosynthesis of phenolic compounds (Aliyu et al., 2011; Agbo et al., 2015).

Conclusion

The DPPH technique was employed to assess the antioxidant activity of *H. sabdariffa* leaves and calyxes (fruits). Leaf and calyx extracts from plants fertilized with chicken manure exhibited strong antioxidant activity. In all cultivars, the contents of total polyphenols, flavonoids, and anthocyanins varied based on the dose of manure and the extraction solvent used. A strong linear correlation was observed between the phenolic compound content and antioxidant power for the different cultivars tested. Therefore, the four *H. sabdariffa* cultivars from Congo can be considered a valuable source of natural antioxidants for medicinal purposes.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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