

IMPACT OF ORGANIC AND FOLIA FERTILIZER APPLICATION ON THE GROWTH, YIELD AND MEDICINAL POTENTIAL OF *HYBISCUS SABDARIFFA* L.**Arowosegbe Sunday**

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ABSTRACT: *The effect of organic (poultry manure) and folia fertilizer application on the growth, yield and phytochemical composition of Hibiscus sabdariffa was investigated in planting pot experiments. Seeds of this plant were sown in soil treated with organic fertilizer at 40, 80 and 120 g separately and also supplemented with folia fertilizer two weeks after germination. Some seedlings with no organic fertilizer application were also sprayed with folia fertilizer, thus making seven treatments in all. No organic or folia fertilizer served as control. Application of 120 g poultry manure in combination with folia fertilizer produced the highest plant height (98.23 cm), dry shoot weigh plant⁻¹ (24.66 g) and number of fruits plant⁻¹ (19.33). The highest leaf number plant⁻¹ (164.00), fresh (28.06 g) and dry (4.43 g) fruit weight was recorded in plants treated with 120 g poultry manure. More so, alkaloids, anthocyanins, flavonoids, phenols, saponins, and tannins were found in the calyces of Hibiscus sabdariffa with or without organic and foliar fertilizer. However, treatments with organic fertilizer singly or in combination with folia fertilizer significantly ($p < 0.05$) increased the phytochemical contents in the calyces of the plant. Interestingly, 120 g poultry manure had the greatest impact. The use of organic fertilizer in the cultivation of this medicinal plant should be encouraged as they could enhance its better growth, good yield and medicinal value under safe agricultural condition.*

KEY WORDS: Growth, Yield, Organic Fertilizer, Folia Spray, Medicinal Value.

INTRODUCTION

Plants are known to have played vital roles in the maintenance of good health of mankind since human existence (Moerman, 1996; Osuagwu and Edeoga, 2012), both as food and for medicinal purposes. However, for plants to grow healthily and have optimal yield, there must be availability of soil nutrients in correct quantity, proportion and in a usable form at the appropriate time (Ibrahim *et al.*, 2013). In meeting these conditions, chemical (inorganic) and/or organic fertilizers are needed to make up for the inadequate soil nutrients and the declining soil fertility as a result of continuous cultivation by peasant farmers. Unfortunately, the use of inorganic fertilizers in excess of plants requirements usually leads to fertilizer lost and environmental pollution. This has led to the renewed interest in the use of organic fertilizers. Organic manures such as farmyard and poultry manure have been reported not only to improve the physical and chemical conditions of the soil, but also help in the improvement of soil biological activities, plant growth and increased yield (Logan *et al.*, 1997; Nithiya *et al.*, 2015). Of recent, the use of foliar fertilizers to improve the performance of plants has become popular. According to Khan *et al.* (2009), foliar fertilizer application is desirable because, less amount of the fertilizer is required with less ground water pollution in addition to its positive effect on plant growth and quality. The authors also reported that folia spraying could have up

to 90% efficiency rate of uptake by plants as opposed to 10% efficiency from soil applied fertilizers.

The introduction of medicinal plants into cultivation is borne out of the increasing global interest and expanding market of herbal drugs. In addition to seeing to an increase in the production of medicinal plants, the aspect of quality end product is very important (Nithiya *et al.*, 2015). Such quality is in turn determined by the presence of secondary metabolites such as saponins, alkaloids, tannins, steroids and phenolic compounds in plants. Interestingly, applications of organic fertilizers to plants have been reported to increase the presence of these bioactive compounds and antioxidants in them. (Okwu, 2004; Ozguven *et al.*, 2008; Kaola *et al.*, 2013).

Hibiscus sabdariffa L. known as Roselle is an annual or perennial erect herbaceous shrub in the family Malvaceae. The plant grows to a height of 2.4 m with smooth or nearly smooth cylindrical red stem. Its mature leaves are alternate and deeply lobed with reddish veins. Flowers are yellow with red calyx, born singly in the leaf axils and turn pink as they wither. The flowers develop into green capsules that turn brown and split open to release the seeds when mature. *H. sabdariffa* is cultivated both in the tropical and subtropical regions of the world for its popular edible calyces, stem fibres, leaves and seeds (Babatunde *et al.*, 2002). In Nigeria, the calyces are processed into a popular non-alcoholic drink called Zobo. The calyces of this plant have been reported to have therapeutic, diuretic and antioxidant properties with many medicinal applications to cure kidney stone, pyrexia, liver damage, hypertension and leukaemia (Abu-Tarboush *et al.*, 1997; Wong *et al.*, 2002; McKay *et al.*, 2010; Akim *et al.*, 2011). The antibacterial activity of *H. sabdariffa* was also reported by Olaleye (2007).

Studies have been conducted on the phytochemical composition of this plant by some researchers (Obouayeba *et al.*, 2014; Okereke *et al.*, 2015), however the effect of organic and folia fertilizer application on the phytochemical contents of this plant have not been fully studied. The objective of this study therefore, was to investigate the impact of organic fertilizer application supplemented with folia fertilizer on the growth, yield and phytochemical composition of *H. sabdariffa* in the light of its medicinal importance.

MATERIALS AND METHODS

Experimental site and materials collection

The experiment was carried out between October 2014 and April 2015, at the experimental site of the Department of Plant Science, Faculty of Science, Ekiti State University, Ado-Ekiti (7° 40'N, 5° 15'E) using experimental pots. Ado-Ekiti is a town located in the South-Western part of Nigeria. The town enjoys a bimodal pattern of rainfall (April to July and September to November) with annual mean of 1400 mm. The organic fertilizer (poultry manure) used for the study was collected from the poultry farm of the University, while the folia fertilizer (Plantzyme®) was purchased from an agro-allied Store in Ado-Ekiti. Seeds of *H. sabdariffa* used were collected from a farmer in Ado-Ekiti. The plant sample was also collected and taken to the herbarium of the Department of Plant Science, Ekiti State University for authentication.

Soil samples were collected from a piece of land at the Teaching and Research Farm of Faculty of Agricultural Sciences in Ekiti State University, Ado Ekiti where maize has just been harvested. The collected soil samples were subjected to routine soil analysis and found to be a

sandy clay loam with an organic matter content of 4.28; 0.28% N; 11.70 mg/kg P; 580 mg/kg K; and a pH of 5.77. The poultry manure was analysed and found to contain 2.50% N; 5.13 mg/kg P; 5856.64 mg/kg K; and a pH of 7.52. The soil samples and the poultry manure were also used for another experiment earlier reported (Ademiluyi *et al.*, 2016)

Planting procedure and fertilizer application

Cultivation of the plant was done using 24 plastic pots each filled with 5 kg sterilized soil. Treatments were carried out in three replicates. The treatments were: 40 g of poultry manure (PM); 80 g of PM; 120 g of PM; 40 g of PM +Folia fertilizer; 80 g of PM +Folia fertilizer; 120 g of PM +Folia fertilizer and Folia fertilizer. No fertilizer served as control. The Poultry manure was applied 2 weeks before seeds were sown by mixing the respective quantity with the 5 kg soil sample to achieve some level of homogeneity.

Four (4) seeds of *H. sabdariffa* were sown per pot and later thinned to two when the seedlings were 2 weeks old. Folia fertilizer (Plantzyme®) was applied to the seedlings as described above according to the manufacturer's recommended rate of 500 ml per hectare at the age of two weeks. The plants were adequately watered. The following data were taken at maturity: plant height (cm), number of leaves per plant, shoot dry weight (g), Number of fruits per plant, fresh and dry fruit weights (g). Harvesting of fruits to get the calyces for phytochemical analysis was done at maturity.

Preparation of calyces samples for analysis

Reasonable quantity of *H. sabdariffa* calyces were collected from representative of each of the treated plant samples and were air-dried for two weeks. The dried samples were ground into powder with an electric blender. The dried samples were later used for the phytochemical analysis.

Qualitative phytochemical analysis of samples

Test for the presence of alkaloids, anthocyanins, flavonoids, anthraquinones, phenols, phlobatannins, Saponins, steroids and tannins were carried out on the different samples using standard phytochemical screening methods as described by Trease and Evans (2002), Sofowora (1993) and Ordonez *et al.* (2006) as follows;

Test for the presence of alkaloids

Each of the ground samples (5 g) was defatted with 5% ethyl ether for 15 min and extracted for 20 min with 5 mL aqueous HCL on a boiling water bath. The resultant mixture was centrifuged for 10 min at 3000 rpm. To 1 mL of the filtrate was added few drops of Mayer's reagent and another 1 mL with Dragendoff's reagent. Creamish/ Brown/ Red/ Orange precipitate shows the presence of alkaloids.

Test for the presence of anthocyanins

- i) To 5 mL of extract of each of the samples was added 2 mL of 10% Sodium hydroxide. Yellow to orange colour shows the presence of anthocyanins.
- ii) To the extract (5 mL) of each of the samples was added 2 mL of conc. H₂SO₄. Yellowish orange colour confirmed the presence of anthocyanins.

Test for the presence of Flavonoids

To 0.5 mL of each of the samples solution was added 0.5 mL of 2% AlCl_3 ethanol solution and left for 1 h at room temperature. A yellow colour indicated the presence of flavonoids.

Test for the presence of anthraquinones

Borntrager's test was used for detecting the presence of anthraquinones. Equal volume (3 mL) of benzene and aqueous extract of each of the samples were shaken together, filtered and 5 mL of 10% ammonia solution was added to the filtrate. This was shaken together and the presence of a pink, red or violet colour in the lower phase indicated the presence of anthraquinones.

Test for the presence of Phlobatannins

Aqueous extract (2 mL) of each of the samples was added to 2 mL of 1% HCL and then boiled in a water bath. Deposition of a red precipitate indicated the presence of phlobatannins.

Test for the presence of Saponins

Each of the powdered samples (2 g) was boiled in 20 mL of distilled water in a water bath and filtered. To 10 mL of the filtrate was added 5 mL of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil, shaken vigorously, and then observed for the formation of emulsion.

Test for the presence of Steroids

Equal volume of concentrated H_2SO_4 and Acetic Anhydride (2 mL) were poured into 5 mL each of the aqueous extract of the samples. The colour changed from violet to blue or green indicating the presence of steroids.

Test for the presence of Tannins

Each of the ground samples (0.5 g) was boiled in 20 mL of distilled water in a test tube and then filtered. A few drop of 0.1% ferric chloride was added and observed for brownish green or blue coloration indicating the presence of tannins.

Quantitative phytochemical analysis of samples

Determination of Alkaloid Contents: Total alkaloid contents of the samples were determined quantitatively using the method of Harborne (2005). A volume of 200 μL of 10% acetic acid was prepared in ethanol and added to 5 g of each of the calyces' samples, covered and allowed to stand for 4 h. The filtrates obtained after filtration were reduced to one-fourth of their original volume over a water bath. Concentrated ammonium hydroxide was added in drops to the extracts until precipitation was completed. The whole solution was allowed to settle and re-filtered after washing with dilute ammonium hydroxide. The residue obtained for each sample was dried, weighed and the percentage composition was determined using the formula: % alkaloid = final weight of the sample/initial weight of the extract $\times 100$.

Determination of anthocyanin contents: Total anthocyanin contents of the samples were determined using the method of Ijeomah *et al.* (2012). A portion of the sample extract, 10 mL (1 mg/ mL H_2O) was diluted to 50 mL with distilled water and divided into two equal parts. These were grouped into two batches, with the pH of one batch adjusted to 1.0, while the second

batch was adjusted to pH of 4.5. The absorbance of each sample was measured at 535 nm, and the difference in absorbance readings at the two pH calculated.

Determination of Flavonoid Contents: The amount of flavonoids in the extract of each of the calyces' samples was determined by using the aluminum colorimetric assay method (Arowosegbe *et al.*, 2012). To 0.5 mL of the sample solution was added 0.5 mL of 2% AlCl_3 ethanol solution. After 1 h at room temperature, the absorbance was measured at 420 nm using UV spectrophotometer. Extract samples were evaluated at a final concentration of 0.1 mg/mL. Total flavonoids were calculated as mg/g of quercetin standard curve using the following calibration: $Y = 0.0255x$; $R^2 = 0.9812$, where x was the absorbance and Y was the quercetin equivalent.

Determination of Total Phenols Contents: The amount of phenols in each of the samples extract was determined spectrophotometrically using the modified method of Oyedemi *et al.* (2012). An aliquot of the extract (1 mg/mL) was mixed with 5 mL Folin-Ciocalteu reagent that was previously diluted with water (1:10 v/v) and 4 mL (75 g/L) of sodium carbonate. The tubes containing all these were vortexed for 15 s and allowed to stand for 30 min at 40 °C to allow for colour development. The absorbance was then measured at 765 nm using the UV spectrophotometer. Results obtained were expressed as mg/g of tannic acid equivalent using the calibration curve from the equation: $Y = 0.1216x$; $R^2 = 0.936512$, where x was the absorbance and Y the tannic acid equivalent.

Determination of Saponin Contents: Saponin contents was determined using the method of Obadoni and Ochuko (2001). The plant sample (20 g) was added to 100 mL of 20% aqueous ethanol and kept in a shaker for 30 min. The samples were heated over a water bath for 4 h at 55 °C and then filtered. The residues were re-extracted with 200 mL of 20% aqueous ethanol. The extracts obtained were concentrated over a water bath at 90 °C to approximately 40 mL. The concentrate was transferred into a 250 mL separatory funnel and extracted twice with 20 mL diethyl ether. The ether layer was discarded and the aqueous layer retained and to which 60 mL *n*-butanol was added. The *n*-butanol extracts were washed twice with 10 mL of 5% aqueous sodium chloride. The samples were dried in the oven at 40 °C to a constant weight after evaporation. The saponins content was then calculated using the formula: % Saponins = final weight of sample/initial weight of extracts \times 100.

Determination Tannin Contents: The method of AOAC (1990) was used, with little modification. To 0.20 g of the sample was added 20 mL of 50% methanol, shaken together and placed in a water bath at 77 to 80°C for 1 h. The extract was then filtered into a 100 mL volumetric flask and 20 mL distilled water was added followed by 2.5 mL Folin-Denis reagent and then 10 mL of 17% aq. Na_2CO_3 . The mixture was made up to 100 mL with distilled water, and allowed to stand for 20 min. The absorbance of the tannic acid standard and the samples were measured at 760 nm. Results were expressed as mg/g of tannic acid equivalent using the calibrated curve from the equation: $Y = 0.0593x - 0.0485$; $R^2 = 0.9826$, where x was the absorbance and Y tannic acid equivalent.

Statistical Analysis

Data were analysed using one-way Analysis of Variance (ANOVA) and the means were separated at $P < 0.05$ using Duncan's Multiple Range Test (DMRT). All statistical analyses were done using SAS software, 1999 version.

RESULTS AND DISCUSSION

Growth and yield

Application of organic and folia fertilizer either singly or in combination, significantly ($P < 0.05$) improved the performance of *H. sabdariffa* in terms of growth and some yield parameters (Table 1). The treated plants performed better than the control in most cases. Application of 120 g poultry manure in combination with folia fertilizer produced the highest plant height (98.23 cm) and dry shoot weight per plant (24.66 g), while the highest leaf number per plant was recorded in plants treated with 120 g poultry manure (164.00). However, the lowest values were recorded for these in the control (50.66 cm, 7.94 g and 44.00, respectively).

Table 1. Impact of organic (Poultry manure) and Folia fertilizer treatments on some growth and yield parameters of *H. sabdariffa*.

Treatment	Plant height (cm)	Leaf number plant ⁻¹	Shoot dry weight Plant ⁻¹ (g)	Fruit number Plant ⁻¹	Fresh weight fruit ⁻¹ (g)	Dry weight fruit ⁻¹ (g)
Control	50.66 ^d	44.00 ^f	7.94 ^c	6.25 ^d	10.36 ^d	1.23 ^b
FF	62.00 ^c	53.34 ^e	8.12 ^c	11.14 ^c	26.10 ^{bc}	3.77 ^a
40 g PM	81.34 ^b	114.00 ^c	20.60 ^{ab}	12.00 ^c	14.83 ^{cd}	1.50 ^b
80 g PM	78.66 ^b	129.34 ^b	21.86 ^{ab}	9.14 ^{cd}	21.37 ^{bc}	3.13 ^a
120 g PM	79.34 ^b	164.00 ^a	22.00 ^{ab}	15.23 ^{bc}	28.06 ^b	4.43 ^a
40 g PM+FF	82.66 ^b	89.34 ^d	18.94 ^b	16.59 ^{ab}	21.97 ^{bc}	3.50 ^a
80 g PM+FF	82.00 ^b	111.34 ^c	21.86 ^{ab}	14.66 ^{abc}	20.70 ^{bc}	3.50 ^a
120 g PM+FF	98.23 ^a	112.00 ^c	24.66 ^a	19.33 ^a	44.97 ^a	4.40 ^a

Values with the same letter(s) within the column are not significantly difference at $P < 0.05$ by Duncan's Multiple Range Test (DMRT). PM: Poultry manure; FF: Folia fertilizer; PM+FF: Poultry manure + Folia fertilizer

The best fruit yield (19.33) measured as number of fruits per plant was also obtained in *H. sabdariffa* plants treated with 120 g poultry manure in combination with folia fertilizer, while the least number of fruits per plant (6.25) was observed in the control (Table 1). Interestingly, application of 120 g of poultry manure alone produced the highest fresh and dry fruit weights (28.06 g and 4.43 g, respectively). Tiamiyu *et al.* (2012) also reported that application of poultry dropping to the soil led to greater plant height in okra. The results obtained in this study is also in line with an earlier research work reported by Adenawoola and Adejoro (2005), that poultry manure was found to increase growth and yield of *Corchorus olitorius*. This was attributed to the availability of nutrients in the soil treated with poultry manure for easy absorption by plant roots (Ajari *et al.*, 2003). According to Warrman (1986), poultry manure tends to be rich in nitrogen (an essential macro element in the soil) due to the diet consumed by chicken. These results also suggested that the response of *H. sabdariffa* to poultry manure treatment is level dependent. The highest level (120 g) of the manure gave the best values, while the least level (40 g) produced very low values in most of the parameters studied. It had been reported that plant height, number of leaves, leaf area as well as N, P and K contents of soil were increased with increase in the level of poultry manure (Ayeni, 2010).

As observed in this study, application of folia fertilizer alone to *H. sabdariffa* had a better effect on the plant than the control in all the variables studied except in the shoot dry weight, but not as much as the impact of poultry manure. However, the combination of poultry manure and folia fertilizer treatments produced the best results. This is in agreement with the findings of Shaalan (2005), who reported that folia application of fertilizer in combination with soil applied organic fertilizer increased the fruit yield and the vegetative growth of *Nigella sativa*. Folia fertilizer therefore, could boost the impact of soil applied organic fertilizers.

Phytochemical composition

The effect of organic and folia fertilizer treatments on the presence of alkaloids, anthocyanins, flavonoids, anthraquinones, phenols, phlobatannins, saponins, steroids and tannins in the calyces of *Hibiscus sabdariffa* is shown in table 2.

Results obtained from this study indicated that there were no anthraquinones, phlobatannins and steroids in the calyces of both treated and the untreated *H. sabdariffa* plants. However, alkaloids, anthocyanins, flavonoids, phenols, saponins and tannins were found in both treated and untreated plants. The implication of these is that organic and folia fertilizer treatments did not affect the presence of phytochemicals in the calyces of *H. sabdariffa*. Osuagwu (2008); Osuagwu and Edeoga (2012) had also reported that treatment with poultry manure and inorganic fertilizer respectively, had no effects on the presence or absence of phenols, flavonoids and steroids in the leaves of *Ocimum gratissimum*. This observation might be due to the fact that phytochemicals are naturally synthesized chemical compounds in plants tissues and are therefore, present in them irrespective of presence or absence of the fertilizer treatments

Table 2. Impact of organic (Poultry manure) and Folia fertilizer treatments on the quantitative phytochemical composition of *H. sabdariffa* Calyces

Phytochemicals	Treatments							
	Control	FF	40 g PM	80 g PM	120 g PM	40 g PM + FF	80 g PM + FF	120 g PM + FF
Alkaloids	+	+	+	+	+	+	+	+
Anthocyanins	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+
Anthraquinone	-	-	-	-	-	-	-	-
Phenols	+	+	+	+	+	+	+	+
Phlobatannins	-	-	-	-	-	-	-	-
Saponins	+	+	+	+	+	+	+	+
Steroids	-	-	-	-	-	-	-	-
Tannins	+	+	+	+	+	+	+	+

(+) Present; (-) Absent; PM: Poultry manure; FF: Folia fertilizer; PM+FF: Poultry manure + Folia fertilizer.

It was observed that application of poultry manure and folia fertilizer had significant effects ($p < 0.05$) on the phytochemical contents in *H. sabdariffa* calyces. (Table 3). Alkaloids content was highest ($2.81 \pm 0.02\%$) under 120 g of poultry manure treatment, followed by 120 g in combination with folia fertilizer treatment ($2.61 \pm 0.01\%$). However, the lowest alkaloids content was found in the control ($1.66 \pm 0.00\%$) and application of folia fertilizer alone ($1.75 \pm 0.05\%$). Interestingly, application of poultry manure and folia fertilizer seemed to cause a

reduction in the anthocyanins content of *H. sabdariffa*, as the highest was found in the untreated plants (12.36 ± 0.02 mg/g). The production of total flavonoids was also highest under 120 g of poultry manure in combination with folia fertilizer (14.23 ± 0.03 mg/g) and treatment with 120 g of poultry manure (13.58 ± 0.02 mg/g), while the lowest was recorded in the control (10.67 ± 0.05 mg/g). The increased flavonoid content due to fertilizer application has also been reported in other plants (Geneva *et al.*, 2008; Osuagwu, 2008; Ibrahim *et al.*, 2013).

Table 3. Impact of organic (Poultry manure) and Folia fertilizer treatments on the quantitative phytochemical composition of *H. sabdariffa* Calyces.

Treatments	Phytochemical contents					
	Alkaloids (%)	Anthocyanins (mg/g)	¹ Total Flavonoids (mg/g)	² Total Phenols (mg/g)	Saponins (%)	² Tannins (mg/g)
Control	1.66 ± 0.0 ^g	12.36 ± 0.02 ^a	10.67 ± 0.05 ^f	0.91 ± 0.00 ^{de}	0.83 ± 0.01 ^d	8.52 ± 0.11 ^g
FF	1.75 ± 0.05 ^{fg}	11.59 ± 0.06 ^{de}	11.13 ± 0.01 ^d	0.80 ± 0.01 ^f	0.84 ± 0.06 ^d	9.10 ± 0.10 ^f
40 g PM	1.83 ± 0.04 ^{ef}	11.83 ± 0.10 ^c	10.83 ± 0.11 ^{ef}	0.84 ± 0.04 ^{ef}	0.93 ± 0.01 ^c	9.02 ± 0.01 ^f
80 g PM	1.95 ± 0.00 ^d	12.15 ± 0.02 ^b	13.58 ± 0.02 ^b	0.72 ± 0.00 ^g	0.99 ± 0.01 ^{bc}	10.44 ± 0.01 ^d
120 g PM	2.81 ± 0.02 ^a	11.72 ± 0.04 ^{cd}	14.16 ± 0.01 ^a	1.65 ± 0.01 ^b	1.27 ± 0.01 ^a	12.53 ± 0.02 ^b
40 g PM+FF	1.90 ± 0.01 ^{de}	11.15 ± 0.01 ^f	10.90 ± 0.06 ^e	0.95 ± 0.01 ^d	1.03 ± 0.05 ^b	9.79 ± 0.01 ^e
80 g PM+FF	2.14 ± 0.03 ^c	12.34 ± 0.00 ^a	12.65 ± 0.07 ^c	1.15 ± 0.01 ^c	0.76 ± 0.02 ^d	10.65 ± 0.01 ^c
120 g PM+FF	2.61 ± 0.01 ^b	11.51 ± 0.01 ^e	14.23 ± 0.03 ^a	1.85 ± 0.04 ^a	1.25 ± 0.02 ^a	13.24 ± 0.04 ^a

Data are expressed as means \pm SE of triplicate experiments. Values in a column not having the same superscripts are different ($P < 0.05$). Mean with the same subscript in the same column are: ¹Expressed as mg quercetin /g of dry plant samples. ²Expressed as mg tannic acid /g of dry plant samples.

PM: Poultry manure; FF: Folia fertilizer; PM+FF: Poultry manure + Folia fertilizer.

The impact of poultry manure and folia fertilizer on the production of saponin in the calyces of *H. sabdariffa* followed the same trend with the total flavonoids content. Application of 120 g of poultry manure in combination with folia fertilizer and treatment with 120 g of poultry manure also gave the highest saponin contents of $1.27 \pm 0.01\%$ and $1.25 \pm 0.02\%$ respectively, while the lowest was recorded in the control ($0.83 \pm 0.01\%$). This is in line with the findings of Ibrahim *et al.* (2013), who reported the highest saponin content in *Labisia pumila* treated with organic fertilizer. The highest total phenols and tannins contents were also recorded under 120 g poultry manure in combination with folia fertilizer (1.85 ± 0.04 mg/g and 13.24 ± 0.04 mg/g, respectively), followed by treatment with 120 g poultry manure (1.65 ± 0.01 mg/g and 12.53 ± 0.02 mg/g, respectively). However, the lowest total phenols (0.08 ± 0.01 mg/g) and tannins (8.52 ± 0.11 mg/g) were found in the calyces of the folia fertilizer treated plants and the control respectively. According to Zheng *et al.* (2006), plants treated with fertilizer tend to have nutrients like nitrogen and phosphorus which are necessary for the synthesis of phenols

in plants, hence the highest content of phenols observed in the calyces of *H. sabdariffa* treated with organic manure with folia fertilizer spray.

CONCLUSION

It was observed in this study that application of poultry manure (organic fertilizer) singly or in combination with folia fertilizer enhanced the growth and yield of *H. sabdariffa*, particularly at highest level. The production of, alkaloids, anthocyanins, flavonoids, phenols, saponins and tannins in the calyces of this plant was also highest under organic fertilizer particularly in combination with folia fertilizer. Thus, it could be concluded that application of poultry manure in combination with folia fertilizer in the cultivation of this plant could enhance better growth, good yield and its medicinal value under safe agricultural condition.

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