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An Evaluation of the Concentration of Six Phytochemicals Present in Seven Assessions of Dioscorea Bubifera

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Abstract **Original Research Article**

The experiment on Evaluation of the concentration of six phytochemicals present in seven assessions of Dioscorea bulbifera was carried out at the research farm of University of Port Harcourt Rivers state Nigeria. It was laid out in a randomized complete block design (RCBD). Various treatments (Ashes amended with sandy soil (AS), Sawdust amended with sandy soil (SAW), sandy soil (SD), and garden soil as control (CNT) were used with seven assessions of Dioscorea bulbifera which includes; assession 3079, 3083, 3084, 3087, 3089, 3094 and 4121 were planted on each of the treatments for 8 weeks. Data was obtained by analyzing the leaves. The results shows that

Phytate; There was asignificant difference increase in assession 3083 of SD and 4121 of sawdust treatments as they were compared to the control

Tanin; We experienced a significant difference increase only in assession 3083 of SD treatment as we compared to the control

Oxalate; There was a significant difference increase only in assessions 3083 of SD and 4121 of sawdust (saw) treatment.

Flavonoid; There was significant difference increase in assessions 3083, 3087 and 3089 of SD, 3087 and 3089 of AS treatments when we compared to the control

Alkaloid; Here we recorded a significant difference increase in assessions 3079, 3087, 3094 and 4121 of SD treatment, 3094 and 4121 of SAW treatment. 3084, 3087, 3094 and 4121 of AS treatment

Phenol; we have an increase in 3083, 3987, of SD and 3087, 3089 of AS treatments.as we compared to the control. This has also shown that assessions 4121 and 3083 has a reasonable amount of phytochemicals present in them than the others and sandy soil (SD) as the best soil after the normal humus soil for optimum production of phytochemical.

Keywords: Dioscorea Bulbifera, Phytochemicals, Phytate, Tannin, Oxalate, Flavonoid, Alkaloid, Phenol, Soil Treatments, Randomized Complete Block Design.

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INTRODUCTION

Dioscorea family. The Dioscorea species popularly known as yam word wide is a prime staple medicinal food substitute for many people. Across different ethnic communities and geographic regions different species of the dioscorea have been adopted within different habitation as a food source due to the high nutritional benefits and values towards treatment and cure of different health challenges Kumar sanjeet *et al*. 2017

. Despite the grate huge benefits derived from the dioscorea family, not much investments/ Research attention is given to them. According to Jue *et, al* , the nutritional value of *Dioscorea bulbifera* is Moisture= 61.6-92.5, Crude protein=0.89-16.8, Crude fat=0.30-8.13, Crude fiber =0.61-18.2, Ash=0.05=8.15, and Starch= 12.5=62.7. (Foods 2020, 9, 1304). It is sad to note that of the over 600 species of yam, only a few are cultivated for food while others are left unexploited (Achy *et, al* 2016). Aerial yam is among the species of yam reported to have originated from Africa and Asia (Ahmed *et, al* 2009, Anthony *et, al* 2011), It is known for its food value (Adewole *et, al* 2011). It is known for its high yielding bulbil bearing propensity with a low capital and labour requirement for propagation when compared with some other consumed yams (Mbaya *et, al* 2013; Afiukwa and Igwe Dan 2015) . *Dioscorea bulbifera* also provides other nutritional benefits like lipids, vitamins, proteins and

minerals (lazzitity *et al* 1998). It is worthy to note that yam's potential as source of food is attributed to its high level of carbohydrate including fiber; starch and sugar contribute about 200 dietary calories per person per day to 300 million people in the tropics (Obidiegwu *et, al* 2020) (mignouna *et al* 2008).

Assessions is collecting and documenting different varieties or strains of *Dioscorea bulbifera* as this is important for studying the plants genetic diversity, understanding its cultivation potentials and beneficial traits for breeding and conservation purpose. Key aspects of *Dioscorea bulbifera* involves collection of multiple assessions as this gives a research room to assess the genetic variation within the species for identifying useful traits for improvement and phytochemical composition

We embarked on this research as to ascertain the concentration of six phytochemicals present in different assessions of *Dioscorea bulbifer*a as this will help to understand their medicinal and other potential uses. It will also help in conservation and agricultural improvement

2.0 MATERIALS AND METHODS

These research work was conducted ai the research farm of the University of portHarcourt , PortHarcourt Rivers state NIgeria between November 2021 and September 2023 cropping season the site is located at (latitude 4º 54"2718MM. seven assessions of Aerial yam (*Dioscorea bulbifera*) bulbils were obtained from Genetic Resource Centre (GRC) of the International Institute of Tropical Agriculture (IITA) Ibadan Nigeria. The treatments' include Saw-dust Amended with sandy soil (SAW) Ashes Amended with sandy soil (AS), Sandy soil (SD) and Garden soil as control (CNT) were used with seven assessions of *Dioscorea bulbifera* which includes; assession 3079, 3083, 3084, 3087, 3089, 3094 and 4121 were planted on each of the treatments for 8 weeks. Data was obtained by analyzing the leave
The phytochemicals analysis of *(Dioscorea bulbifera*.) was carried out on all the assessions to determine the phytochemicals present in the *Dioscorea bulbifera*. The following phytochemicals: Phytate, tannin, oxalate, alkaloids, flavonoids and phenolic compounds were analyzed.

2.1 Phytate

We used the method of (Okonwu *et, al* 2017), Oberlease *et al*. (1973).to know the concentration of phytate, we use 0.2 N HCl to extract the material such that 3-30 µg/mL phytate solution was gotten. The plant material (0.5 ml) was pipetted into a test tube fitted with a ground-glass stopper and 1 mL of ferric solution was added (Okonwu *et, al* 2017) we heated the test tube in very hot water bath for about 30 minutes. We made sure the Sample was cooled in ice water for not more than 15 minutes and allowed to settle to room temperature. We made sure the content of the tube was mixed and centrifuged for not more than 30 minutes at 3000 rpm. Then we transferred the supernatant (1 m) to another and 1.5 mL of 2, 2-Bipyridine solution. The Absorbance of the solution is 519 nm when compared to distilled water. We had to calibrate it with the reference solutions as a substitute for the sample solution with each set of analyses. We prepared the calibration curve by plotting the concentrations of the reference solutions against their corresponding absorbance. The absorbance of the test sample was now used to know the concentration level from the calibration curve (Okonwu et, al 2017).

2.2 Determination of Tannin

We carried out this with the Folin-Denis spectrophotometric method. The method was described by Pearson (1976) and (Okonwu *et, al* 2017 (Okonwu *et, al* 2017). The tannin content was as shown below;.
% Tannin = An/As x C x 100/w x 5
Where, An = absorbance of test sample; As = absorbance of standard solution; C = concentration of standard solution; W= weight of the sample we used (Okonwu *et ,al* 2017).

2.3 Oxalates

The process we used here in oxalate determination was same with the method used by (Okonwn *et al* 2017) It takes three major steps which includes; digestion, oxalate precipitation and permanganate titration (Okonwu *et ,al* 2017).
(Ijesanmi *et, al* 2016).

2.4 Flavonoids (extraction and analysis)

The flavonoids in the plant were known by using the procedure used by IIesanmi *et al*.2016). Plant sample (1.5g) were weighed into a measured set of extraction tube(s) and 20ml of boiled ultra-pure water transferred into all the extraction tubes. We allowed it to stand for 1.5 hours and we allowed it for 5 minutes before we now transferred the solution to a clean sets of centrifuge tubes, shook for 15 minutes and centrifuged for 5 minutes at 3000 rpm. After which, a set of vials were used to collect the supernatants before determination on water 616/626 HPLC. The way we did the analysis of the flavonoids are as follows: (i) An auto sampler (ii) An automated gradient controller (iii) Gradient elution HPLC pump (iv) Reverse-phase HPLC column were thermostatically heated in a room that was temperature-controlled (v) Detector by fluorescence (vi) Carrier gas: Nitrogen gas at flow rate of 60 ml/min. (vii) Temperature: Detector- 147oC; Injector port- 166oC and Column: 115oC (viii) Computer facilities for storing data. (ix) Printer for results reporting (Okonwu *et, al* 2017.

2.5 Alkaloids

We were able to know the alkaloids in the plant following the procedure adopted by Ilesanmi *et al*. (2016).. The conditions of HPLC (Water 616/626) for the analysis of alkaloids were as follows: (i) An auto sampler (ii) An automated gradient controller (iii) Gradient elution HPLC pump (iv) Reverse-phase HPLC column, thermostatically heated in a temperature-controlled room. (v) Detector by fluorescence (vi) Carrier gas: Nitrogen gas at the rate of 40 mL/mins. (vii) The Temperature: Detector- 170oC; Injector port-90oC and Column- 125oC (viii) The Computer facilities for storing data. (ix) Printer was for

results reporting (Adebola 2023).

2.6 Phenolic extraction

The process we used in analyzing Phenolic compounds extraction in the plant was according to the procedure used by Okonwu and Akonye (2019) using 616/626 HPLC.. The conditions we adapted to in analysis of phenolics were (i) An auto sampler (ii) An aut. (Ilesanmi *et al* 2016).omated gradient controller (iii) we used Gradient elution HPLC pump (iv) Reverse-phase HPLC column, thermostatically heated in a temperature-controlled room. (v) Detector by fluorescence (vi) Carrier gas: Argon gas at flow rate of 60ml/mins. (vii) Temperature: Detector- 120oC; Injector port- 155oC and Column- 117oC (viii) we also used Computer facilities for storing accurate data. (ix) Printer was also used for results reporting.(llesanmi *et al* 2016).

RESULTS

Phytate Concentration of all Assessions of *Dioscorea bulbifera.* Grown on the Treatments Sample

The phytate concentration of all the assessions of *Dioscorea bulbifera* grown on control (normal soil), SAW (saw dust), SD (Sandy Soil) and AS (Ashes) are shown in Figure 1 below. There were significant difference decrease in phytate concentration in assessions 3079, 3084, 3087 and 3089 of SAW, 3079, 3087, 3089, 3094 and 4121 of SD and 3079, 3083, 3084, 3087, 3089, 3094 and 4121 of AS treatments when they were compared to the control except assession 3083 of SD which shows significant increase. Also, there were significant difference in some of the assessions treated within control, SAW, SD and AS.

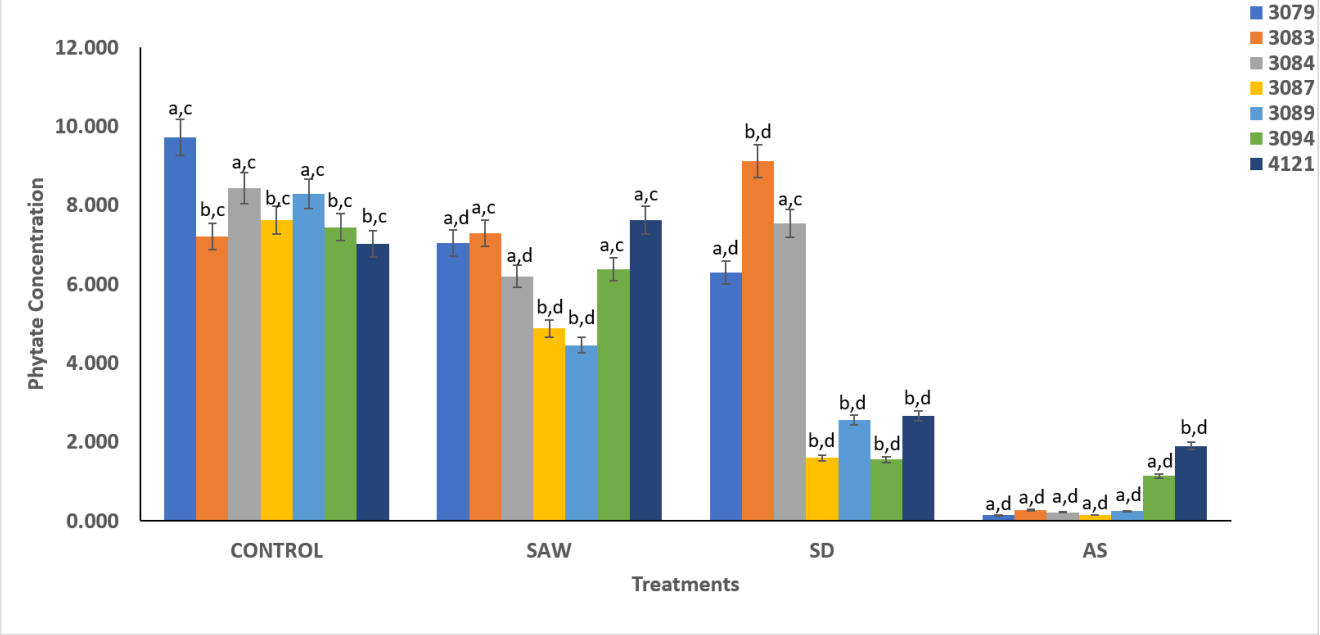

Figure 1: Phytate Concentrations of different assessions of *Dioscorea bulbifera* (3079, 3083, 3084, 3087, 3089, 3094 and 4121) grown on normal soil Control (CNT), Sawdust (SAW), Sandy soil (SD) and Ashes (AS).

Values are means ± Standard Error Mean (SEM). Values with different superscripts are statistically different at ($p \leq 0.05$). Superscript (a,b) compares assession 3083, assession 3084, assession 3087, assession 3089, assession 3094 and assession 4121 to assession 3079 (1st letters) within the group while Superscript (c,d) compares SAW, SD and AS to Control of the same assession across the treatments (2nd letters).

Tanin Concentration of all Assessions of *Dioscorea bulbifera.* Grown on the Treatments Sample

The Tanin concentration of all the assessions of *Dioscorea bulbifera* grown on control (normal soil), SAW (saw dust), SD (Sandy Soil) and AS (Ashes) are shown in Figure 2 below. There were significant difference decrease in tanin concentration in assessions 3079, 3084, 3087 and 3089 of SAW, 3079, 3087, 3089, 3094 and 4121 of SD and 3079, 3083, 3084, 3087, 3089, 3094 and 4121 of AS treatments when they were compared to the control except assession 3083 of SD which shows significant increase. Also, there were significant difference in some of the assessions treated within control, SAW, SD and AS.

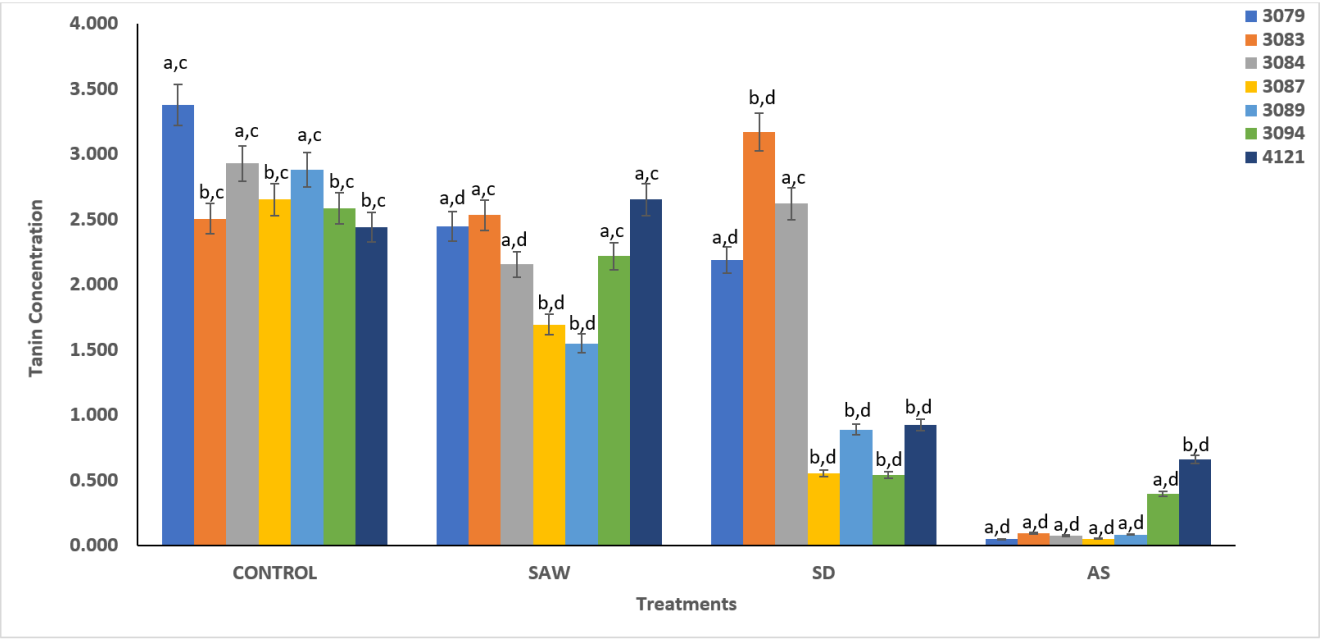

Figure 2: Tanin Concentrations of different assessions of *Dioscorea bulbifera* (3079, 3083, 3084, 3087, 3089, 3094 and 4121) grown on normal soil Control (CNT), Sawdust (SAW), Sandy soil (SD) and Ashes (AS).

Values are means ± Standard Error Mean (SEM). Values with different superscripts are statistically different at ($p \leq 0.05$). Superscript (a,b) compares assession 3083, assession 3084, assession 3087, assession 3089, assession 3094 and assession 4121 to assession 3079 (1st letters) within the group while Superscript (c,d) compares SAW, SD and AS to Control of the same assession across the treatments (2nd letters).

Oxalate Concentration of all Assessions of *Dioscorea bulbifera.* Grown on the Treatments Sample

The oxalate concentration of all the assessions of *Dioscorea bulbifera* grown on control (normal soil), SAW (saw dust), SD (Sandy Soil) and AS (Ashes) are shown in Figure 3 below. There were significant difference decrease in oxalate concentration in assessions 3079, 3084, 3087 and 3089 of SAW, 3079, 3087, 3089, 3094 and 4121 of SD and 3079, 3083, 3084, 3087, 3089, 3094 and 4121 of AS treatments when they were compared to the control except assession 3083 of SD which shows significant increase. Also, there were significant difference in some of the assessions treated within control, SAW, SD and AS.

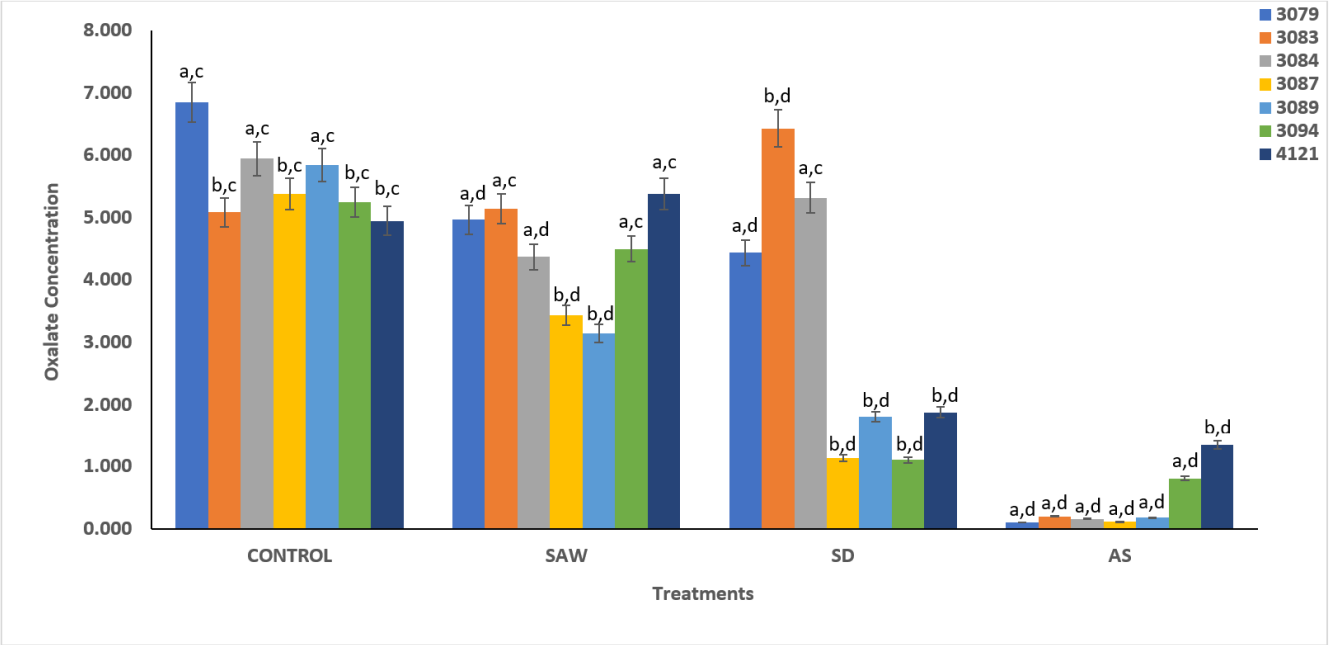

Figure 3: Oxalate Concentrations of different assessions of *Dioscorea bulbifera* (3079, 3083, 3084, 3087, 3089, 3094 and 4121) grown on normal soil Control (CNT), Sawdust (SAW), Sandy soil (SD) and Ashes (AS).

Values are means ± Standard Error Mean (SEM). Values with different superscripts are statistically different at ($p \leq 0.05$). Superscript (a,b) compares assession 3083, assession 3084, assession 3087, assession 3089, assession 3094 and assession 4121 to assession 3079 (1st letters) within the group while Superscript (c,d) compares SAW, SD and AS to Control of the same assession across the treatments (2nd letters).

Total Flavonoids Profile Concentration of all Assessions of Dioscorea bulbifera. Grown on the Treatments Sample

The total flavonoids profile concentration of all the assessions of Dioscorea bulbifera grown on control (normal soil), SAW (saw dust), SD (Sandy Soil) and AS (Ashes) are shown in Figure 4. below. There were significant difference decrease in total flavonoids profile concentration in assessions 3079, 3084, 3087, 3094 and 4121 of SAW, 3079, 3084, 3094 and 4121 of SD and 3079, 3083, 3084, 3089 and 4121 of AS treatments when they were compared to the control except assessions 3087 and 3089 of SD and AS which shows significant increase. Also, there were significant difference in some of the assessions treated within control ,SAW ,SD and AS.

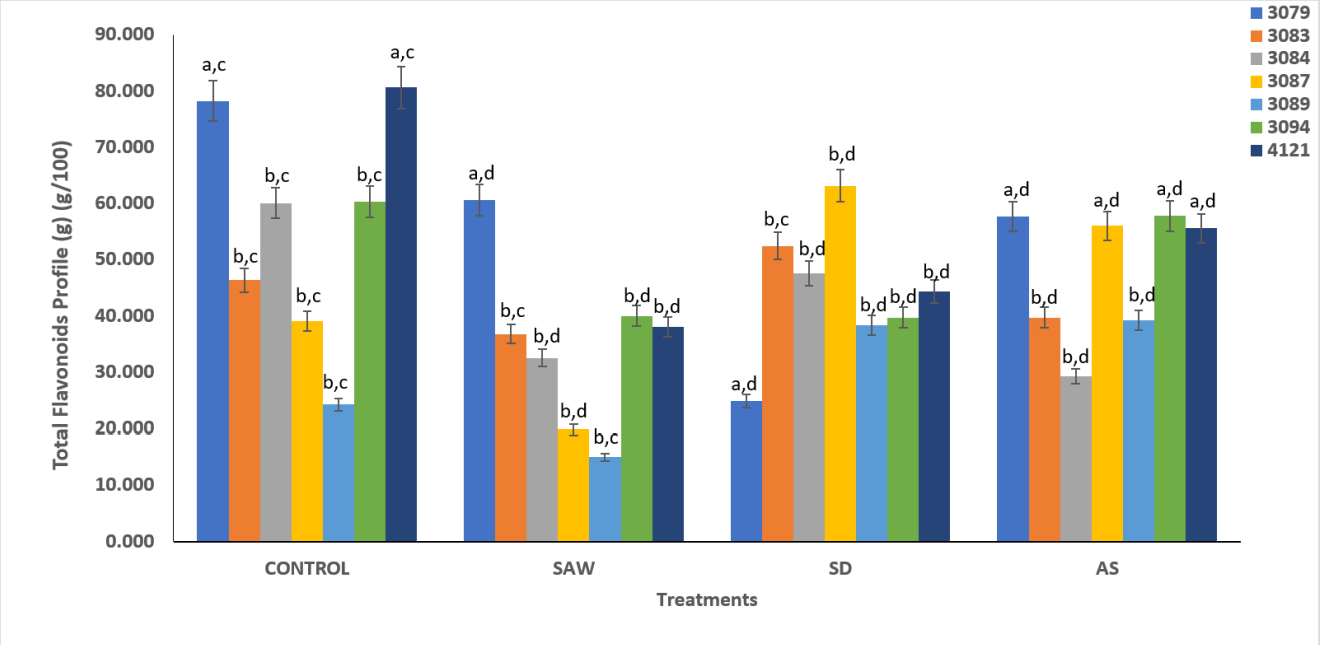

Figure 4: Total Flavonoids Profile Concentrations of different assessions of *Dioscorea bulbifera* (3079, 3083, 3084, 3087, 3089, 3094 and 4121) grown on normal soil Control (CNT), Sawdust (SAW), Sandy soil (SD) and Ashes (AS).

Values are means ± Standard Error Mean (SEM). Values with different superscripts are statistically different at ($p \leq 0.05$). Superscript (a,b) compares assession 3083, assession 3084, assession 3087, assession 3089, assession 3094 and assession 4121 to assession 3079 (1st letters) within the group while Superscript (c,d) compares SAW, SD and AS to Control of the same assession across the treatments (2nd letters).

Total Alkaloids Profile Concentration of all Assessions of Dioscorea bulbifera. Grown on the Treatments Sample

The total alkaloids profile concentration of all the assessions of Dioscorea bulbifera grown on control (normal soil), SAW (saw dust), SD (Sandy Soil) and AS (Ashes) are shown in Figure 5 below. There were significant difference increase in total alkaloids profile concentration in assessions 3094, 4121of Sawdust SAW and 3079,3087, 4121 of SD and 3084, 3087, 3094 and 4121 of AS treatments when they were compared to the control except in assessions 3083, 3089 of SAW and 3083 of AS which shows significant decrease. Also, there were significant difference in some of the assessions treated within control, SAW, SD and AS.

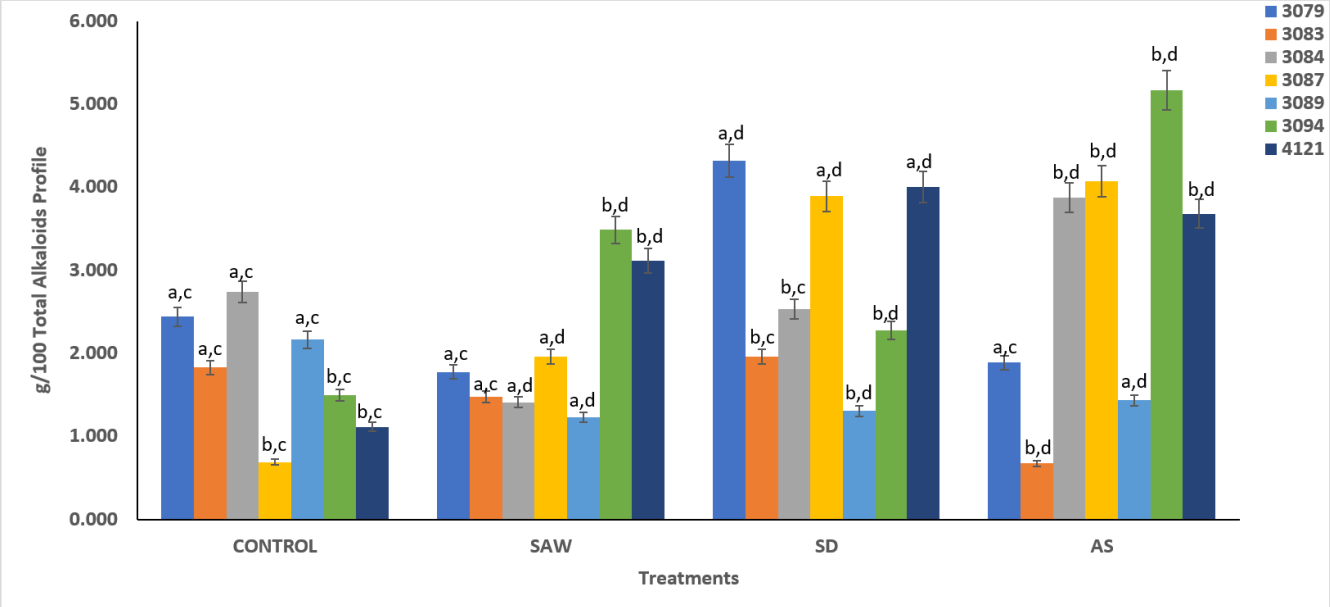

Figure 5: Total Alkaloids Profile Concentrations of different assessions of Dioscorea bulbifera (3079, 3083, 3084, 3087, 3089, 3094 and 4121) grown on normal soil Control (CNT), Sawdust (SAW), Sandy soil (SD) and Ashes (AS).

Values are means ± Standard Error Mean (SEM). Values with different superscripts are statistically different at ($p \leq 0.05$). Superscript (a,b) compares assession 3083, assession 3084, assession 3087, assession 3089, assession 3094 and assession 4121 to assession 3079 (1st letters) within the group while Superscript (c,d) compares SAW, SD and AS to Control of the same assession across the treatments (2nd letters).

Total Phenolics Compounds Profile Concentration of all Assessions of Dioscorea bulbifera. Grown on the Treatments Sample

The total phenolics compounds profile concentration of all the assessions of Dioscorea bulbifera grown on control (normal soil), SAW (saw dust), SD (Sandy Soil) and AS (Ashes) are shown in Figure 6 below. There were significant difference decrease in total phenolics compounds profile concentration in assessions 3079, 3084, 3087, 3094 and 4121 of SAW, 3079, 3084, 3094 and 4121 of SD and 3079, 3084 and 4121 of AS treatments when they were compared to the control except assessions 3087 and 3089 of SD and AS which shows significant increase. Also, there were significant difference in some of the assessions treated within control, SAW, SD and AS.

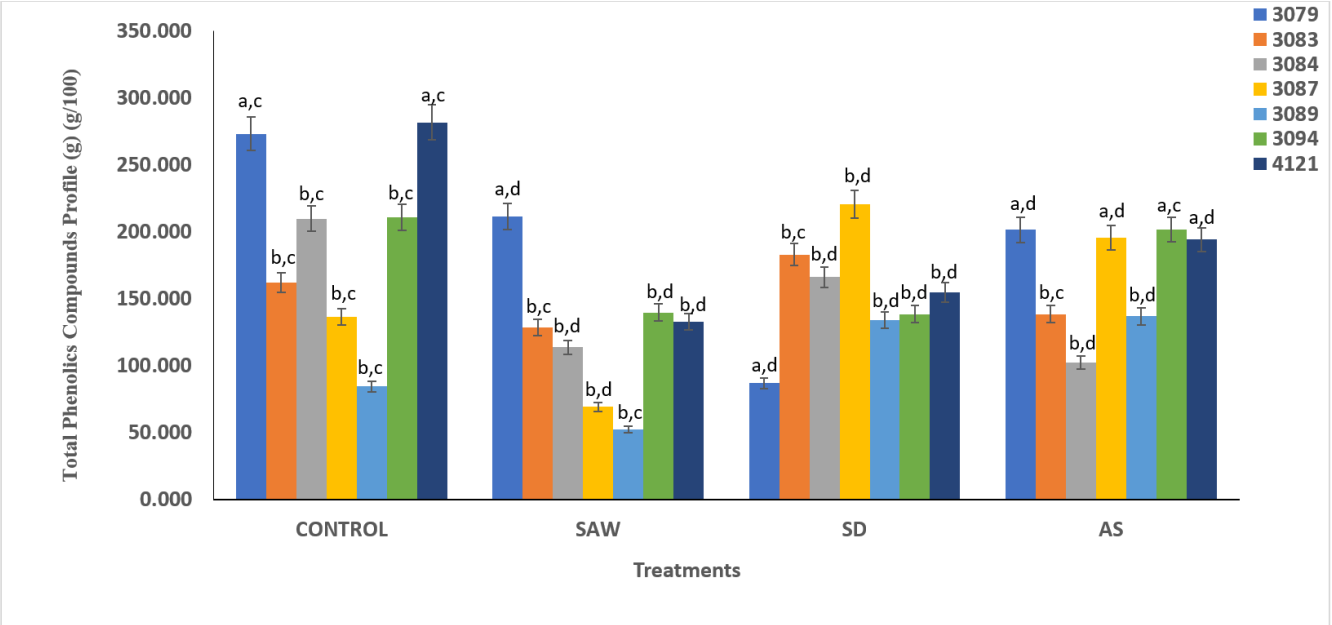

Figure 6: Total Phenolics Profile Concentrations of different assessions of *Dioscorea bulbifera* (3079, 3083, 3084, 3087, 3089, 3094 and 4121) grown on normal soil Control (CNT), Sawdust (SAW), Sandy soil (SD) and Ashes (AS).

Values are means ± Standard Error Mean (SEM). Values with different superscripts are statistically different at ($p \leq 0.05$). Superscript (a,b) compares assession 3083, assession 3084, assession 3087, assession 3089, assession 3094 and assession 4121 to assession 3079 (1st letters) within the group while Superscript (c,d) compares SAW, SD and AS to Control of the same assession across the treatments (2nd letters).

DISCUSSIONS AND CONCLUSION

Phytate;The result of the concentration of phytate from the treatments level shows that sandy soil SD and the humus soil is the best for optimum production of phytate in *Dioscorea bulbifera*. It was in assessions 3084 of SD and 4121 of SAW that we recorded a significant difference increase in phytate concentration. This is shown in **Figure 1.**

Tanni; in assession 3079 there was a significant difference decrease in tannin concentration in all the treatments when compared to the control CNT . In assession 3083 it was a different dimension as there was a significant difference increase in tannin concentration as we compared to the control CNT. We also experience a decrease in tannin concentration in assessions 3084, 3087, 3089 and 3094. In assession 4121 there was a slight increase of Tanin in sawdust treatment and a decrease in SD and AS treatments as we compared to the control This is shown in **Figure 2..**

Oxalate; In assession 3079, there was a significant difference decrease in all the treatments as compared to the control with AS as the lowest. In assession 3084,3087,3089, 3094, there is also a decrease in the concentration of oxalate when compared to the control CNT . in assession 4121 we have a slight increase in SD and AS treatment. This is shown in **Figure** 3

Flavonoid; There was a decrease in flavonoid concentration in some of the assessions when they were compared to the control. In assession 3087, we recorded the highest concentration in the sawdust treatment followed by the Ashes and the lowest was in sandy soil treatment. In assession 3083, the highest concentration was recorded in the SD treatment which shows an increase in Flavoniod. it was also same in assession 3084. In assession 3087 we have a significant difference increase in both the SD and AS treatments, it was also same in assession 3089 but we have the lowest decrease in SAW treatment. In assessions 3094 and 4121 we recorded the highest concentration in the AS treatment as they were compared to the control. This is shown in **Figure 4.**

Alkaloid; there was significant difference increase in total Alkaloid concentration in assession 3094,4121 of sawdust, 3087, 4121 of SD and 3084, 3087, 3094 and 4121of AS when they were compared to the control except in assessions 3083,3089 of sawdust and 3083 of AS which shows a significant decrease when they were compared to the control. This is shown in **Figure 5**.

Phonol; In assession 3079 there was a significant decrease in all the treatments with the SD treatment as the lowest as we compared to the control. In assession 3093, there was a slight increase in SD treatment and a decrease in SAW and AS treatments. In assession3084, we recorded a significant difference decrease in all the treatments with the SD treatment higher than the others. In assession 3087, we experienced an increase in SD and AS treatments with SAW treatment as the lowest. In assession 3094, there was an increase in the concentration of phenol in the SD and AS treatments. In 3094, all the three treatments recorded a decrease in concentration and this was also same in assession 4121 as we compared to the control. This is shown in **Figure 6.**

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