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Investigation into Proximate, Phytotoxicity and Antimicrobial Functions of Active Agent Saponin Extracted from *Momordica charantia* (A Local Herbal Leaf)

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Abstract

Momordica charantia is a medicinal bitter leaf, which has been in use for ages by our fore fathers, hence the need to investigate its biological activities in order to elucidate its medicinal values. The proximate and mineral compositions of *M. charantia* were determined and its protein, fibre, phosphorus, calcium, potassium, sodium, manganese, iron, copper and zinc contents were 25.09, 5.01, 0.45, 1.04, 2.72, 72.6, 1.99, 1.77, 70.7 and 44.9 mg/100 g, respectively. The result further showed that the sodium content in the *M. charantia* is higher than all other elements present, hence helping in the maintenance of acid base balance in the body. The phytotoxic assay of saponin extracted from *M. charantia* on maize seedlings was concentration-dependent and showed an inhibitory effect on growth of root and shoot of the maize seedlings. The phytochemical assay of the sample showed that *M. charantia* contained saponin (80.32%), phytic acid (60.40%), tannin (24.10%) and cyanide (15.5%), respectively. The antimicrobial activities of the saponin extracted from *M. charantia* was observed to have inhibitory effect on the microbial growth such as; *E. coli*, *Streptococcus pyrogens*, *Klebsiella* and *Enterobacter*. The study therefore, concluded that *M. charantia* is fully enriched with saponin levels that could be exploited for as functional food and antimicrobial agent in the areas of food and health applications.

Keywords: *Saponin, Phytotocicity, Antimicrobial, Momordica charantia, Proximate*

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1. Introduction

Momordica charantia is an annual herb, climbing vines growing to 3-4 cm in length with five angled slender stem longitudinally furrowed, tendrils simple or branched leaves with foetid odour when rubbed (Gill, 1992). Its seeds are brown, with scarlet aril, flattened oval, l-1.5 cm in length containing 32% oil with concise weights. It adapted to a wide variation of rainfall, normally grown in hot, humid areas at elevations up to 500 m soil with a high organic content and water-retaining capacity are required for optimum yields. Propagation and planting of the seeds are directly done on beds of ridges, 60-75 cm apart, 30-38 cm between plants or 50 cm x 50 cm each way while requiring support by poles or trellis. The seed required is 4.5.5 kg/ha for a density of 40,000 plants/ha, while irrigation is applied during dry periods to maintain an adequate reserve of soil moisture (Gill, 1992). The organic manure, NPK is required before sowing, followed by applications of nitrogenous fertilizer at intervals during the growing period. The immature fruits may be harvested 50-70 days from sowing to have yields of up to 15t/ha but an average yield would be ~8-l0t/ha. The plants selected for seed production normally have optimum fruit characters according to ideal local preference and not be grown with crops intended for consumption or marketing. Although the seeds are rarely stored for any appreciable period but its fruits may be maintained at a temperature of 1-20 °C in a relative humidity of 85-90% for up to 20-30 days (Wiedenfied and Braverman, 1992).

The immature fruits are boiled or fried often after steeping in salt water to remove the bitter taste and used in curries and pickles, mostly the young shoots and leaved are sometimes cooked. Its stem is grooved while two acidic resins and a bitter substance called momordicine in the leaves have been reported along with vitamin C with or without carcine depending on the sample. The presence of d-aminobutric acid in the leaf has

been estimated to contain about 12.84% ash, with major elements like silicon, calcium, phosphorus, strontium, copper lead, zinc, sodium, iron and soluble pectin but contained no free pectic acid (Wiedenfield and Braveman, 1992). *M. charantia* contained saponins, 5 hydroxy l tryptamine and the alkaloid momordicine but the fruit contained 0.3% total alkaloid and 0.035% isolated charantin in pure state as a neutrolines from fresh immature fruits (Wiedenfied and Braverman, 1992). The presence of steroids glycosides in the fruits and mixture of D glucose of *b*-sistosterol and D^5, 25 stigmastadine 3B-01 was confirmed in *M. charantia* (Sucrow, 1968). The seeds contained 32-35% of a purgative fixed oil that made up of stearic acid, oleic acid (45%), albumin, globulin, glutelin rich and essential amino acids as well as vitamin B, carotene and alpha amino butyric acid (Hokputsa *et al*, 2004).

The dried plant contained 0.038% of alkaloid with the total carotenoid pigments of 8.35 mg/g and an activity of 2.4-5.6 m/g (Breyer and Watt, 1962). Moreover, the results of a screening on the entire Congolese plant species showed the presence of trace amounts of alkaloids and saponins but the absence of flavonoids orthophthalic acid was also characterized from the Brazilian species. A related species, *M. foctida* sehum was found to contain foctidin, which is similar to charantin (Olaniyi and Fatope, 1975)**.** In India peninsula, the roots are used as an aborti-facients, which are being used for the same purpose and sometimes as ingredients in aphrodisiac preparation in Nigeria and Ghana (Olaniyi and Fatope, 1975). In congo, it is used for colic while the juice of the leaves and fruits in certain part of Nigeria are used an anthelmintic, purgative and vermifuges in conjunction with root or the leaves for the treatment of some gastro intestinal disorders. In the Philippines the plant is used in making poisoned arrow while the pulverized plant is applied externally against malignant ulcer (Watt *et al*., 1962)**.** Hypoglycaemic effect of a crystalline fraction, which was obtained from an alcoholic extract of the fruit on rabbit was established (Rivera, 1981). An orally administered charantin in the proportion of 50 mg/kg body weight of rabbit proved to be appreciable when compared with tolbutamide in reducing the blood sugar level (Lotlikar and Rajarama, 1966).

A remarkable anlicancerpus action on mice with transplantable sarcoma 180 proved effective in reducing the tumur using the aqueous extract of the leaf and showed no oestrogenic activity (Abbot and Lotlikar, 1966). Besides, the extracts of the root had oxytoxic-action on the mice (Sucrow *et al.*, 1985). The aqueous extract of the root and leaves reportedly have appreciable antibacterial activity against *E. coli*. The extract of the leaf showed insecticidal activity while the alcoholic or aqueous extracts of both the leaves and the whole plant of the seed mesocarp or epicarp caused a relaxation of guinea pig ileum and rat jejunum with contractions (Watt *et al.,* 1989). Another study (Sofowora, 1980) reported that aqueous decoction of *M. charantia* collected in Nigeria produced significant purgative effect in rats and the decoction prepared as used in traditional medicine in Nigeria was the most potent purgative out of the various solvent extracts tested. For instance, an oral administration of an alcoholic extract of seed produced abortion in rat at a given dose of extract per kg body weight and the effect appeared to be more pronounced in early pregnancy (Sofowora, 1980). Besides, an intra-uterine application of the same extract also produced a vigorous contraction of the rat uterus. This is because a decoction of the leaf has been reported to inhibit the growth of *Bacillils subtilis*, Salmonella species and *E. coli*, respectively (Sofowora, 1980). Therefore, this study aimed to investigate the proximate, elemental compositions and assess the antimicrobial activities of the leave extracts of *M. charantia*, as well as determining its phytotoxic effects on the roots and shoots of maize seedlings.

2. Materials and Methods

2.1 Sources of Materials

The plant material, *M. charantia* was collected from farm at Odo lse quarters in Ise Ekiti, Nigeria and was authenticated at herbarium of the Department of Crop, Soil and Pest Management of the Federal University of Technology, Akure, Nigeria. The viable maize seeds used for this assay were obtained from School of Agriculture and Agricultural Technology, Federal University of Technology, Akure, Nigeria. All the chemicals and standard reagents used were of analytical grade. The plant material was sundried, blended in order to reduce its sizes for analysis and then stored in polythene bag at 4 ℃ until use. Note: the sample was not originally washed with water in order to avoid the loss of its saponin contents.

2.2 Determination of Proximate Composition of *Momordica charantia*

The proximate composition was determined using standard methods of Association of Analytical Chemist (AOAC, 2012). The analyses were performed in triplicate for each proximate component. The carbohydrate content was determined by difference (i.e., addition of all the values of moisture, crude, fat, ash, crude protein and crude fibre was subtracted from 100%).

2.3 Determination of free fatty acid (FFA) of *Momordica charantia*

M. charantia leaf was milled using laboratory bench top electric blender. Briefly, 5 g of the sample was mixed with light petroleum ether (40-60 ^oC Bp), for a contact time of 10 min inside a stainless-steel container. The mixture was immediately filtered using a muslin cloth and the oil was recovered from the filtrate by distillation method. The fat obtained was further dried in the oven at a temperature of 95 ^oC for 30 min to rid the fat of traces of petroleum-ether. The oil extracted was analyzed for percentage free fatty acid, thiobarbituric acid number and peroxide value. The sample of the oil extracted was poured into 250 ml conical flask, 50 ml of 95% ethanol was measured into 150 ml glass beaker and allowed to boil. Eight drops of phenolphthalein indicator and one drop of 0.1 M NaOH were introduced into the ethanol to neutralize the alcohol. The ethanol was poured into the flask containing oil sample, and the sample was heated to boiling again, two drops of phenolphthalein indicator were added again and quickly titrated against 0.1 M NaOH, solution with constant swirling to a faint pink end point which persisted for about 15 s.

% FFA = T x M x C x100 / weight of sample used; T = Titre value; M = Molarity of the NaOH; C = Oleic acid (constant) Acid value = FFA x 2; 0.002M = Molarity of Sodium thiosulphate used.

2.4 Determination of peroxide value, thiobarbituric acid value (TBA) and mineral elements

The peroxide value of the extracted oil was determined by using the AOAC (2012) methods. The TBA was determined as described by AOAC (2012). The metal composition of *Momordica charantia* was detected by using flame photometry and spectrophotometer as previously described (AOAC, 2012)

2.5 Tannin determination

The method of AOAC (2012) was used thus: one gram of the dried ground sample was placed into a 500 ml round bottom flask and for about five hours and then allowed to cool it was diluted to 500 ml with distilled water and the mixture was allowed to stand overnight and the supernatant was used for the determination of tannin. 50 ml of the supernatant was pipetted into 100 ml volumetric flask and 5 ml each of buffer at pH 5.0 and iron reagent were added into the flask and the mixture was allowed to stand for 30 min and absorbance was taken at 550 nm in the spectrophotometer.

2.6 Phytic acid determination

The phytic acid of *Momodica charantia* was determined by using AOAC (2012) procedures. 2.0 g of the sample was placed in a conical flasks and 100 ml of HCl was added, then the flask was shaken thoroughly and left for three hours after which the content was filtered, then the filtrate 25 ml was placed in a beaker containing few drops of NaOH and 4 ml of ferric chloride solution was added and boiled for 15 min in a water bath after cooling the content was centrifuged and supernatant was poured away then the precipitate was mixed with 5 ml of HCl and heated in a water bath for 10 min and stirred. 2 ml of NaOH was added and it was heated for another 15 min and precipitated sodium Phytate from ferric chloride was washed with hot water the wash was then added to the filtrate, l ml of concentrated H_2SO_4 and 1.2 ml of perchloric acid 60% were added and the mixture was digested in a low flame after evaporation strong heating at 300 ℃ followed for 30 min in order to get rid of perchloride acid residue 10 ml of distilled water was added and the resultant solution-was neutralized with 20 ml NaOH, then the volume was made up to 50 ml with distilled water the digest five ml was pipetted and mixed with l0ml of ammonium - molybdate vanadate reagent the volume was made up to 48 ml with distilled water the l ml of freshly diluted tetrachloride solution was added and volume made up to 50 ml then it was placed in the sample cell of spectrophotometer and the absorbance was taken at 480 nm after 10 min.

2.7 Cyanide contents analysis

The cyanide content of *Momordica charantia* was determined by the method of AOAC (2012). Briefly, 20 ml of distilled water was poured into a 500 ml round bottom flask and 200 ml phosphate buffer at pH 7.0 and 10 ml 20% mercury (H) chloride solution were added and mixed thoroughly. 20 g of Momordica charantia sample was transferred quantitatively to the flask and soaked overnight to allow glycosidic cyanide to form, then 5 g of hydrated tin (II) chloride was added to the soaked mixture and the flask was connected to a steam distillation unit, a conical flask containing 50 ml of 1% alcoholic NaOH was attached to the collecting end of the distillation unit and the distillate was passed into the alkaline medium.

$$NaOH + HCN \longrightarrow NaCN + H_2O$$

The amount of cyanide was determined titrimetrically using 0.02m silver nitrate, 1 ml of freshly prepared 0.5% w/v dithizone in ethanol was used as indicator at the end point the indicator colour changed from red to purple.

2.8 Extraction of saponin

200 g sample of the grounded Momordica charantia was put into the extraction thimble and covered at the top with filter paper, the plant materials was extracted with 500 ml portion of boiling petroleum ether for 16 h, and the solvent was then changed to methanol and the extraction was left for another sixteen hours to remove the saponin along with other compounds such as simple sugars, oligosaccharides and the flavonoids. The first extraction was a form of cleaning exercise and this removed lipids pigment then the solvent was changed to methanol and the extraction continued for a further sixteen hours to remove the simple sugars, oligosaccharides and flavonoids. To remove other constituent of the extract, the mixture was poured into separating funnel and butanol and distilled water was added in a ratio 2:1 in which 300 ml of butanol to 150 ml of distilled water was added and shaken thoroughly and then left to stand overnight the butanol the added distilled water removes sugars, flavonoids and oligosaccharides present in the crude extract. The crude saponin extract was purified by adding diethyl ether, it was noticed that when the reagent was added the colour changes to greenish black which indicated the end point, and the saponin appeared in dense black semi solid substance at the bottom of the beaker and the remaining diethyl ether at the top of the purified saponin was poured away and the saponin was left at the bottom of the beaker.

Foaming test: Little quantity of saponin obtained from the sample leaf was mixed with water and shaken and this produces foam which is one of the properties of saponin.

Thin layer chromatography: The chromatography tank containing the mixture of N. butanol, glacial acetic acid and distilled water in ratio 60:30:10 the saponin extract was dissolved in methanol and a spot was made on a slurry plate which serves as the stationary phase. When solvent front has almost reached the end of the plate the plat was removed and allowed to dry then the spot is identified using lieberman-Burchard reagent which contained methanol, H_2SO_4 and acetic acid in ration 50:5:5 respectively and the plate is placed inside oven for 2-3 min at 60 ℃ to allow colour separation and the resetting' band of colour was obtained.

2.9 Antimicrobial actions of momordica charantia

This experiment was carried out in order to test the inhibitory effect of the sample on certain microorganism four types of the sample on certain microorganism were used they include; *E. coli*, streptococcus, pyrogens, *Klebsiella* and *Enterobacta*. ***Preparation of aqueous extract:*** crude dried sample was weighed into five beakers, in the following proportions:

beakers one contains 20 g, beaker two contain l5 g, beaker three contain l0 g, beaker four contain 5 g and the last beaker contain 2.5 g of the dried sample, in each beaker 100 ml of distilled was added to soak the sample and left for 24 h and then the mixture was filtrate was poured into petri-dishes and put in the oven to dry and this was scrapped and reconstituted with distilled water. ***Paper disc bioassay*:** 0.2 g of saponin was weighed and put into a test tube and 2 ml of methanol was added in order to dissolve the Saponin then 1 ml was pipetted out of the 2 ml of the second test tube and 2 ml of methanol was added to the second test tube, these was done till the fourth test tube in which the first test tube has 1 mg/ml, second has 0.5 mg/ml, third test tube has 0.25 mg/ml and the last test tube contain 0.125 mg/ml. 0.2 g aqueous extract was also reconstituted with 2.0 ml of distilled water in the first test tube and serial dilution was carried out to the last test tube which is the fourth test tube. In paper disc bioassay, sterilized disc made of paper was numbered 1 to 4 and poured into the solutions prepared above in Saponin extract and another four discs in the prepared aqueous each test tube contain four discs making a total 16 discs. The discs were allowed to soak in the solution by leaving it overnight

Preparation of medium

2.8 g of nutrient agar was weighed and suspended in 1000 ml of distilled water and then boiled to dissolve completely when it has fully dissolved it was transferred into autoclave for sterilization at 12l ℃ for 15 min and then removed and allowed to cool to 45 ℃ then it was poured into sterilized petri dishes. A total of eight petri dishes was used at a depth of 2.3 mm then the media was allowed to set at 4° C.

Culturing of microorganism

The culturing of the microorganism into the nutrient agar media was done by flaming method. A loop that has been flamed was used to pick the microbes and spread on the solidified agar media, then the disc in the test tube containing different concentration of saponin was picked and placed in the media of cultured microorganism and this was also done for the discs in aqueous extract solution and the media was incubated for 24 h.

2.10 Phytotoxic effect of saponin isolated from *Momordica charantia*

The purpose of this assay is to determine if saponin extracted from *M. charanti*a was phytotoxic toward maize seeds by using method described by (Adanlawo, 1998). 2 g saponin extracted from *M. charantia* was dissolved in distilled water at 10 mg/ml, 2.5 mg/ml concentration was obtained by serial diluting the 10 mg/ml to get 5, 2.5 and 1.25 mg/ml, respectively. Cotton wool was placed at the bottom of fifteen dishes, which was prepared in triplicate, ten maize seedlings were then embedded and the distances between the seed was ~1.5 cm A control of three replications was made with distilled water. In petri dish A, 10 mg/ml of the test solution was transferred to it to soak the cotton wool, in petri dish B, 5 mg/ml of the test solution was added and in petri dish C 1.25 mg/ml of the saponin was added. In the control only 10 ml of distilled water was added daily to increase its humidity. 'The experiment was allowed to continue for a total of five days during which the root lengths and coleoptiles length were measured, this was compared with those in the control group as an index of inhibition of stimulation of seed germination and of the growth of radicles any seedling signs of visual fungal or bacteria contamination was discarded and means per dish and per treatment were calculated.

2.11 Statistical Analysis

Analysis was carried out in triplicates. The error was determined as the standard deviation from the mean obtained from the Statistical Package for Social Sciences (SPSS, Version 21). Analysis of variance (ANOVA) was used to determine the level of significant difference while the means were separated by using New Duncan Multiple Range Test (NDMRT) at 5%. The student test was used to analyze the data collected from the phytotoxic assay.

3. Results and Discussion

3.1 Proximate composition of *M. charantia*

The proximate composition of *M. charantia* showed in Table 1 revealed that the sample contained many nutrients which are required for the normal functioning of body systems. The result further revealed that the leaf is a good source of protein and carbohydrate, which helped in body building and provision of energy for metabolic activities. The combination of both carbohydrate and protein can be termed as glycoprotein, which are conglomerate of bioactive sugars that may give the leaf its medicinal properties as previously reported (Hokputsa *et al*, 2004).

3.2 Mineral composition of *M. charantia*

The result in Table 2 showed that the sample contain many mineral elements such as sodium potassium, calcium, zinc, magnesium iron, manganese and copper. The result further showed that the sample is a good source of sodium and potassium, which possessed several functions that included but not limited to the maintaining acid/base balance in the body. Therefore, consuming the leaf as vegetable could help in preserving the normal irritability of muscle and permeability of the cells. Calcium played a vital role in muscle contraction and in the formation of bones and teeth and also helped in blood clotting.. The abundant of iron in the leaf sample is an important element needed in haeme-formation in blood, which is needed for transportation of oxygen and CO_2 during respiration (Ahmed and Chaudhary, 2004). The presence of zinc in the sample helped in prevention and treatment of diarrhoea and also helped in proper functioning of immune system (Adeleye, 1991).

3.3 Antinutrient factors of *M. charantia*

Table 3 showed the various anti-nutritional factors in the sample and it can be seen from the table that the sample contained more phytic acids than tannin and cyanide. However, the percentage of the antinutrient did not have inhibitory effects on the other nutrients because the value is still within the stipulated or recommended standard limits (WHO, 1991).

3.4 Antimicrobial effects of isolated and purified saponin from *M. charantia*

The purity of the isolated saponin extract was shown by a single spot noticed on the separation plate in the thin layer chromatography (Plate 1). The antimicrobial effects of the

saponin in the sample is shown in Table 4. The result further revealed that the zone of inhibition when aqueous extract was used. It clearly shown that the saponin from *M. charantia* has the greatest inhibition on the growth of *Klebsiella*, which is gram positive and more inhibitory effect on the growth of *E. coli* (Table 4). Hence, as a result of this antimicrobial action, the decoction of the leaf can be used as a chemotherapeutic agent against microbial infection and this is in line with the reports of previous studies (Watt *et al.* 1962; Sofowora, 1980) on the appreciable activities aqueous decoction of *M. charantia* against *E. coli* and salmonella species, respectively.

Table 1: Proximate composition of *Momordica charantia* (mg/100 g)

Moisture	Ash	Fat	Fibre	Protein	Carbohydrate
10.74	16.06	2.91	5.01	25.44	36.30

Table 2: Mineral composition of *Momordica charantia* (mg/100 g)

Minerals	Dried	Undried
Magnesium	199.4±0.1	201 ±0.02
Calcium	25.44 ± 0.02	32.4±0.01
Phosphorus	0.450±0.01	531±0.1
Potassium	27.16 ± 0.01	661±0.2
Zinc	44.9±0.01	5.36±0.1
Iron	17.65± 0.1	6.01 ±0.01
Copper	70.7±0.02	26.8±0.1
Sodium	72.6±0.01	325 ± 0.01

Table 3: Antinutrient factors (mg/100 g)

Tannin	Phytic acid	Cyanide
24.10	60.40	1.55

Table 4: Antimicrobial effect of *Momordica charantia*

	Zone of inhibition			
Concentration of Saponin (mg/ml)	**Streptococcus**	***E. Coli.***	**Enterobacter**	***Klebsiella***
1.0	1.00	1.60	0.40	1.80
0.5	0.80	0.90	0.20	1.20
0.25	0.40	0.60	-	0.80
0.125	0.20	0.40	-	0.70

Plate 1: Antimicrobial effect of saponin from *Momordica charantia*

3.5 Phytotoxicity of saponin isolated from *M. charantia*

The phytotoxic assay carried out on isolated saponin from *M. charantia* on maize seedlings showed that the saponin has inhibitory effect on both shoot and root of the seedling as shown in Plate 2. It was revealed that the saponin at different concentration strongly inhibit the growth of shoot and root of the maize seedling as shown in Table 5. The inhibition is more pronounced with the highest concentration of saponin, which is in agreement with the report of past study (Adanlawo, 1998) on crude saponin from *Tetracapidium conophorum* and *Phaseous lunatus*.

Table 6 showed that the root is more affected than the shoot, which clearly showed that it is the root that is mostly sensitive than the shoot to the saponin and the rate of growth inhibition of the saponin on the root and the shoot of the seedling was concentration dependent.

Table 5: Phytotoxicity of isolated saponin from *M. charantia*

Concentration	Shoot (mm)	Root (mm)
10 mg/ml	0	0
5 mg/ml	12	9
2.5 mg/ml	13	12
1.25 mg/ml	22	17

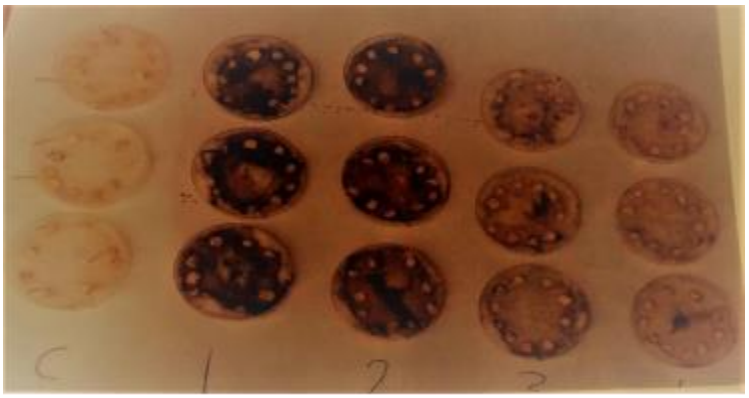

Plate 2: Phytotoxic effect of saponin isolated from *M. charantia*

Table 6: Percentage of shoot and root growth inhibition at different concentration

Concentration (mg/ml)	Shoot (%)	Root (%)
10	0	0
5	3.0	1.8
2.5	3.3	2.64
1.25	5.5	3.5

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4. Conclusion

Various assays on *Momordica charantia* had revealed some of its medicinal value, which could be viewed that the leaf sample could actually be a good source of raw material for the food and pharmaceutical industries due to the presence of bioactive compounds in the sample. However, further research work is needed to elucidate the *in-vivo* chemotherapeutic value of the leaf. The effect of the sample leaf on microorganisms clearly showed that the *Momordica charantia* leaf could be incorporated into antibiotic drugs as well. Also, the numerous nutrients and minerals found in the leaf could boost body nutrients and immunity after consumption. The phytotocicity effect on maize revealed that the higher concentration of the saponin from the leaf is poised to inhibit growth of plant, therefore its incorporation into herbicides for agricultural use should be fully explored.

Declaration

Author Contributions:

Author ADO performed the experiment, collected and analyzed the data as well as prepared the draft of the manuscript. Author OOO performed the experiment and analyzed the data. Author SAM supervised the study and edited the manuscript.

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Conflicts of Interest: The authors declare that they do not have any conflict of interest.

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