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NUTRIENT AND PHYTOCHEMICAL PROFILE OF AQUEOUS EXTRACTS OF GARLIC (*ALLIUM SATIVUM*) AND SOURSOP (*ANNONA MURICATA*) LEAVES FOR USE AS HYPOGLYCEMIC AGENTS

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ABSTRACT

Introduction and objective: Diabetes mellitus (DM) is one of the most prevalent diseases globally. The current mode of treatment of DM, which is based on synthetic drugs is expensive and causes genetic and metabolic alterations. However, safe mode of treatment involving use of materials and/or extracts of plant origin is needed to mitigate the disease development and progression, which will also constitute a cost-effective strategy in DM treatment and management. Thus, the study was aimed to determine nutrient and phytochemical profiles in aqueous extracts of *A. sativum* and *A. muricata* leaves use in DM management. **Materials and methods:** Experimental study design was adopted for the study. Fresh leaves of A. sativum and A. muricata were harvested, sorted, washed and dried at room temperature. The leaves were processed into aqueous gummy extracts using standard method. About 100mg/mL each of the extracts was used to profile nutrients and phytochemicals using standard methods. One-way analysis of variant was used to analyze data. **Results:** *A. muricata* and *A. sativum* leaves had 3.42% and 2.02% of protein, 0.35% and 0.03% of fibre, 2.17% and 0.97% of ash, respectively. Iron and calcium contents were 4.12% and 14.82% in *A. muricata*; and 0.87%

and 4.63% in *A. sativum*, respectively. Vitamin B1 and C contents were 0.23% and 2.08% in *A. muricata*; and 0.33% and 52.40% in *A. sativum*, respectively. The aqueous extracts contained alkaloids, flavonoids and terpenoids. **Conclusion:** The study showed that the aqueous gummy extracts of the leaves were rich in vital nutrients and bioactive compounds that could exhibit anti-hyperglycaemic effects in DM.

KEYWORDS: Aqueous extract, *A. sativum*, *A. muricata*, Nutrients, Phytochemicals, Diabetes mellitus.

INTRODUCTION

Garlic (*Allium sativum*) is observed, among others, to be a vital vegetable in the globe. Garlic as a normal plant, has been reported to be useful in herbal medicine in treating diabetes mellitus and other diseases.[1] Earlier researches had revealed that intake of *A. sativum* extract orally, led to declined blood glucose level in rats that has diabetes.[2,3] Sangeetha & Quine[4] proposed that antidiabetic potentials of garlic was maybe as a result of increased measure of S-allyl cysteine sulfoxide (allicin), which is an active compound. *Annona muricata* (soursop) is a member of *Annonaceae* family and the leaves are the greatest helpful aspects of the tree. Soursop contains acetogenins compounds known as squamosin, asimisin and bulatacin.[5] Sawant & Dongre[5] reported that nutrients in *A. muricata* leaves were observed and accepted to have a stability effect to bringing blood glucose level to normal values needed in managing diabetes mellitus. Many studies revealed that *A. muricata* leaves have hypoglycaemic effects and regenerative capacity for pancreatic islet.[6,7,8] Diabetes mellitus (DM) is one of the most prevalent diseases globally. It is described as a chronic disease caused by inherited and/or acquired deficiency of pancreatic insulin production, or as a result of insulin resistance. The dilapidating action of DM makes it a disease of major public health importance. It has been projected to become the foremost cause of morbidity and mortality within the next 25 years especially in Africa and Asia. The estimated prevalence of diabetes in Africa is 1% in rural areas, 5-7% in urban sub-Saharan Africa, and 4.3% in Nigeria.[9] Diabetes mellitus as a prolonged disease has affected a larger population globally. Thus, active natural therapeutic agent will be highly helpful in reducing drastically complications of diabetes and compliment the healthy lifestyle of patients with diabetes. The current mode of treatment of DM, which is based on synthetic drugs is expensive and causes genetic and metabolic alterations. However, safe mode of treatment involving use of materials of plant origin is needed to mitigate the disease development and progression, which will also constitute a cost-effective strategy in

DM treatment and management. Furthermore, some nutrients especially micronutrients and phytochemicals have been implicated in the management of chronic diseases such as diabetes mellitus. Micronutrients such as zinc, selenium, calcium, chromium, vitamins C, E and B1 have been useful in the insulin secretion and action through sensitization of pancreatic beta cells, scavenging free radicals thereby inhibit the formation of oxidative stress caused by impaired carbohydrate metabolism. Thus, there is need to profile some nutrients and phytochemicals present in the aqueous extracts of *Allium sativum* and *Annona muricata* leaves vital in diabetes mellitus management. Therefore, the study was to determine the nutrients and phytochemical profile in aqueous extracts of A. sativum and A. muricata leaves important in diabetes mellitus management.

MATERIALS AND METHODS

Experimental study design was used to carry out the study.

EXPERIMENTAL MATERIALS

Fresh leaves of *A. sativum* and *A. muricata* were harvested from a home-garden in Abuja, and identified in Plant Science and Biotechnology Department, University of Nigeria Nsukka, Enugu State, Nigeria.

Preparation of the samples

Sukhder et al.[10] method for extraction of leaves was used with little modification. The leaves of *A. sativum* and *A. muricata* were harvested fresh, sorted, washed and dried with air at ambient temperature for 3 days. The leaves were chopped into small pieces and then milled to fine powder, ready for further treatment. One hundred and fifty grams (150g) each of the powdered leaves were immersed in 4500ml of distilled water and agitated on a mechanical shaker for 30 minutes. The mixtures were allowed to stand for 5 hours and then drained using a muslin cloth into a 5L dry stainless steel bowl. The liquid extracts were concentrated to dryness in a Gallenkamp hot air oven (Size One-Oven BS) at 60^0C until a gummy extract was obtained. The gummy extracts (concentrates) were then recovered into sample bottle and stored in the refrigerator until use for further analysis and study. One grams (1g) each of the extracts (*A. sativum* and *A. muricata*) was dissolved in 10ml of distilled water to make a stock of 100mg/ml of extract. The stock was then used for determination of nutrients and phytochemicals of interest in diabetes mellitus management.

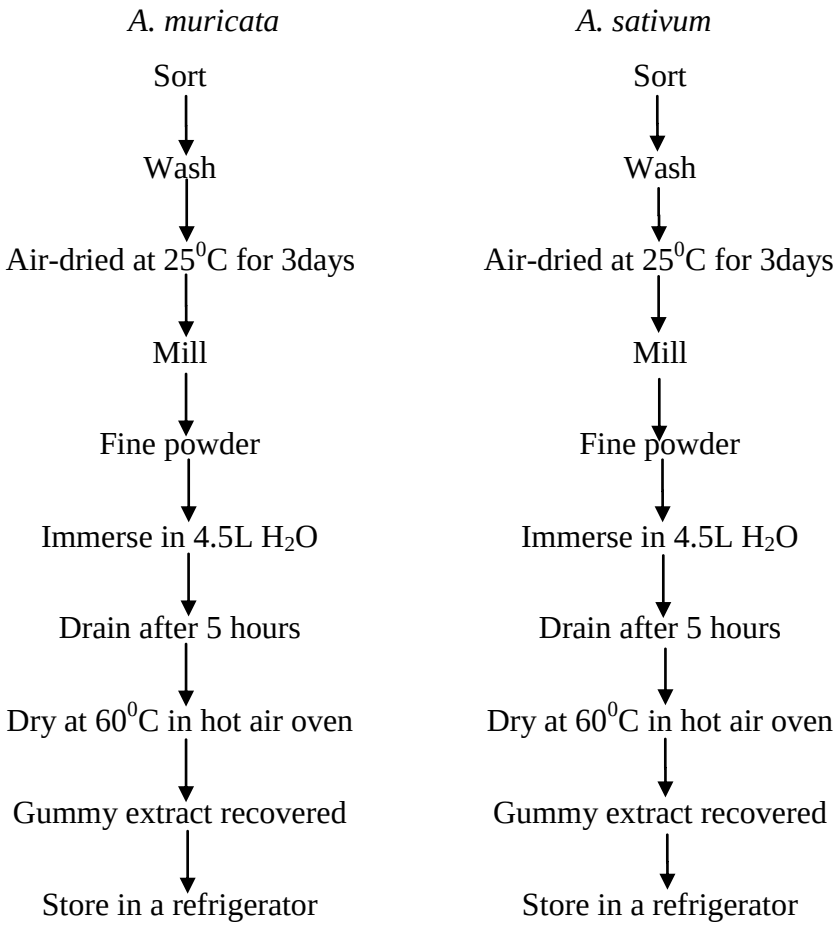

Fig. 1: Flow chart for the leaves extraction.

Proximate analysis was carried out, and nutrient and phytochemicals of interest were zinc, calcium, iron, potassium, selenium, vitamin A, C, E and B1 and flavonoids, alkaloids and terpenoids. These nutrients and phytochemicals are thought to be needed in diabetes management and treatment.

Proximate evaluation of aqueous extract of *A. sativum* and *A. muricata* leaves

Labouratory analysis of *A. sativum* and *A. muricata* extracts were carried out to evaluate their proximate properties. The labouratory analysis was done in triplicates, in order to validate the observed results.

Moisture content of the extracts (samples) were evaluated using method of hot air oven developed by Association of Official Analytical Chemist (AOAC).[11]

The ash content of the samples was investigated using method of muffle furnace detailed by AOAC.[11]

The micro kjeldhal method was used as described by Onwuka.[12] The total nitrogen (N) content was ascertained by multiplying the constant (6.25) to get the value of protein for each sample.

Crude fibre was determined using the method described by AOAC.[11]

The study adopted soxhlet method of fat determination elucidated by AOAC.[13]

The quantity of total carbohydrate in the samples was ascertained by adding the values of protein, fat, fibre, ash, and moisture and then subtracted from one hundred (100).

Total carbohydrate = 100 – (%moisture + %fat + %fiber + %protein + %ash).

Determination of micronutrients in aqueous extract of *A. sativum* and *A. muricata* leaves

The spectrophotometric method elucidated by Pearson (14) was adapted for analysis of pro-vitamin A (beta-carotene). The samples were analyzed for vitamin C content using spectrophotometric method. Vitamin E contents of the extracts were determined adding pyrogallol (10 ml of 6% (w/v)) into ethanol (95%) to the samples, mixed, then, flushed with N_2. According to AOAC (2010) the content of iron in the samples was ascertained using method of phenonthroline. The content of calcium was ascertained using the method elucidated by AOAC.[11]

Potassium, selenium, chromium and zinc were analyzed thus; about 0.2g of each of the ashed samples were weighed into digesting glass tube. Then, added 20ml of nitric acid (HNO_3) into the weighed samples in the beaker and the mixtures were kept over the night at ambient temperature. Thus, addition of about 4ml HClO (perchloric acid) was made to the mixtures and heated using hot plate in the fume cupboard for digestion. The temperature of the hot plate was increased gradually (50–300^{0}C). The digestion ended at about 70–85mins immediately white fumes were observed. After the mixtures had cool off, the contents of the tubes were moved to both 100ml of water in volumetric flasks of same quantity. The wet digested solutions were moved into bottles (plastic) and labelled correctly. The samples were loaded into test-tubes and centrifuged at 300rpm for 10 minutes and the supernatants were used for potassium, iodine and selenium determination using atomic absorption spectrophotometer. Thus, the mineral concentrations were noted unit of ppm and later conversion to milligrams (mg) of the minerals was done, as shown below:

$$\mathbf{MW = \frac{absorbency \times dry\ wt \times D}{Wt\ of\ sample \times 1000}\ (mg/g)}$$

Determination of phytochemicals in aqueous extract of *A. sativum* and *A. muricata* leaves

The analysis of alkaloids in the samples was ascertained using improved method by Ngounou et al.[15] The analysis of flavonoids in the samples was ascertained using improved method by Onwuka.[12] One hundred grams (100g) (Wi) of the samples were collected, soaked in 9ml of ethanol for 24hours as described by Indumathi, Durgadevi, Nithyavani & Gayathri[16] for terpenoid determination.

Statistical analysis

Data were presented as mean and standard deviation (mean ± SD). The data on proximate, micronutrients and phytochemicals of the aqueous extracts of the leaves were analyzed using one-way analysis of variant (ANOVA).

RESULTS

Proximate composition of the aqueous extracts of the leaves

Table 1 reveals the proximate compositions of the aqueous extracts of the leaves. The moisture content of the leaves extract ranged from 72.76 mg/100g to 78.62 mg/100g. The aqueous extract of soursop (*Annona muricata*) leaf had the highest (78.62 mg/100g) moisture content, while aqueous extract of *A. sativum* (garlic) leaf had 72.76 mg/100g moisture content. The ash content of the leaves extracts ranged from 0.97 mg/100g to 2.17 mg/100g. The ash content of aqueous extract of soursop leaf was 2.17 mg/100g, whereas aqueous extract of garlic leaf had 0.97 mg/100g.

The result revealed that, the content of the fat in the aqueous extracts studied varied from 0.02 mg/100g to 0.12 mg/100g. The aqueous extracts of *A. muricata* and *A. sativum* leaves had, 0.12 mg/100g and 0.02 mg/100g, respectively. The crude fibre content of the aqueous leave extracts varied from 0.03 mg/100g to 0.35 mg/100g. The content of crude fibre in aqueous extract of soursop leaf was 0.35 mg/100g, while that of aqueous extract of garlic leaf was 0.03 mg/100g. The content of crude protein in aqueous extracts varied from 2.02 mg/100g to 3.42 mg/100g, whereby the aqueous extract of *A. muricata* leaf had 3.42 mg/100g crude protein, while *A. sativum* leaf aqueous extract had 2.02 mg/100g. The content of carbohydrate in aqueous extracts varied from 15.32 mg/100g to 24.22 mg/100g. Thus, the aqueous extracts of *A. sativum* leaf had 24.22 mg/100g, while that of *A. muricata* leaf had 15.32 mg/100g content of carbohydrate.

Table 1: Proximate composition of aqueous leaves extracts as administered to the animals.

Parameters (mg/100g)	*A. muricata* leaf extract	*A. sativum* leaf extract
Moisture	78.62 ± 0.03	72.76 ± 0.02
Ash	2.17 ± 0.02	0.97 ± 0.02
Fats	0.12 ± 0.02	0.02 ± 0.01
Crude fibre	0.35 ± 0.02	0.03 ± 0.01
Crude protein	3.42 ± 0.02	2.02 ± 0.01
Carbohydrate	15.32 ± 0.02	24.22 ± 0.04

Table 2 described mineral compositions of the aqueous extracts of *A. muricata* and *A. sativum* leaves. The content of iron (Fe) in the aqueous extracts ranged from 0.87 mg/100g to 4.12 mg/100g, whereby Fe content in aqueous extract of *A. muricata* leaf was 4.12 mg/100g, while that of *A. sativum* leaf was 0.87 mg/100g. The calcium content in aqueous extract of *A muricata* leaf was 14.82 mg/100g while that of *A. sativum* leaf was 4.63 mg/100g. The content of potassium in aqueous extract of *A. sativum* leaf was 2.10 mg/100g while potassium content in aqueous extract of *A. muricata* was 15.56 mg/100g. Aqueous extract of *A. muricata* leaf had about 0.77 mg/100g of zinc whereas, *A. sativum* leaf had 0.04 mg/100g of zinc. More so, the quantity of selenium in aqueous extracts of *A. sativum* and *A. muricata* leaves were 0.04 mg/100g and 1.44 mg/100g, respectively. The content of chromium in aqueous extract of *A. muricata* leaf was 0.02 mg/100g, while chromium content of aqueous extract of *A. sativum* leaf was 0.03 mg/100g.

Table 2: Mineral compositions of aqueous leaves extracts as administered to the animals.

Parameters (mg/100g)	*A. muricata* leaf extract	*A. sativum* leaf extract
Iron	4.12 ± 0.02	0.87 ± 0.02
Calcium	14.82 ± 10.41	4.63 ± 0.02
Potassium	15.56 ± 0.03	2.10 ± 0.02
Zinc	0.77 ± 0.02	0.04 ± 0.01
Selenium	0.04 ± 0.01	1.44 ± 0.02
Chromium	0.02 ± 0.01	0.03 ± 0.02

Table 3 shows some vitamin composition studied. Pro-vitamin A content in aqueous extract of *A. sativum* leaf was 0.08 mg/100g, while aqueous extract of *A. muricata* leaf had 3.23 mg/100g of pro-vitamin A. Vitamin B1 content of aqueous extract of *A. muricata* leaf was 0.23 mg/100g, whereas aqueous extract of *A. sativum* leaf had 0.33 mg/100g. The vitamin C content of aqueous extract of *A. muricata* leaf was 2.08 mg/100g, while that of aqueous extract of *A. sativum* leaf was 52.40 mg/100g. the aqueous extract of *A. muricata* leaf had

about 0.45 mg/100g of vitamin E, while aqueous extract of *A. sativum* leaf had 1.03 mg/100g of vitamin E.

Table 3: Vitamin compositions of aqueous leaves extracts as administered to the animals.

Parameters (mg/100g)	*A. muricata* leaf extract	*A. sativum* leaf extract
Pro-vitamin A	3.23 ± 0.03	0.08 ± 0.02
Vitamin B1	0.23 ± 0.03	0.33 ± 0.02
Vitamin C	2.08 ± 0.02	52.40 ± 0.02
Vitamin E	0.45 ± 0.02	1.03 ± 0.03

Table 4 reveals phytochemicals evaluation carried out on the aqueous extracts of the leaves. The content of alkaloids in aqueous extract of *A. muricata* leaf was 1.13 mg/100g, while aqueous extract of *A. sativum* leaf had about 1.02 mg/100g. The content of flavonoids in aqueous extract of *A. sativum* leaf was 2.90 mg/100g, whereas aqueous extract of *A. muricata* leaf had 0.84 mg/100g of flavonoids. Aqueous extract of *A. sativum* leaf had about 0.52 mg/100g of terpenoids, while aqueous extract of *A. muricata* leaf had about 0.05 mg/100g of terpenoids.

Table 4: Phytochemical composition of aqueous leaves extracts as administered to animals.

Parameters (mg/100g)	*A. muricata* leaf extract	*A. sativum* leaf extract
Alkaloids	1.13 ± 0.03	1.02 ± 0.02
Flavonoids	0.84 ± 0.02	2.90 ± 0.02
Terpenoids	0.05 ± 0.01	0.52 ± 0.02

DISCUSSIONS

Table 1 showed the proximate compositions of the aqueous extracts of *A. sativum* leaf and *A. muricata* leaf. The moisture contents of the aqueous leaves extracts revealed high contents, which indicated that the extracts were analyzed in a reconstituted form (that is, 1000mg of each extract dissolved in 10ml of distilled water). This explains that the extracts would have limited storage time. This is because moisture content greater than 12% could promote both biochemical and microbial spoilage.[17] Therefore, the moisture content of extracts may be used as an index for its storage potential, the reason being that water has been seen to be a means of many biochemical activities. Mercy[17] reported that water content more than 12% in any food samples are susceptible to microbial activities, which could lead to short shelf life. The high moisture content seen in the study validates earlier report by Dalhat, Adefolake & Musa[18] on moisture content (86.67 mg/100g) of some plant extracts.

The ash content observed in the study, suggested that the aqueous extracts of the leaves may be good sources of mineral. Dalhat et al.[19] opined that any inorganic residues that remains when organic matter in a sample is ignited or completely oxidized is referred to as ash content, and this reveals mineral content in the samples. More so, minerals play vital part in carbohydrate, fat and protein metabolism.[18] Majorly, ash contents of the aqueous extracts studied were encouraging, which could be implicated as good sources of minerals that are required in macronutrient metabolism. Thus, the aqueous extracts could paramount in management of diabetes mellitus. The ash content reported in the study was less compared to that (5.86 mg/100g) reported by Muhammad & Ibrahim[20] in their study on some plant leaves extracts. This could also be attributed to variations in method adopted during extraction of the different leaves. The fat contents of the aqueous extracts were relatively low, which indicated that the aqueous extracts may not be presented as good sources of dietary fat. High fat content may indicate increased caloric values and likely presence of fat-soluble vitamins (vitamins A, D, E and K), as reported by Dalhat et al.[18] However, extracts from plant leaves could be known to be rich in essential oils which could account for its characteristic aroma. Thus, the content of fat observed in the study was not in line with that (0.73% to 0.78%) reported by Dalhat et al.[18], and in conformity with the study by Harsha et al.[21] Low fat foods have been reported to reduce cholesterol level, while diets high in fat could cause glucose intolerance and promote obesity, dyslipidaemia and heart disease.[22] Thus, the low fat contents obtained in the study could mean that the aqueous extracts of the leaves could have the potentials of anti-hypercholesterolaemia activity.

The crude fibre of the aqueous extracts of the leaves reported in the study showed that the extracts especially that of *A. muricata* leaf could be seen as good source of crude fibre and may contribute to the healthy state of the digestive system to aid its normal functions. However, the crude fibre content was low compared to that (0.58% to 0.69%) reported by Dalhat et al.[18] The variation could be as a result of method adopted during extraction of the leaves and/or the form at which the labouratory analysis was perform. Thus, fibre are said to be polysaccharides whereby human digestive enzymes are unable to breakdown, however, both soluble and in-soluble fibre modulate physiologic functions and inhibit some diseases due to metabolism in human. Mensah et al.[23] reported that fibre sanitizes the digestive tract by taking out carcinogenic activities in the body, inhibits the assimilation of excessive cholesterol and add bulk to the diet and then, reduces starchy food intake in excess. The crude protein obtained in the study was encouraging and thus, combination of aqueous extract of *A.*

sativum leaf and *A. muricata* leaf if consumed regularly in food, may serve as sources of plant protein in the diet, and this could contribute to reducing serum cholesterol. Some evidence suggests that intake of vegetable protein contributes to reducing serum cholesterol compare to animal protein.[24] Protein content varies widely in food. Protein is a structural block and essential constituent of cells that produces amino acids needed by the body. The content of protein (8.54%) reported by Dalhat et al.[18] is greater than the protein content (3.42%) reported in the study. High amount of protein is essential for growth and development. Protein is a macromolecule, an alternative source of energy when other macromolecules are limited in supply.[25] Production of important hormones, vital chemicals for brain, digestive enzymes, antibodies and requisite elements for manufacturing DNA are made possible by food rich in protein.[25]

The carbohydrate result suggested that *A. sativum* and *A. muricata* could contribute more carbohydrate (energy), which may be useful in making the extracts good sources of energy, especially in controlling post-prandial hyperglycemia. The range of carbohydrate content (15.32 to 24.22 mg/100g) in the study was higher compared to that (9.89 to 20.46%) reported by Dalhat et al.[18] Carbohydrate is a major source of energy to cells of the body, specifically to the brain, organ depended on carbohydrate in the body.[26] Therefore, moderate intake of carbohydrate food is necessary in the treating and/or managing diabetes.

The mineral compositions of the aqueous extracts of the leaves were shown in Table 2. The iron content in aqueous extract of *A. muricata* leaf was high, and could suggest to serve as a better non-haeme iron source than aqueous extract of *A. sativum* leaf, and thus, would require vitamin C for its absorption. Iron is a crucial constituent of haemoglobin, which helps the transportation of oxygen and carbon dioxide. The iron content obtained in the study was high compared to that (0.026 mg/100g to 0.0028 mg/100g) reported by Dalhat et al.[18] This could be due to processing methods adopted and/or the form in which the extracts were analyzed. Iron is vital in preventing anaemia and other related diseases. Deficiency of iron in the bloodstream in diabetes especially type 2 diabetes which may be a consequence of other micronutrient deficiencies could lead to death. Poorly controlled diabetes is linked to increased accumulation of end products of oxidation.[27] Increased oxidative stress in diabetes leads to elevated blood glucose and thereby reduce antioxidant defense mechanism.[28] However, sequestration of iron transporting and storage proteins could contribute to defensive mechanism of antioxidants.[28]

Calcium intake from sources is vital because its impairment in diabetes could contribute to defect in cell function in regulating cardiac muscles, erythrocytes, platelets and skeletal muscles.[29] This impairment could further worsen its significant capacity in regulating both insulin secretion and action properly, and affect diverse vascular problems.[30,29] In the study, aqueous extract of *A. muricata* leaf indicated to be good source of calcium. However, administration of the extracts could contribute and/or play a role in modulating immune system and secretion and action of insulin in the pancreatic beta cells. Hyperglycaemia could further show adverse effect on glucose metabolism.[31,29] Calcium is therefore, required for regulation of several organs internally, including both the liver and heart. The result of the study is not conformity with that (83.83 mg/100g to 122.40 mg/100g) reported by Dalhat et al.[18] Several physiological potentially probity, such as proper functioning of muscles in the heart, skeletal system and cell membrane, transmission of nerve signal, blood clotting and enzymes and hormone regulation requires calcium.[32]

Potassium content was relatively high in aqueous extract of *A. muricata* leaves. Oral administration of the aqueous extracts may contribute in the proper functioning of cell membrane and cardiac muscle, and also in reducing blood pressure and water retention, and thus, may help protect against stroke. Intracellular fluid has a main cation refer to as potassium. Potassium functions in maintaining acid-base balance, osmotic pressure regulation, nerve impulse conduction, cardiac muscle contraction and functioning of cell membrane.[33]

Zinc displays an important role in cell signaling and both in cell division and cell apoptosis. However, disruption in zinc homeostasis could be connected to insulin resistance and diabetes.[29] The study suggested some quantity of zinc in the aqueous extracts of the leaves. Inadequate zinc intake may encourage the occurrence of vascular changes in atherosclerosis and its outcome for diabetes. Thus, oral intake of the aqueous leaves extracts and other foods rich in zinc could contribute in activating the enzymes necessary for carbohydrate metabolism. The zinc content obtained in the study was lower than that (3.14 mg/100g to 3.15 mg/100g) reported by Dalhat et al.[18] Zinc is a vital micronutrient in the structure of insulin, it stabilizes and preserves damage caused by oxidation. Deficiency in zinc could lead to reduced insulin-receptors synthesis and to glucose intolerance and decline in insulin sensitivity.[34] Therefore, increased intake of zinc may be advantageous in the management of hyperglycaemia and/or diabetes.[35]

Selenium is required to synthesize selenoproteins for its biological functions as antioxidant and cytoprotective nutrient in order to delay the onset of type 2 diabetes mellitus.[36] The aqueous extract of *A. sativum* leaf had a high content of selenium and oral administration of the aqueous leaves extracts could contribute in modulating oxidative stress caused by impaired carbohydrate and lipid metabolism. Studies have shown the importance of soil content of selenium as a sole contributor in the quantity of selenium found in plants materials.[37,38] It has also been reported that selenium plays key part in glutathione peroxidase (GPx) enzyme activity and this (GPx) in turn is vital in defending cells against reactive oxygen species (free radicals) caused by diabetes.[29] Therefore, sustained adequate consumption of selenium as an antioxidant could aid regulation of metabolism of carbohydrate in order to prevent development of diabetes. Selenium is also known to prevent cardiomyopathy, muscle degeneration and immunologic dysfunction.[39]

Proper chromium nutrition has been said to enhance blood glucose and lipid profile levels, and insulin action, however, depended on the dosage.[40] The result on chromium, indicated that the aqueous leaves extracts may also act as sources of chromium due to its availability in the leaves extracts. Sufficient intake of chromium has been implicated to support insulin action and sensitivity in the pancreatic beta cells, thereby encourage insulin expression. Chromium also enhances internalization of insulin by binding insulin receptor enzymes, elevating sensitivity of both insulin and beta cell.[41,29] Thus, deficiency of chromium could result in glucose intolerance and disorder in the utilization of glucose.[41]

The pro-vitamin A (beta-carotenoid which is converted to vitamin A in the liver) contents of the aqueous extract of the leaves could be enough to supply required vitamin A in the body and/or in the management of hyperglycaemia, because there was no evidence of vitamin A risk amongst diabetics. Vitamin A is an essential vitamin needed by the immune system to function maximally, for good vision and also play a vital role in cell differentiation.[42,43] The vitamin B1 content of the aqueous extracts in the study suggests that the aqueous extracts of the leaves may also been observed as sources of vitamin B1 (thiamin), and could be adequate to the point of assuming a focal point in protein, carbohydrate and lipid metabolism, since vitamins are needed in smaller quantity. Studies demonstrate that most people with type 1 and type 2 diabetes have deficient supplies of vitamin B1 and weakened metabolism of thiamin.[44] A tissue-specific vitamin B1 deficiency could expand the danger of vascular disorders (such as nephropathy).[45] Guaranteeing adequate provision of thiamin using

aqueous extracts of the leaves sought to be a focal point of treatment, because adequate supply of vitamin B1 upholds sensory system capacity and forestalls neuropathies resulting from diabetes.

The vitamin C content was high in aqueous extract of *A. sativum* leaf and could be seen as a better source of vitamin C. The vitamin C as a water-soluble vitamin may not have been affected and/or leeched out during reconstitution of the aqueous extracts. This further confirmed that the extracts could contribute to improving antioxidant status, thereby help reduce glycosylation of protein by ambitiously supplanting glucose from protein amino groups. Thus, vitamin C inhibit damage of the endothelium occasioned by reactive products of glycosylation and enhances the function of endothelium.[46] Vitamin C also decrease aldose enzyme (reductase) and thus, decelerate the build-up of sorbitol (sugar alcohol) within the cell, which could aggravate destruction of kidneys, eyes and nerves.[47] The vitamin C content obtained in the study was high compare to that (4.30 mg/100g) reported by Dalhat et al.[18] and also high when compared to that (3.48 mg/100g) reported by Effiong et al.[48] More so, the vitamin E content obtained in the study was not line with that (277.12 to 143.14 mg/100g) reported by Dalhat et al.[18] However, the aqueous leaves extracts could still serve as sources of vitamin E, and may be able to play a major role in protecting enzymes and fighting free radicals in diabetes. This is because the concentration in plasma of vitamin E is lower in diabetics, however, regeneration by vitamin C could help.[49] Low density lipoprotein (LDL), biological membranes of polyunsaturated fatty acids, enzymes and hormones are protected against oxygen radicals (oxidation) by vitamin E. To this end, oxidation and regeneration of vitamin E is performed by vitamin C and/or flavonoids. Hence, the vitamin fights degradation of fatty acids oxidation, especially by modifying LDL oxidation that have been implicated in the occurrence of atherosclerosis.[49] Furthermore, through enzymes inhibition, vitamin E delays procedure of inflammation and the spread of connective tissue in the blood vessels, thereby decreases the complications of diabetes.[49] Vitamins in general are required daily in foods, however, in little quantity. Vitamins does not contribute energy but assists in the metabolism of energy-giving molecules and help in body's maintenance, growth and development.[50]

The result of the phytochemical evaluation revealed that the leaves extracts contain alkaloids, flavonoids and terpenoids. Phytochemicals are biological active components generally involved in improving several clinical problems and ailments.[51,52,53] Alkaloid content

obtained in the study was not in conformity with that (7.20 mg/100g) reported by Muhammad & Ibrahim.[20] Alkaloids are very useful in medicine and are used in the production of several valuable drugs.[18] The presence of alkaloids indicated that the aqueous leaves extracts could be used to reduce glycaemia and control diabetes, and may also be used as antioxidants and analgesic. Previous studies revealed that alkaloids found in various species of plant displayed glycaemic control.[52,54,53] In *in vivo* and *in vitro* studies, alkaloids inhibited glycogen phosphorylase, thereby, stimulate basal glucose uptake rate in rat adipocytes.[52,53] Alkaloids can also mediate the insulin-signal transduction pathway, reversing molecular defects that causes glucose intolerance and insulin resistance and reducing diabetes complications, both *in-vitro* and *in-vivo.*[55]

The result of the study revealed that, various aqueous leave extracts studied contained a significant content of flavonoids. Flavonoid is a known strong antioxidant that is soluble in water and fight against free radical, which inhibit cell damage caused by oxidation and also display potent anticancer effect.[56] Thus, the aqueous leave extracts, when consumed, could contribute and/or make a vital impact in managing diabetes induced oxidative stress and hypoglycemic effect by increasing insulin secretion due to the presence of flavonoids. The flavonoid content obtained in the study was in conformity with that reported by Muhammad & Ibrahim (20) (2.18 mg/100g) in their study. More so, flavonoids have also been reported to stimulate take-up of Ca_{2+} from the isolation of islet cells, however, it was also productive in type 2 diabetes.[57] Therefore, oral administration of the aqueous leaves extracts among other things, could prevent many cardiovascular diseases, including hypertension and atherosclerosis. Castell, Baiges & Ard,[58] reported positive effect of flavonoids in increasing insulin secretion, as well as alleviate beta-cell apoptosis and modulation of its proliferation.

The terpenoid content of the aqueous leave extracts studied was relatively low both in quality and quantity. This could be an indicative that the terpenoid content in the aqueous leave extracts may have been lost during extraction processing, reconstitution of the extracts and/or the aqueous leave extracts may not have been a very good sources of terpenoids. Muhammad & Ibrahim (20) had earlier reported low (0.4 mg/100g) terpenoid content, and this further conformed with the present study that the aqueous leave extracts studied does not possess sufficient quantity of terpenoid due to its low content. However, terpenoids could be helpful therapeutically in preventing many ailments, including diabetes. Terpenoids are also known to exhibit antifungal, anti-parasitic, antimicrobial, antiallergenic, and antifungal potentials,

and also display antiviral, antispasmodic, anti-hyperglycemic, immunomodulatory and anti-inflammatory activities.[59]

CONCLUSION

It was discovered that the aqueous extracts of *A. sativum* leaf and *A. muricata* leaf were loaded with nutrients and bioactive components that are capable of demonstrating anti-hyperglycaemic potentials and also their availability will contribute to cost-effective strategy in diabetes treatment and/or management. The administration of the aqueous extracts will therefore be beneficiary both in healthy and disease state.

There is no conflicting interest.

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