

ORIGINAL CONTRIBUTION

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Phytochemicals and *in vitro* anti-apoptotic properties of ethanol and hot water extracts of Cassava (*Manihot esculenta* Crantz) peel biogas slurry following anaerobic degradation

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Abstract

Background: Wastes emanating from cassava (*Manihot Esculenta* Crantz) processing in African countries significantly contribute to environmental pollution, besides, such toxic wastes contribute to greenhouse gas emission. Although cassava peel has been successfully used as a raw material in mushroom cultivation, feedstock for livestock, biogas production but the bio-transformed products recovered from the anaerobic digestion of cassava wastes, especially the peels have often been overlooked. Therefore, this research aimed at quantifying the secondary metabolites in the slurry recovered from ethanol and hot water extraction of cassava peel subjected to biogas production, *in vitro*, for anti-apoptotic properties.

Methods: Fresh cassava peels were allowed to ferment anaerobically to produce three states of matter; gas, solid, and liquid/slurry. The slurry was extracted using 95 % ethanol and 100 °C hot water to obtain crude extracts, which were then subjected to anti-apoptotic screening using the mitochondrial swelling assay. The qualitative phytochemical analysis of the crude extracts was done using standard methods. Further characterization of the crude extracts was done by FTIR for the chemical elucidation of the functional groups present.

Results: The qualitative phytoconstituents revealed that the slurry extracts are naturally enriched with alkaloids, steroids, flavonoids, and saponins. The infrared spectrum of the crude extracts revealed the presence of hydroxyl, alkane, carboxyl groups in the ethanol extract, and hydroxyl, alkene, amide, carbonyl groups in the hot water extract. In the presence and absence of exogenous Ca^{2+} , both extracts of the slurry induced liver mitochondrial permeability transition pore opening albeit at low amplitude swelling as the mean absorbance was less than one (at 540 nm).

Conclusions: Based on these results obtained, the crude extracts of cassava peel biogas slurry have been proven to possess bioactive compounds that could induce liver mitochondrial permeability transition pore opening, *in vitro*.

Keywords: Cassava peel, Mitochondrial permeability transition pore, FTIR

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Background

Organic waste generation is an unavoidable component of our daily life which has posed serious environmental pollution to humans due to their degradable nature and lack of technology to convert them into a profitable raw material that is eco-friendly [1]. The major agricultural product in many countries in Africa, Cassava (*M. esculenta*) is known for its resistance to diseases and drought and its noticeable not only to the quota of carbohydrate content for human consumption but also as a cheaply available food for the poor. Increase in demand for the cassava products have led to the rapid growth of cassava plant and therefore makes cassava processing waste such as the cassava peels and cassava wastewater linger around the environment [2]. According to [3], annually, approximately 350 million metric tons of cassava solid residue was generated from 550 million metric tons of cassava crop.

Cassava waste leaked into the environment and therefore, the toxic effect emancipated from biodegradation of cassava waste increases the growth of pathogenic organisms, obstruction of waterways and drains, contaminating water resources for aquatic animals, and also caused depletion in the ozone layer due to toxic gases emission. Current researches have been defining how to convert solid waste and liquid waste resulting from cassava processing into a profitable substance that is eco-friendly. The nutrient profile present in cassava, its various parts, including the residues have been biotechnological harnessed into by-products for both higher (humans) and lower animal consumptions [4]. Recently, [5] reviewed the genetically modified uses of the cassava crop as a potential way-out to the treatment of some chronic diseases like anticancer, antidiabetic, antimicrobial, antiulcer, and anti-hyperlipidemic. More cassava crop and waste uses are currently under study by researchers. Towards identifying the medicinal components, chromatography and spectroscopic techniques have been the major tools in estimating the phytonutrients in the plant, vis-à-vis their structure-activity relationship [6].

Over the years, the extracts from plants and the products of bio-transformation by microorganisms characterized fascinating metabolic pathways that favour the innovation of prospective drugs, while their by-products have been used to mediate biological processes or offset the risk of causation of chronic diseases in humans [7]. The use of Fourier Transform Infrared Spectroscopy has been known for identifying bio-molecular compositional information in plants [8]. Cassava (*M. esculenta*) is not left out, it is also a good source of some phytochemicals. Cassava (*M. esculenta*) is a good source of antioxidant, anti-haemorrhoid, anti-inflammatory, analgesic, anthelmintic, antiseptic, cyanogenic, demulcent, diuretic and antibacterial activities [5].

However, the balancing of cell survival and death is under tight genetic control and induction of the mitochondrial membrane permeability transition (MMPT) pore has been implicated in the cascade of events involved in apoptosis also known as programmed cell death [9]. The process of the Mitochondrial permeability transition is calcium-dependent [10]. Moreover, Ahmed et al. [11] confirm the use of phytochemicals compounds as a modulator to alternate apoptosis pathways. Conversely, the present of some important phytochemicals such as flavonoids, carotenoids and phenolic compounds in cassava serves as a defensive agent against cancer, diabetes and cardiovascular disease [12]. Onodu et al. [13] confirm the presence of secondary metabolites such as alkaloids, flavonoids, tannins, cardiac glycosides, anthraquinone, phlorotannins, saponins and anthrocyanosides in the aqueous and ethanolic extracts of raw tubers, leaves and peels of cassava (*M. esculenta*), while Ehuehi et al. [4] additionally reported the presence of reducing sugars and anthocyanosides, but not cardiac glycosides, anthraquinone, phlobatinnins and saponins in the aqueous and ethanolic extracts of raw cassava tuber.

Most researchers ascertain that the physicochemical composition of the solid component of cassava wastes (peels and bagasse) confirms its potential usage in mushroom cultivation, as an additive in feedstock for livestock, compost material, biosolids and substrate for production of fuel such as bioethanol, biogas, biochar, bio-oil etc. [14, 15]. Sawyerr et al. [16] reported that cassava peel has been successfully used as feedstock material in the production of biogas with other fruit wastes. However, the anaerobic digestion of cassava peel produces two main outputs: biogas and bio-slurry - the by-products of anaerobic decomposition from a biogas plant.

Unfortunately, while biogas is used to produce energy, the bio-slurry majorly is being focused on biofertilizers for agricultural yield products. However, the large potential and the biotransformation of anaerobic organic wastes by microorganisms have often been overlooked [17]. Islam et al. [18] reported that most of the anaerobic microorganisms isolated from bioslurry are germane to human health. But limited information is provided on the biotransformational extract recovered from the bio-slurry. This study, therefore, assessed, *in vitro*, the secondary metabolites recovered from the bio-transformed products of anaerobic digester of cassava peel waste for anti-apoptotic properties.

Materials and methods

Collection and Preparation of Cassava Slurry

Fresh cassava peels used as substrate were obtained from Oyo town, Oyo State, Nigeria using a sterile plastic bag. The Cassava peels were identified by Dr Mahboob Jimoh at the herbarium of the Department of Biological

Sciences, Osun State University, Osogbo, Osun State, Nigeria, with a voucher number: UNIOSUN/PBO/JM/04/198/Met. They were completely sun-dried, and a total of 15 g dried peels were mixed with water (1:1 w/v) and anaerobically stored with clean water in a biogas digester for a month, at room temperature and away from direct sunlight, to form a slurry [19].

Preparation of Extracts

The slurry was cold-macerated with 6 volumes of 95 % ethanol for 38 h at room temperature and another different portion of the slurry was extracted in a similar volume of boiled (100 °C) distilled water over a heating block for 30 min in two rounds, the exudates were pooled. The ethanol crude extract obtained was filtered and evaporated to dryness in a rotatory evaporator while the hot water extract was also evaporated to dryness in a rotatory evaporator (LIDAi.DNA XMT – J7000/RE52-3). The pastes (50 %) were weighed and used to prepare the stock solution for experiments.

Experimental Animals

Eight male Wistar rats weighing between 120 and 180 g were used for this experiment. The rats were obtained from the Animal House, College of Health Sciences, Osun State University, Nigeria. The rats were housed in a standard environmental condition of temperature (30 ± 1 °C), humidity (60 ± 0.2 %) and 12 h light and 12 h dark cycle, and fed with commercial grower's mash and water, ad libitum in the Animal House, Department of Biochemistry, Osun State University. The rats were acclimatized for three weeks before the excision of the liver. The ethical approval was obtained from the University's College of Health Sciences and Research Ethics Committee, Osun State University, Osogbo (UNIOSUN/HREC/2019/A/008).

Experimental Design

Two hundred milligram per millilitre (200 mg/ml) stock solution of ethanol and hot water extracts of Cassava Peel Biogas Slurry (about twelve weeks old) were used to assess *in vitro*, phytochemical content, and effects on the mitochondrial membrane permeability transition pore assay both in the presence and absence of exogenous Ca^{2+} .

Qualitative Phytochemical Tests

The phytochemical screening of ethanol and hot water extracts of cassava peel biogas slurry was carried out using standard methods to detect the presence or the absence of secondary metabolites such as alkaloids, saponins, tannins, terpenes, flavonoids, steroids, and carbohydrates [20], viz.

Test for Alkaloids

One millilitre of 1 % HCl was added to 3 ml of each extract in different test tubes. Each mixture was heated for 2 min in a water bath while stirring continuously. It was cooled and filtered. 1ml of the filtrate was added to 0.5ml of Mayer's reagent in the different test tubes. The turbidity of the extract filtrate on the addition of Mayer's reagent was taken as evidence of the presence of alkaloids in the extracts [20].

Test for Steroids

Five drops of acetic anhydride, and then a drop of concentrated H_2SO_4 were added to 0.5 g of each extract. The mixture was steamed for 1 h and neutralized with sodium hydroxide (NaOH), followed by the addition of chloroform. The appearance of a blue-green colour indicated the presence of steroids [20].

Test for Flavonoids

The total flavonoid content was determined by the colourimetric method described by Ordon et al. (2006) with some modifications. Two drops of NaOH solution was added to 1 ml of each extract in different test tubes. Two drops of AlCl_3 solution were added to the mixture, followed by the addition of concentrated H_2SO_4 [21].

Test for Saponins

Each extract (0.5 g) was mixed with water in a test tube. Foaming which persisted in warming was taken as evidence for the presence of saponins [20].

Test for Glycosides

Each extract (0.5 g) was dissolved in 2 ml of chloroform. Tetraoxosulphate VI acid (H_2SO_4) was carefully added to form a lower layer. A reddish-brown colour at the interface indicated the presence of a steroidal ring, that is, the aglycone portion of the cardiac glycosides [20].

Test for Tannins/Phenols

Each extract (0.5 g) was separately stirred with 10 ml of distilled water and then filtered. Few drops of 5 % FeCl_3 reagent were added to the filtrate. Blue-black or blue-green colouration or precipitation was taken as an indication of the presence of phenolics and tannins [20].

Test for Reducing Sugar

This was determined according to the method of Oyaizu (1986). 5 ml of an equal volume of Fehling solution A and B were added to 5 ml of each extract and boiled for 5 min. The development of red precipitate/ rusty brown colour indicated a positive result [22].

Test for Terpenoids

Two ml of each extract was dissolved in 2 ml of chloroform, and evaporated to dryness. 2 ml of concentrated sulphuric acid was then added and heated for about 2 min. The development of a greyish colour indicates the presence of terpenoids [20].

Identification of the Organic Composition of Crude extracts

Characterization by Fourier Transformed Infrared Spectrometer (FTIR)

The metabolites formed from the biotransformation of cassava peels in the slurry were identified using the FTIR to determine the functional groups of the product composition.

Mitochondrial Membrane Permeability Transition Pore Assay

Preparation of Rat Liver Homogenate from Normal Rat for Mitochondria Membrane Permeability Test

Sanz et al. [23] method was used for the assessment of the low ionic strength mitochondria assay. Two normal Wistar rats were sacrificed by cervical dislocation. The livers were excised and minced in ice-cold homogenization buffer pH 7.4 (210 mM mannitol, 70 mM sucrose, 5 mM HEPES-KOH, pH 7.4 and 1 mM EGTA (Sigma Aldrich, USA) and centrifuged (Allegra X-30R centrifuge) twice at $1000 \times g$ for 10 min at 4°C to remove cellular debris and the nuclear fraction. The supernatant was re-centrifuged at the same speed and time to remove unbroken cells. The supernatant thus obtained was centrifuged at $10,000 \times g$ for 10 min to sediment the mitochondria. The brown mitochondria pellet obtained was resuspended in isolation buffer (210 mM mannitol, 70 mM sucrose, 5 mM HEPES-KOH, pH 7.4, 0.5 % BSA and the whole solution made up to 100 ml mark and stored at 4°C in a refrigerator) and then centrifuged at $10,000 \times g$ for 10 min. This washing stage was done twice to eliminate extraneous debris. The mitochondria were immediately suspended in a solution

of ice-cold suspension or MSH Buffer (3.83 g mannitol, 2.3 g sucrose, 0.12 g HEPES-KOH, 0.12 g Tris, pH 7.4) then dispensed in Eppendorf tubes as aliquots and placed on ice for immediate use. The protein concentration of the liver homogenate was determined according to Lowry's method [24] using bovine serum albumin (BSA) as standard.

Assessment of Mitochondrial Membrane Permeability

Transition in Normal Rat Liver mitochondria

The sensitivity of the mitochondrial membrane transition pore (swelling) was determined by studying the rate of change in absorbance at 540 nm under energized and de-energized conditions using the Camspec M106 spectrophotometer [25]. Mitochondria that have accumulated Ca^{2+} can be induced to undergo a permeability transition. The inner membrane becomes non-selectively permeable to small (1500 Da) solutes. Isolated mitochondria undergoing Permeability Transition (PT) show colloid osmotic, that is, large-amplitude swelling which results in a decrease photometric absorption at 540 nm. Several experimental PT was assessed by measuring the swelling of mitochondria by monitoring the associated decrease in light scattering.

The principle is based on the fact that when the mitochondria swell, their refractive index changes and thus less light is passed across the cuvette resulting in a decrease in the light absorbance measured with a spectrophotometer. In a means to avoid any complications that changes in the redox state of respiratory chain components might cause, the wavelength of the incident light should be at the isosbestic point from the cytochromes (540 nm) as used in several studies on isolated mitochondria.

In the absence of exogenous CaCl_2 , the mitochondria were allowed a 3.5-minute incubation with the extracts in varying concentrations; whereas in the presence of exogenous CaCl_2 , they were allowed a 3-minutes incubation, following which CaCl_2 was added to trigger the pore opening within 30 s, then the mitochondria were energized via the Complex II of the electron transport

Table 1 Procedure for the assay of mitochondrial membrane transition pore in rat liver mitochondria (MMPT)

Sample	MSH buffer (μl)	Rotenone (μl)	Spermine (μl)	Extract (μl)	MIT (μl)	Calcium (μl)	Succinate (μl)
NTA	2385	25	---	---	30	---	50
TA	2360	25	---	---	30	25	50
I	2297.5	25	62.5	---	30	25	50
E10 ($\mu\text{g}/\text{ml}$)	2330	25	---	10	30	25	50
E30 ($\mu\text{g}/\text{ml}$)	2330	25	---	30	30	25	50
E50 ($\mu\text{g}/\text{ml}$)	2310	25	---	50	30	25	50

NTA No triggering agent, TA Triggering agent, I Inhibitor, E Extract at different concentrations, MSH Buffer Swelling buffer, MIT Isolated mitochondria. Absorbance was read at 540 nm at 30-sec intervals over 12 minutes, and the assay was conducted in duplicates (24 readings, $n=3$).

$\Delta \text{Abs} = \text{Abs at } T_{(30 \text{ s, 1 min, 1 min } 30 \text{ s } \dots 12 \text{ min})} - \text{Abs at } T_0 \text{ sec}$

Table 2 Qualitative phytochemical screening of crude ethanol and hot water extract of Cassava peel biogas slurry

S/ N	Chemical Group	Specific Test	CPE Observation	CPH Observation
1	Test for Alkaloids	Mayer's test	+	+
2	Test for Steroids	a) Sulphuric acid test b) Salkowski test	+ +	+ +
3	Test for Flavonoids	Sulphuric acid test	++	+
4	Test for Saponins	General test	+	+
5	Test for Glycosides	General test	+	+
6	Test for Tannins/Phenol	Ferric chloride test	-	-
7	Test for Reducing Sugar	Benedict test	-	-
8	Test for Terpenoids	Chloroform test	+	-

CPE Crude ethanol extract of Cassava peel biogas slurry, CPH Crude hot water extract of Cassava peel biogas slurry

system by the addition of sodium succinate (rotenone blocks the Complex I). The mitochondrial swelling was recorded spectrophotometrically as the decrease in extinction at 540 nm (Table 1).

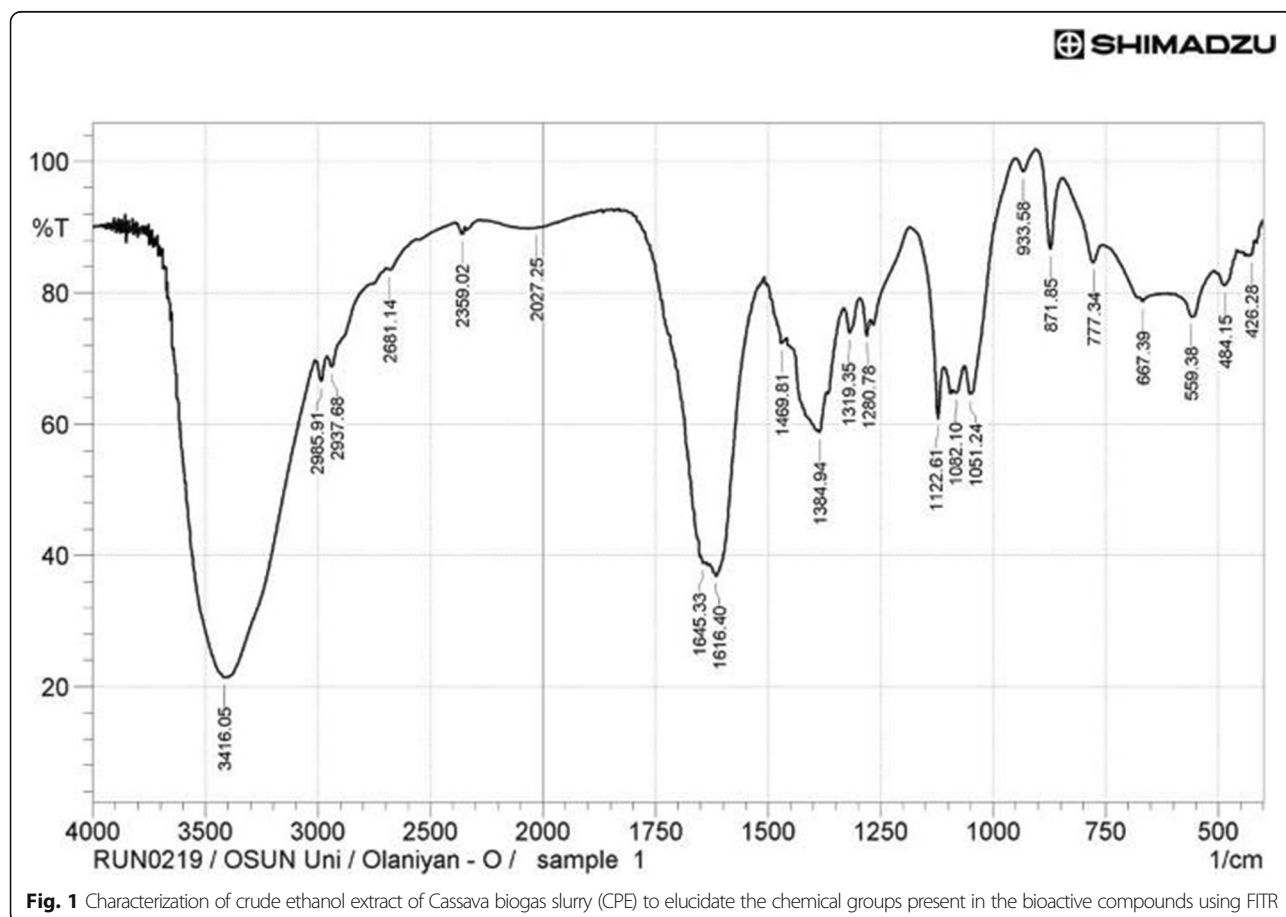
Statistical Analysis

The results were analysed using SPSS Version 12 to determine the difference between mean using One Way Analysis of Variance (ANOVA).

Results

Phytochemical constituents of crude ethanol and hot water extracts of Cassava biogas slurry

The qualitative screening of Crude Ethanol and Hot Water Extract of Cassava Peel Biogas Slurry revealed the presence of alkaloids, steroids, flavonoids, saponins, glycosides, terpenoids whereas terpenoids are absent in the Hot Water Extract of Cassava Peel Biogas Slurry as shown in Table 2.



Characterization of the bioactive compounds in the slurry extracts by FITR

The ethanol extract of cassava biogas slurry peak at 3416.05 cm^{-1} revealed the presence of the Hydroxyl group (O-H stretch). The peak at 2985.91 and 2937.68 cm^{-1} refers to the presence of alkanes (C-H stretch). The peak at 1645.33 and 1616.4 cm^{-1} corresponds to the Carboxyl group (C=O stretch) as shown in Fig. 1. Similar results were obtained from the Hot water extract of Cassava Peel Biogas Slurry and the peak at 2924.18 and 2850.88 cm^{-1} shows the presence of the alkane group (C-H stretch). The peak at 2359.09 , 1616.4 , 3446.91 cm^{-1} corresponds to amide (C=N stretch), carbonyl group (C=O stretch), and Hydroxyl group (O-H stretch) (Fig. 2). The peaks around $3373\text{--}3422\text{ cm}^{-1}$ may be due to the presence of bonded N-H/C-H/O-H stretching of amines and amides.

The Assessment of the *in vitro* effect of crude ethanol and hot water infusion extracts of Cassava biogas slurry on Rat liver mitochondrial membrane permeability transition pore in the absence and presence of Calcium (Ca^{2+})

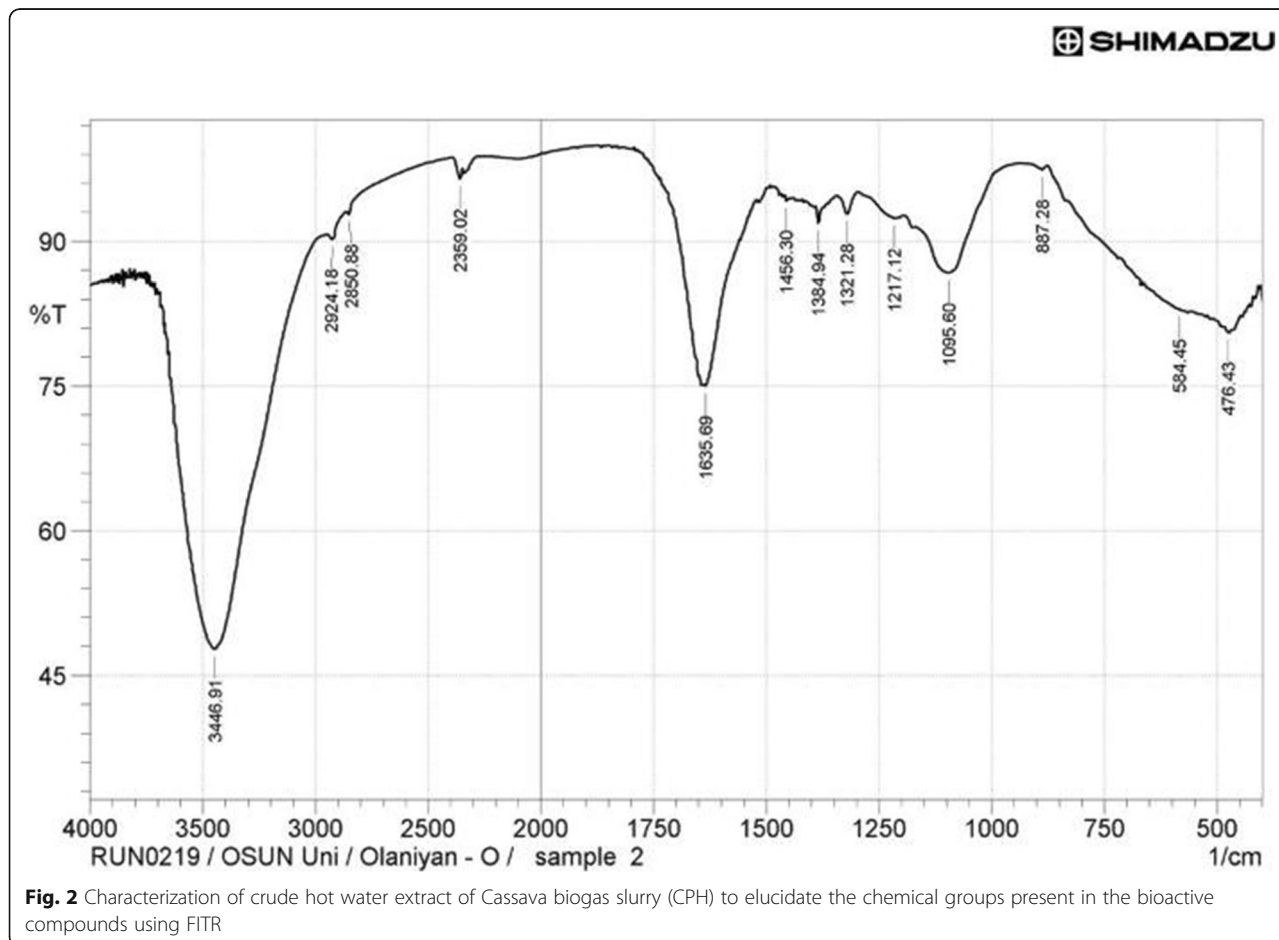
The absence and presence of calcium (Ca^{2+}) revealed that both the ethanol and hot water extract was able to

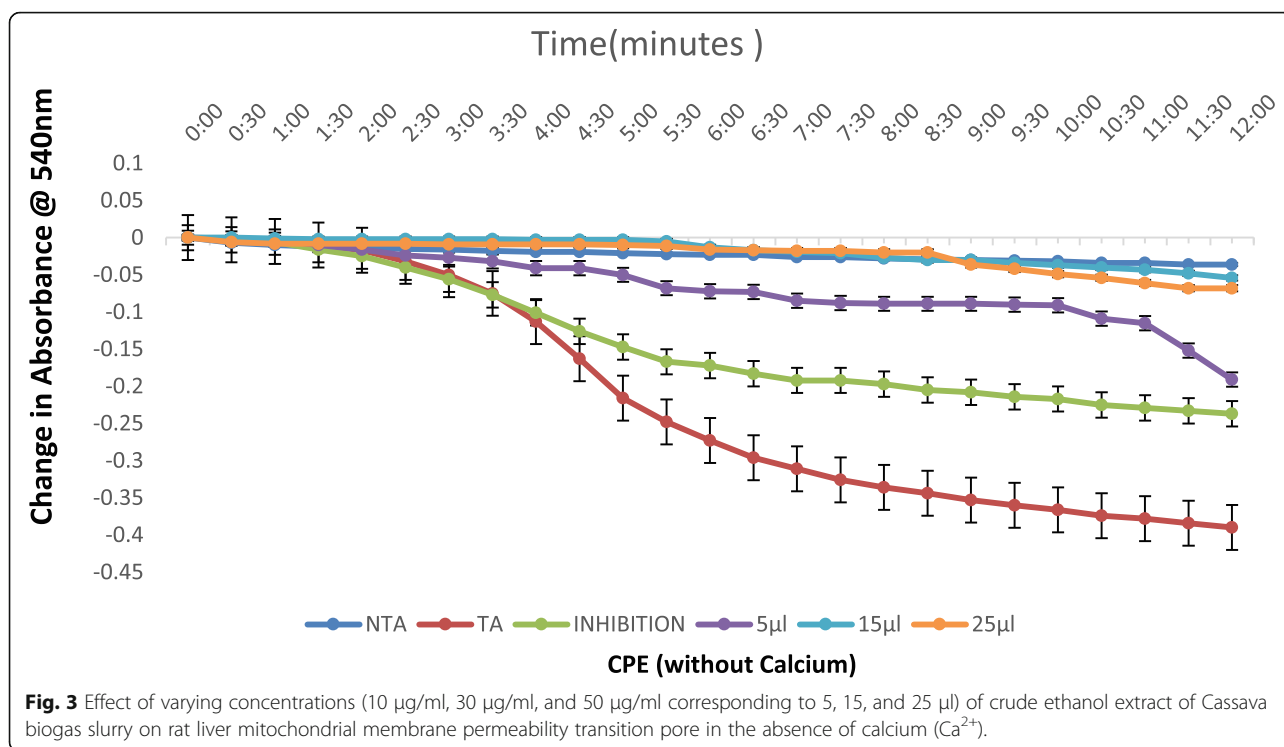
cause a slight amplitude swelling of the MMPT pore (Figs. 3 and 4) while it further opened in the presence of exogenous calcium (Figs. 5 and 6) in an increasing concentration-dependent manner.

Discussion

Cassava (*M. esculenta*) plantation has been identified as a promising staple crop that can reduce food security, poverty and hunger [24]. Cassava products are not only a source of energy but also a cheaply available food product for the poor [26]. Charles et al. [5], suggested that this explains the high demand for cassava (*M. esculenta*), leading to a high yield of cassava waste.

Conversely, various parts of cassava contain some important nutrients including carbohydrates and protein, which have been biotechnologically harnessed into by-products for both higher (humans) and lower animal consumption. Several kinds of research confirm that the physicochemical composition of the solid component of cassava wastes (peels and bagasse) exhibit a potential usage in mushrooms cultivation, as an additive in feed-stock for livestock, compost material, biosolids and substrate for production bioenergy [15]. Other uses include



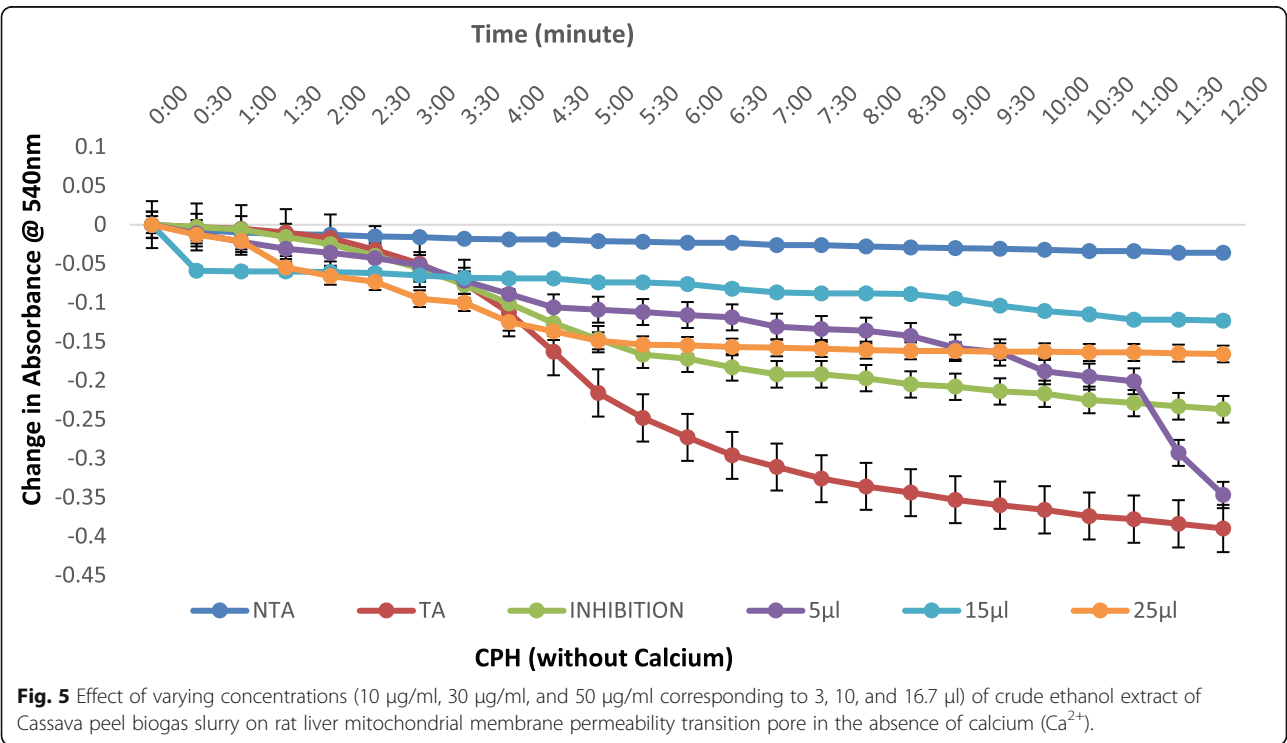
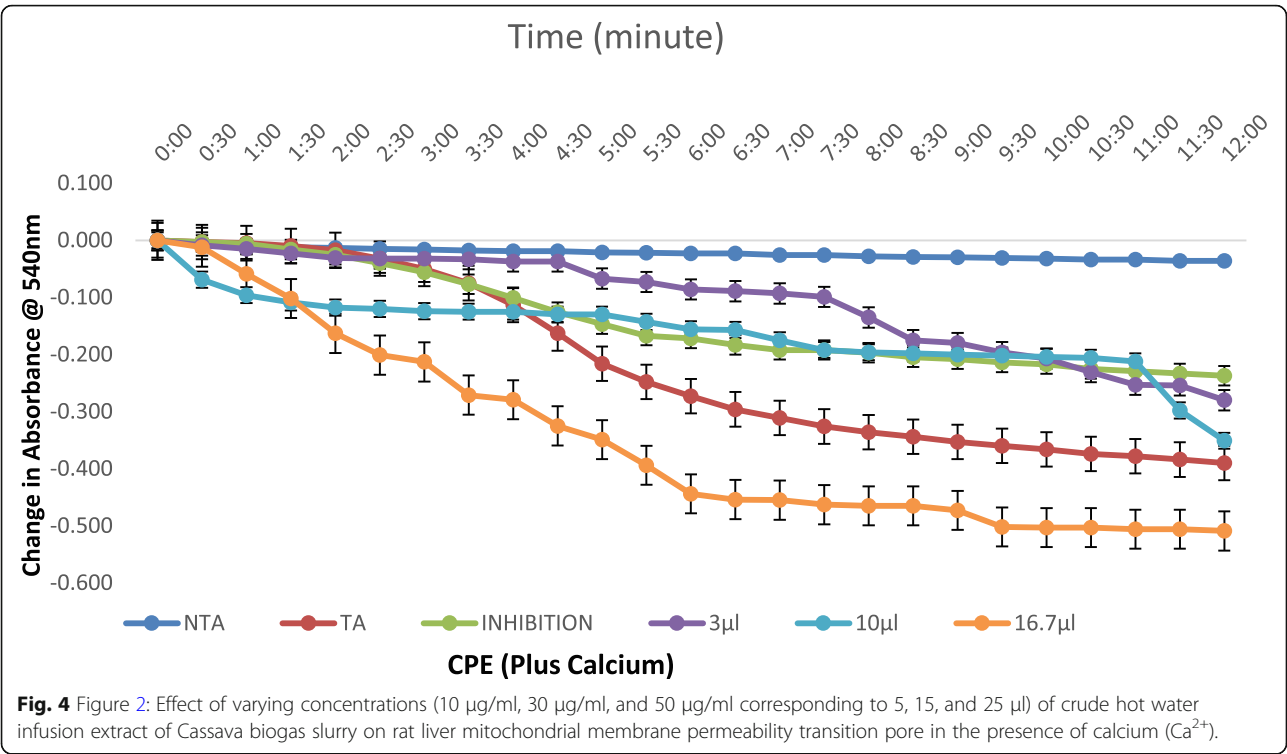


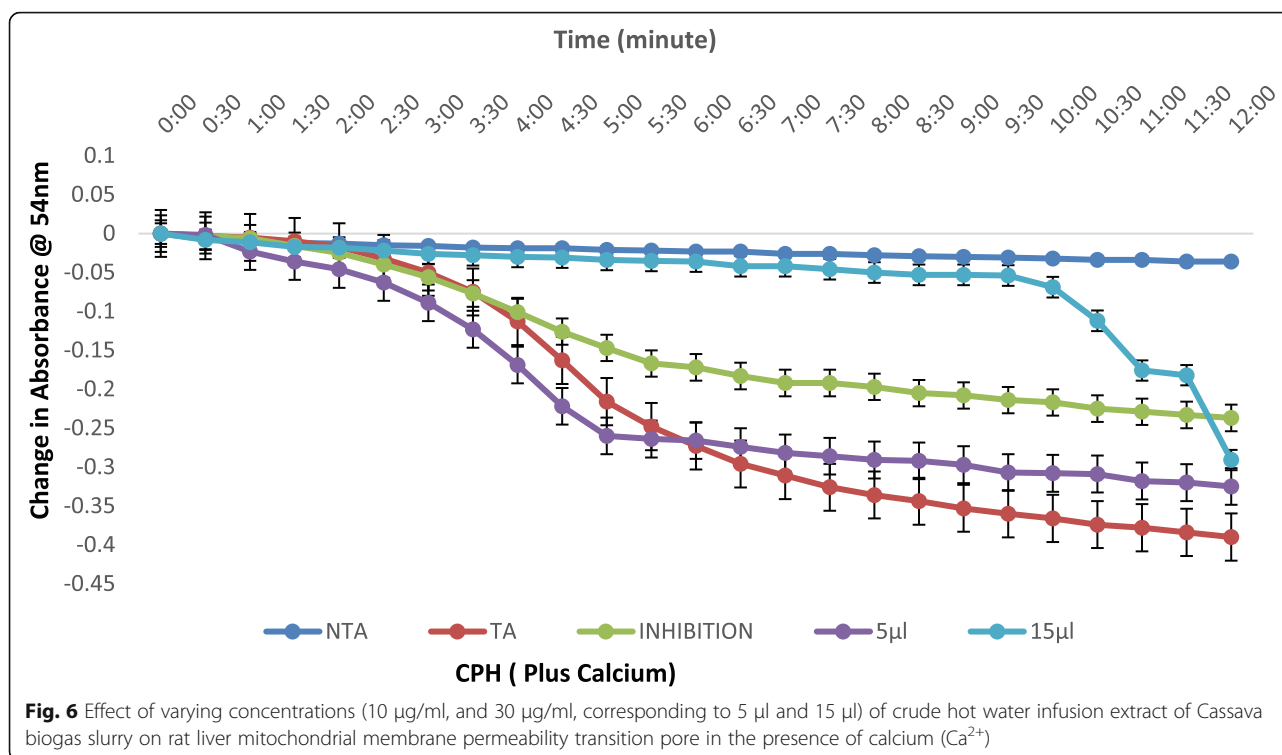
the production of mushroom, biogas and animal additives using cassava peel [14]. Also, Sawyerr et al. [16] confirmed that cassava peel has been successfully used as a substrate in the production of biogas with other organic wastes. Indeed, technological interest has improved towards the advancement of converting cassava waste into fuel such as bioethanol, biogas, biochar, bio-oil etc. [14]. Whereas the transformation of cassava (*M. esculenta*) has been aimed at by many researchers for decades, there still exists an information gap regarding the biotransformation products of the cassava waste especially the cassava peel by the microorganisms.

Unfortunately, while cassava peel has been confirmed for energy production especially through use in biogas production, the slurry has been focused on as a source of biofertilizer for agricultural products. However, this study applied the slurry from the microbial biotransformation of cassava peel to the modulation of mitochondrial apoptosis in diabetic rats. Phytochemicals are secondary metabolites of plants, which include flavonoids, flavones, isoflavones, catechins, anthocyanidins, isothiocyanates, carotenoids, and polyphenols capable of eliciting biological activities [27]. Liu et al. [28] asserted that certain extracts of some plants have been shown to play therapeutic roles in some diseases like cancer, diabetes, and heart disease among others, through their ability to induce apoptosis and cassava (*M. esculenta*) has also been confirmed to contain bioactive compounds that play important therapeutic roles [5].

This study confirmed the presence of certain phytochemical constituents in cassava peel biogas slurry extracts. As presented in Table 2, alkaloids, steroids, flavonoids, saponins, glycosides, and terpenoids were present in the ethanol extract of cassava peel biogas slurry, except tannins/phenol, and reducing sugar. This was in line with the findings of Uka nwoko and Nwachukwu [29] which confirms the presence of tannins in dry cassava peels. A review by Panche et al. [30] elucidated the role of flavonoids as very effective in preventing lipid peroxidation which is responsible for various diseases such as cancer, diabetes, hepatotoxicity, and inflammation. The hot water extract of cassava peel biogas slurry contained alkaloids, steroids, flavonoids, saponins, and glycosides. Also, Onodu et al. [13] confirmed the presence of alkaloids, flavonoids, tannins, cardiac glycosides, anthraquinone, phlorotannins, saponins and anthocyanosides in the aqueous and ethanolic extracts of raw tubers, leaves and peels of cassava (*M. esculenta*). The presence of these compounds in both extracts may account for their therapeutic activities. It also appeared that the ethanol extract of cassava biogas slurry contained more phytochemical constituents than the hot water extract. This also supports the potential benefit of the use of cold maceration with ethanol (at room temperature) for the extraction of secondary metabolites in cassava peels slurry [31].

The elucidation of the functional groups present in the cassava slurry extract was done using Fourier-Transform





Infra-Red Spectrophotometer (FTIR). The Fourier-Transform Infra-red Spectrophotometer (FTIR) is a spectroscopic technique that elucidates functional groups or chemical bonds present in a compound based on the Infrared radiation peaks value region [6]. The FTIR chromatograph (as shown in Figs. 1 and 2) indicate the presence of hydroxyl (OH) at the absorption peak of 3446.91 cm^{-1} and 3446.91 cm^{-1} , alkane (CH_3) at the absorption peak of 2985.91 cm^{-1} , 2924.18 cm^{-1} , and 2850.88 cm^{-1} , and carboxyl groups ($\text{C}=\text{O}$) at the absorption peak of 1616.4 cm^{-1} and 1616.4 cm^{-1} in both ethanol and hot water extract as shown in Tables 3 and 4, although amide ($\text{C}=\text{N}$) at the absorption peak of 2359.09 cm^{-1} was only present in the hot water extract. The peaks confirmed that both extracts contain pharmaceutically useful secondary metabolites, including the aromatic compound O-H stretching vibration signifying the presence of flavonoids, polyphenol and other phenol compounds, which interestingly play important role in the control of oxidative damage by reactive oxygen species

[32]. The carbonyl groups ($\text{C}=\text{O}$) detected in the hot water extract agreed with the study of Rajiv et al. [33] (Table 4). Also, the aliphatic compound C-H group present in both extracts indicate terpenes that function as antiseptic [32]. The compounds present in both extracts correspond with the secondary metabolites profiles for medicinal plants [6].

Suhaili et al. [34] reported that death signals on the mitochondria also led to the permeabilization of the outer and inner mitochondrial membranes, causing the opening of the MMPT pore, loss of mitochondrial membrane potential, inactivation of ATP synthase, mitochondrial matrix swelling and the release of proapoptotic proteins that drive cascades of reactions that ultimately lead to cell death in a programmed fashion. But calcium (Ca^{2+}), inorganic phosphate, alkaline pH, and reactive oxygen species (ROS) are a few of the many agents that promote the MPT, whereas the immunosuppressive drug, cyclosporin A (CsA), Mg^{2+} , acidic pH, and phospholipase inhibitors, including trifluoperazine,

Table 3 Characteristic FTIR absorption frequency of organic functional group in crude ethanol extract of Cassava peel biogas slurry

S/N	Class of compounds	Type of vibration	Absorption, cm^{-1}	Intensity	Chemical Formula
1	Hydroxyl group	Stretch	3416.05	Single bond	O-H
2	Alkane	Stretch	2985.91	Single bond	C-H
3	Alkane	Stretch	2937.68	Single bond	C-H
4	Carboxyl group	Stretch	1645.33	Double bond	$\text{C}=\text{O}$
5	Carboxyl group	Stretch	1616.4	Double bond	$\text{C}=\text{O}$

Table 4 Characteristic FTIR absorption frequency of organic functional group in crude hot water infusion extract of Cassava biogas slurry

S/N	Class of compounds	Type of vibration	Absorption, cm ⁻¹	Intensity	Chemical Formula
1	Hydroxyl group	Stretch	3446.91	Single bond	O-H
2	Alkane	Stretch	2924.18	Single bond	C-H
3	Alkane	Stretch	2850.88	Single bond	C-H
4	Amide	Stretch	2359.09	Double bond	C = N
5	Carbonyl group	Stretch	1616.4	Double bond	C = O

dibucaine, and quinacrine, block the opening of the PT pores [35]. In this study, assessments were made of the *in vitro* effects of the crude ethanol and hot water extracts of Cassava Peel Biogas Slurry on rat liver mitochondrial membrane permeability transition pore (Figs. 3 and 5). The readings confirmed the report of Tait and Green [36] that apoptosis occurs by the induction of the opening of the mitochondrial membrane permeability transition pore, followed by the concomitant release of cytochrome c from the mitochondria intermembrane space. This study confirms that both crude extracts possess certain bioactive compounds that may modulate hepatic mitochondrial membrane opening, leading to the progressive induction of the liver mitochondrial membrane permeability transition pore opening in normal rats, *in vitro* [37, 38], in the presence of exogenous calcium, whereas the integrity of the membrane was ascertained *ab initio* in the absence of exogenous calcium. The hot water extract of cassava biogas slurry induced low amplitude swelling of the liver mitochondrial membrane permeability pore at low concentration in the presence of exogenous calcium compared to the ethanol extract that induced the same at each concentration tested in the presence of exogenous calcium.

Conclusions

While cassava peels have been successfully used as a raw material in edible mushroom cultivation (as manure), feedstock for livestock, and in bioenergy production; this study has shown that the ethanol and hot water extracts of the products recovered from the anaerobic digester of bio-transformed cassava waste especially the peel possess certain bioactive compounds that can induce liver MMPT pore opening in normal rats, *in vitro*, especially in the presence of exogenous Ca²⁺. This research confirms the *in vitro* therapeutic importance of microbial biotransformation of cassava peel as a source of potential anti-apoptotic drugs. However, further screening on the anaerobic biotransformation of cassava wastes are therefore encouraged in the search for new chemical entities.

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Authors' contributions

AEIO and OOP conceived the experiment. OOP performed and AEIO supervised the experiment. OOP wrote the manuscript, AEIO proof-read the manuscript. Both authors read and approved the final submission of this manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable as no humans were recruited for the study.

Consent for publication

All authors approved the final copy of the manuscript for submission.

Competing interests

The authors declare that they have no competing interests.

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References

1. Oparaku NF, Ofomatah AC, Okoroigwe EC. Biodigestion of cassava peels blended with pig dung for methane generation. *Afr J Biotechnol*. 2013. doi: <https://doi.org/10.5897/AJB2013.12938>.
2. Olukanni DO, Olatunji TO. Cassava waste management and biogas generation potential in selected local government areas in Ogun State, Nigeria. *Recycling*. 2018. doi: <https://doi.org/10.3390/recycling3040058>.
3. Sivamani S, Chandrasekaran AP, Balaji M, Shanmugapriakash M, Hosseini-Bandegharai A, Baskar R. Evaluation of the potential of cassava-based residues for biofuel production. *Rev Environ Sci Biotechnol*. 2018. doi: <https://doi.org/10.1007/s111157-018-9475-0>.
4. Ebuechi O, Ahmed Z, Bablola O. Phytochemical, the nutritive and anti-nutritive composition of cassava (*Manihot esculenta* L.) tubers and leaves. *NIFOJ*. 2006. DOI: <https://doi.org/10.4314/nifo.v23i1.33597>.
5. Adetunji CO, Akram M, Imtiaz A, Bertha EC, Sohail A, Olaniyan OP, et al. Modified cassava; the last hope that could help to feed the world-recent advances. In: Kayi Kishor PB, Rajam MV, Pullaiah T, editors. *Genetically modified crops*. Singapore: Springer; 2021. pp. 203–13.
6. Dhivya SM, Kalaichelvi K. Screening of primary and secondary metabolites, UV-Vis spectrum and FTIR analysis of *Acmella calva* (DC.) R.K. Jansen.

- Carmona retusa* Vahl. and *Leptadenia reticulata* W. & A. *Int J Recent Sci Res*. 2017;8(6): 17952-56.
7. Mauerhofer L, Pappenreiter P, Paulik C, Seifert A, Bernacchi S, Rittmann S. Methods for quantification of growth and productivity in anaerobic microbiology and biotechnology. *Folia Microbiol*. 2019. doi:<https://doi.org/10.1007/s12223-018-0658-4>.
 8. Pakkirisamy M, Kalakandan SK, Ravichandran K. Phytochemical screening, GC-MS, FT-IR analysis of methanolic extract of *Curcuma caesia* roxb (Black Turmeric). *Pharmacog J*. 2017;9(6):952–6.
 9. Bryan N, et al. Reactive oxygen species (ROS) – a family of fate deciding molecules pivotal in constructive inflammation and wound healing. *Eur Cell Mater*. 2012;24:249–65.
 10. Bauer TM, Murphy E. Role of mitochondrial calcium and the permeability transition pore in regulating cell death. *Circ Res*. 2020;126:280–93.
 11. Ahmed K, Zaidi SF, Cui Z, et al. Potential proapoptotic phytochemical agents for the treatment and prevention of colorectal cancer. *Oncol Lett*. 2019. doi:<https://doi.org/10.3892/ol.2019.10349>.
 12. Sharifi-Rad M, Anil Kumar NV, Zucca P, Varoni EM, Dini L, Panzarini E, et al. Lifestyle, oxidative stress, and antioxidants: back and forth in the pathophysiology of chronic diseases. *Front Physiol*. 2020. doi:<https://doi.org/10.3389/fphys.2020.00694>.
 13. Onodu B, Culas RJ, Nwose EU. Health values of cassava compared to wheat and yam about diabetes and dyslipidaemia management. *IJCMPH*. 2020. doi:<https://doi.org/10.18203/2394-6040.ijcmph20200469>.
 14. Odeiran OF, Ashimolowo OR, Sodiya CI, Sanni LO, Adebayo K, Ojebiyi WG, Adeoye AS. Awareness of cassava peel utilization forms among cassava processors in rural communities of Southwest, Nigeria. *IJAAR*. 2015;11(1–2): 93–102.
 15. Akinrinola-Akinyemi AO, Asagbra AE, Asiru WB, Lalemi MO, Okere VO, Ouwawole OF, et al. Viability of cassava peels spawn's production and mushroom cultivation. *Open Agric*. 2017. doi:<https://doi.org/10.1515/opag-2017-0027>.
 16. Sawyerr N, Trois C, Workneh T. Identification and characterization of potential feedstock for biogas production in South Africa. *J Ecol Eng*. 2019. doi:<https://doi.org/10.12911/22998993/108652>.
 17. Nyang'au J, Gatebe E, Nyagah C, Ahenda S. Evaluation of biogas slurry as an alternative organic fertilizer a case study in Kenya. *Int J Exten Res*. 2016;9:10–4.
 18. Islam MA, Biswas P, et al. Microbial load in bio-slurry from different biogas plants in Bangladesh. *J Adv Vet Anim Res*. 2019. doi:<https://doi.org/10.5455/javar.2019f357>.
 19. Olaniyan OP, Bamigboye OO, Adediji TA, Bamigboye FO, Ajediti BO. Biogas production from kitchen waste and cow dung using a locally fabricated bioreactor. *AUJET*. 2018;1(1):10–5.
 20. Odeja O, Obi G, Ogwuche CE, Elemike EE, Oderinlo Y. Retracted article: Phytochemical screening, antioxidant and antimicrobial activities of senna occidentalis (L.) leaves extract. *Clin Phytoscience*. 2015;1(1):1–6.
 21. Ordon Ez AAL, Gomez JD, Vattuone MA, Isla MI. Antioxidant activities of *Sechium edule* (Jacq.) Swart extracts. *Food Chem*. 2006;97:452–8.
 22. Ismail GA. Biochemical composition of some Egyptian seaweeds with potent nutritive and antioxidant properties. *Food Sci Technol Campinas*. 2017;37(2):294–302.
 23. Sanz A, Pamplona R, Barja G. Is the mitochondrial free radical theory of ageing intact? *Antioxid Redox Signal*. 2006. doi:<https://doi.org/10.1089/ars.2006.8.582>.
 24. Jelili TO. Growth analysis of genetically modified cassava (*Manihot esculenta* Crantz) plants expressing NPT II marker and GUS reporter genes. *Agri Res Tech: Open Access J*. 2018. doi:<https://doi.org/10.19080/ARTOAJ.2018.14.555921>.
 25. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem*. 1951;193(1):265–75.
 26. Ceballos H, Iglesias CA, Pe' rez JC, Dixon AGO. Cassava breeding: opportunities and challenges. *Plant Mol Biol*. 2004. doi:<https://doi.org/10.1007/s11103-004-5010-5>.
 27. Banerjee P, Nath D. A phytochemical approach to synthesize silver nanoparticles for non-toxic biomedical application and study on their antibacterial efficacy. *Nanosci Technol*. 2015. doi:<https://doi.org/10.15226/2374-8141/2/1/00123>.
 28. Liu XY, Wei B, Shi HX, Shan YF, Wang C. Tom70 mediates activation of interferon regulatory factor 3 on mitochondria. *Cell Res*. 2010. doi:<https://doi.org/10.1038/cr.2010.103>.
 29. Ukanwoko AI, Nwachukwu J. Nutrient and anti-nutritional composition of crop residues and kitchen wastes fed to small ruminants in Choba, Port Harcourt. *Greener J Agric Sci*. 2017. doi:<https://doi.org/10.15580/GJAS.2017.2.032317044>.
 30. Panche AN, Diwan AD, Chandra SR. Flavonoids: an overview. *J Nutr Sci*. 2016. doi:<https://doi.org/10.1017/jns.2016.41>.
 31. Hijazi A, Bandar H, Rammal H, Hachem A, Saad Z, Badran B. Techniques for the extraction of bioactive compounds from Lebanese *Urtica dioica*. *Am J Phytomed Clin Ther*. 2013;1(6):507–13.
 32. Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A. Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: an overview. *Medicines (Basel)*. 2018;doi:<https://doi.org/10.3390/medicines5030093>.
 33. Rajiv P, Deepa A, Vanathi P, Vidhya D. Screening for phytochemicals and FTIR analysis of *Myristica dactyloids* fruit extracts. *Int J Pharm Pharm Sci*. 2017. doi:<https://doi.org/10.22159/ijpps.2017v9i1.11053>.
 34. Suhaili SH, Karimian H, Stellato M, Lee T, Aguilar M. Mitochondrial outer membrane permeabilization: a focus on the role of mitochondrial membrane structure organization. *Biophys Rev*. 2017. doi:<https://doi.org/10.1007/s12551-017-0308-0>.
 35. Gunter TE, Pfeiffer DR. Mechanisms by which mitochondria transport calcium. *Am J Physiol*. 1990. doi:<https://doi.org/10.1152/ajpcell.1990.258.5.C755>.
 36. Tait SWG, Green DR. Mitochondrial regulation of cell death. *Cold Spring Harb Perspect Biol*. 2013. doi:<https://doi.org/10.1101/cshperspect.a008706>.
 37. Han H, Long H, Wang H, Wang J, Zhang Y, Wang Z. Progressive apoptotic cell death triggered by a transient oxidative insult in H9c2 rat ventricular cells: A novel pattern of apoptosis and the mechanisms. *Am J Physiol Heart Circ Physiol*. 2004. doi:<https://doi.org/10.1152/ajpheart.00199.2003>.
 38. Khan N, Adhami VM, Mukhta H. Apoptosis by dietary agents for prevention and treatment of prostate cancer. *Endocr Relat Cancer*. 2010. doi:<https://doi.org/10.1677/ERC-09-0262>.
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