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Bioassay-guided Fractionation of *Carica papaya* Seed Extracts against Potassium Bromate-induced Nephrotoxicity Detected Fatty Acid-rich Compounds and Prevents Oxidative Stress in Rat's Kidney

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Authors' contributions

This work was carried out in collaboration among all authors. Author MAK designed the study, performed the statistical analyses, wrote the protocol and wrote the first draft of the manuscript. Authors AMW and AIA managed the analyses of the study. Authors AI, IUM and AM managed the literature searches. All the authors read and approved the final manuscript.

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ABSTRACT

Aim: To evaluate the effect and identify the bioactive constituents of *Carica papaya* seed with potency against potassium bromate –induced nephrotoxicity and oxidative stress in renal tissue of rat.
Study Design: For each state of polarity, twenty male Wistar rats were divided into four groups, five rats per group; normal control, $KBrO_3$ control, *papaya* fraction control and $KBrO_3$ group administered with required concentration of extract of *C. papaya* seed for 48 hours.
Place and Duration of Study: Department of Biochemistry Laboratory, Faculty of Basic Medical Sciences, Bayero University Kano, Nigeria.

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Methodology: A bioassay-guided screening of powdered *C. papaya* seed and its fractions was carried out against $KBrO_3$ –induced nephrotoxicity and oxidative stress. The tests carried out include serum urea, creatinine, uric acid and electrolytes. Also the following markers of oxidative stress were assayed in renal homogenates; superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH) and malondialdehyde (MDA). Spectroscopic analysis of the most active fraction was also carried out.
Results: Fractionation of *C. papaya* seed yielded fractions and sub-fractions that prevent $KBrO_3$ – induced increases in serum urea, creatinine, uric acid and electrolytes as well as the level of MDA. Furthermore there were increases in activities of SOD, CAT, GPx and level of GSH. F1 was the most active fraction. Spectroscopic analysis of F1 identified six functional groups and ten compounds. Seven of the compounds have been previously reported to possess antioxidant activities: 9-octadecenoic acid (z)- 2- hydroxyl-1- (hydroxymethyl) ethyl ester, 17-octadecynoic acid, Hexadecanoic acid methyl ester, 1,2-benzenedicarboxylic acid butyl 2-ethylhexyl ester, 9,12-octadecadienoic acid (z,z) methyl ester, 10-octadecenoic acid methyl ester and 9,17-octadecadienal (z).
Conclusion: Fractions of *C. papaya* seed contain bioactive compounds that could prevent $KBrO_3$ – induced nephrotoxicity and oxidative stress in rats however isolation and administration of each compound was recommended for a more convincing result.

Keywords: Bioassay- guided fractionation; Carica papaya seed; nephrotoxicity; oxidative stress.

1. INTRODUCTION

The use of herbal preparation for medicinal purposes appears to be gaining international attention by the day, particularly in developing countries including Nigeria. The world Health Organization (WHO) has recognized herbal medicine as an alternative treatment to several diseases [1]. Plant contain several secondary metabolites which when harnessed can prevent or cure diseases, or promote general wellbeing [2]. It is estimated that natural products and their derivatives contributes over 50% of all drugs in clinical use and that the pharmaceutical industry is mainly reliant on the diversity of secondary metabolites in medicinal plants for discovery of new drugs [3,4]. The scientific procedures for harnessing medicinal plant requires phytochemical screening of plant extracts, isolation and identification of active principles, evidence of non-toxicity and the study of its mechanism of action [5].

Potassium bromate, a white crystalline powder used as food additive in bread to improve flour and condition dough and also used in cosmetic as a component of hair weaving solutions has been reported to cause multiple organ toxicity with the kidney being the most affected organ [6]. Nephrotoxic single oral doses of $KBrO_3$ can lead to increased serum urea and creatinine and can induce oxidative stress in the kidney 48 hours after administration, leading to impaired glomerular filtration and tubular cell toxicity [7-9].

Although the actual mechanism by which $KBrO_3$ induces nephrotoxicity has not been elucidated, previous workers have reported that increased production of reactive oxygen species and oxidative stress are strongly suspected for the toxic renal effect of the substance [6,7]. However data on the preventive effect of medicinal plants on $KBrO_3$ -induced oxidative stress and nephrotoxicity are quite few despite the advances made by herbal medicine and such data appears uncoordinated. *Carica papaya* seed, a medicinal plant material with several therapeutic applications is known to possess phytochemicals with potent effect against oxidative stress and nephrotoxicity caused by $KBrO_3$ [10,11]. We have also demonstrated the relative safety of this plant material even at high dose of 5000mg/ kg body weight [12]. The present study goes further to identify the functional groups and bioactive constituents in methanol extract of *C. papaya* seed with potency against nephrotoxicity and oxidative stress.

2. MATERIALS AND METHODS

2.1 Chemicals and Assay Kits

Potassium bromate, Dichromate solution, hydrogen peroxide, reduced glutathione, sodium azide, Epinephrine, tris (hydroxymethyl) aminomethane (Tris), [2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid], HEPES, Tricholoacetic acid (TCA), hydrogen peroxide, H_2O_2, Thiobarbituric Acid (TBA) were supplied by Labtech Chemicals Lagos, Nigeria. The assay kits for GGT, ALP, maltase and LAP were

obtained from Spectrum diagnostics Germany, Dialab Production Neudorf Austria, Elabscience biotechnology USA and Bioway Nanjing China respectively. All other chemicals used meet the requirements of the American Chemical Society Committee on Analytical reagents.

2.2 Plant Sample and Preparation

Twenty five matured *C. papaya* was bought from Na'ibawa market Kano, Nigeria and identified by the department of Plant Biology, Bayero University Kano, Nigeria with an accession number, BUKHAN 0012. Each of the plant samples was cut into two to remove the seeds which was washed with tap water, shade-dried and ground into fine powder using an electric blender.

2.3 Preparation of Extracts

The dried powdered *C. papaya* seed (312.5 g) was soaked in 900 ml of hexane, chloroform, ethyl acetate, methanol and water sequentially for 24 hours and shaken at regular intervals [13]. In each case, the extracts were then sieved first with cheese cloth and then with Whatman filter paper No 1. The filtrate was concentrated to dryness in a water bath preset at 45^{o}C.The procedure was repeated three times for each of the extraction solvents. The weight of each crude extract was measured and is shown on Fig. 1. Methanol seed extract was then fractionated because of its active properties against $KBrO_3$ – induced nephrotoxicity.

2.4 Fractionation Procedures

12g of methanol seed extract of *C. papaya* was partitioned on silica gel by column chromatography using gradients of ethyl acetate (EtOAc) / n-hexane (Hex) and chloroform (CH_2Cl_2) / methanol (MeOH). Twenty stages of polarities were used: 100% EtOAc, 90% EtOAc/ Hex 10%, 80% EtOAc/ Hex 20%, 70% EtOAc/ Hex 30%, 60% EtOAc/ Hex 40%, 50% EtOAc/ Hex 50%, 40% EtOAc/ Hex 60%, 30% EtOAc/ Hex 70%, 20% EtOAc/ Hex 80%, 10% EtOAc/ Hex 90%, Hex 100% and 100% CH_2Cl_2, 90%CH_2Cl_2 / 10% MeOH, 80%CH_2Cl_2 /20% MeOH, 70%CH_2Cl_2 / 30% MeOH, 60%CH_2Cl_2 / 40% MeOH, 50%CH_2Cl_2 / 50% MeOH, 40%CH_2Cl_2 /60% MeOH, 30%CH_2Cl_2 /70% MeOH, 20%CH_2Cl_2 / 80% MeOH, 10%CH_2Cl_2 /90% MeOH, 100% MeOH. A total of 267 aliquots of 50 cm^3 each were collected and later pooled to eight sub-fractions according to their chemical profiles analyzed by thin layer chromatography. All the eight fractions were recovered from the solvents by using a rotary evaporator preset at 45ºC and later stored at 4ºC pending use. Fig. 1 depicts the general scheme of fractionation.

2.4.1 Thin Layer Chromatography (TLC)

TLC was performed to select suitable solvent system for column chromatography and to pool similar fractions after isolation. Pre-coated TLC plates were prepared by drawing the baseline and solvent front on the plate. A thin capillary tube was dipped into the sample solution and spotted onto the baseline. The plate was then put into the developing chamber saturated with non polar and polar solvents at different ratios. The spot developed was visualized under ultra-violet lamp with both short and long wavelengths 254 and 365 nm respectively [14].

2.5 Experimental Animals

Healthy young male Wistar rats were raised for the study until each weighs between 120-150 g. The study was carried out at the animal house unit of the department of Biological Sciences, Bayero University Kano, Nigeria. All animal procedures were performed according to the guide for the care and use of laboratory animals of the National Institute of Health as well as the Animal Welfare Act.

2.6 Experimental Design

At each stage of extract's polarity, the animals were randomly divided into four groups into metal-plastic cages as shown below. Each group contains five rats. Solution of $KBrO_3$ was administered orally as a single dose of 100mg/kg body weight of rats to the test and $KBrO_3$ control groups while animals in the normal control and *papaya* fraction control groups were administered equivalent volume of distilled water and the concerned *C. papaya* seed fraction respectively. All the animals were observed for 48 hours.

Group one, normal control: Given equivalent volume of distilled water.

Group two, $KBrO_3$ control: Given $KBrO_3$, 100 mg/kg bw.

Group three, *papaya* control: Given required volume of *papaya* fraction.

Group four, treatment: Given 100 mg/ kg bw $KBrO_3$ + required volume of *papaya* fraction.

2.6.1 Collection of blood sample

All the animals were sacrificed at the elapse of the 48 hours experimental period and blood samples were collected in lithium heparin tubes and centrifuged at 4000 rpm for 5 minutes to collect the serum which is stored at 4ºC.

2.6.2 Preparation of renal homogenates

After the animal sacrifice, the kidneys of each rat was removed, horizontally cut into two equal parts and kept in ice-cold 154 mM NaCl and 5 mM Tris-HEPES buffer, pH 7.5. The cortex and medulla were carefully separated using a sharp scalpel and homogenized separately in a glass Teflon homogenizer in 2 mM Tris-HCl, 50mM mannitol buffer, pH 7.0, to get a 10% (w/v) homogenate. These homogenates were diluted to 5% with Tris-mannitol buffer followed by high speed homogenization (20,000 rpm) in an Ultra Turrex homogenizer [15]. Brush border membrane vesicle (BBMV) was isolated from renal cortex at the elapse of the experimental period [16]. The renal homogenates and the BBMV were frozen immediately after preparation pending analysis.

2.7 Determination of Biochemical Parameters

2.7.1 Serum urea, creatinine and uric acid

Urea was determined in serum by the diacetyl monoxime method using kit from Randox Laboratories Ltd, UK. Creatinine level was assayed in deproteinized serum based on its reaction with saturated picric acid to give a yellow-red complex using kits from Randox Laboratories Ltd, UK while uric acid level was determined by the measurement of quinoneimine dye complex using kit from Linear Chemicals Barcelona, Spain.

2.7.2 Serum electrolytes

Na^+, K^+, Cl^- and HCO_3^- were all estimated in serum by spectrophotometric measurement using kits from Teco Diagnostics Anaheim, USA. Na^+ determination was based on its reaction with excess uranium and ferrocyanide to produce a chromophore that is measured sphectrophotometrically. K^+ determination was based on the measurement of the turbidity formed when K^+ react with ferric ion to form a complex that is measured spectrophometrically while Cl^- determination was based on the formation of mercuric thiocyanate which then react with ferric ion to form a complex that is measured using spectrophotometer and HCO_3^- determination is based on the reaction catalyzed by phosphoenol pyruvate carboxylase to form oxaloacetate which undergoes further reactions to form a complex that is measured spectrophotometrically.

2.7.3 Antioxidant activity

The following parameters that indicate induction of oxidative stress were assayed in homogenates of cortex and medulla; catalase (CAT) (EC 1.11.1.6), superoxide dismutase (SOD) (EC 1.15.1.1), glutathione peroxidase (GPx) (EC 1.11.1.9), reduced glutathione (GSH) and malondialdehyde (MDA). CAT activity in renal tissues was determined by the quantitation of chromic acetate formed at pH 7.0 [17] while SOD activities were determined by the inhibition of auto oxidation of epinephrine at pH 10.2 [18]. GPx activity was determined by the splitting of H_2O_2 with oxidation of GSH at pH 7.4 [19] while the level of GSH was quantified in deproteinised samples by measurement of 5', 5'-dithiobis-(2-nitrobenzoic acid) (DTNB) [20]. Malondialdehyde was determined by the measurement of thiobarbituric acid reactive substances (TBARS) [21].

2.8 Spectroscopic Identification of Functional Groups and Bioactive Compounds

The identification of the main functional groups in the most active fraction of methanol extract of *C. papaya* seed (F1) was carried out using Fourier Transform Infrared Spectroscopy (FTIR) detection system [22] while the identification of the main bioactive compounds of F1 was carried out using GC-MS detection system. 1µL of the extract was subjected to analysis using Agilent Technologies 6890N GC system coupled with JEOL Mass spectroscopy. The sample was injected into the Agilent J&W HP-5 capillary column (30 m x 0.2 mm x 0.25 µm) fused with silica. The injection temperature was maintained at 220ºC. The oven temperature of GC was programmed with an initial temperature of 50ºC and increased up to 250ºC at the rate of 10ºC per minute. Helium (He) was used as the carrier gas system with the flow rate of 1ml/min. GC-MS interface temperature was maintained at 250^{o}C. Identification of compounds was based on the comparison of the spectral values with the

National Institute of Standards and Technology (NIST) Chemical Web book database. In addition the peak area percentage contributed by each of the compounds detected was calculated [23].

2.9 Statistical Analysis

Results are expressed as mean ± SDM and n =5 for all readings. One-way analysis of variance (ANOVA) was used to analyze data and a difference of ($P<0.05$) was considered significant.

3. RESULTS

3.1 *In vivo* Nephroprotective and Antioxidant Activity of Extracts

The results for the biochemical tests carried out on serum and renal homogenates of rats for the most active fraction (F1) alone are highlighted below;

3.1.1 Serum urea creatinine and uric acid

Administration of $KBrO_3$ resulted in significant increases ($P<0.05$) in serum levels of urea, creatinine and uric acid as compared with normal control however co-administration of the most active fraction of partially purified methanol extract of *C. papaya* seed decreased these changes in rats receiving the dual therapy. There was no significant change ($P>0.05$) in the serum levels of these kidney function parameters in animals that were administered the most active fraction of partially purified methanol extract of *C. papaya* seed only.

3.1.2 Serum electrolytes

There was significant increases ($P<0.05$) in the serum levels of Na^+, K^+, Cl^- and HCO_3^- when $KBrO_3$ was administered to rats as compared with normal control however when $KBrO_3$ was concurrently administered with the most active fraction of partially purified methanol extract of *C. papaya* seed it resulted in decreases in these electrolytes towards normal control values. In rats administered with only F1, no significant ($P>0.05$) change was observed.

3.1.3 Antioxidant activity in homogenates of renal cortex and medulla

$KBrO_3$ induced a considerable decreases ($P<0.05$) in the activities of antioxidant enzymes studied namely CAT, SOD, GPx and level of GSH and significantly increases ($P<0.05$) MDA concentration in the homogenates of renal cortex and medulla of rats. The severity of $KBrO_3$ toxicity was more in cortex than medulla. However co-administration of most active fraction of *C. papaya* seed extract significantly ($P<0.05$) prevented these effects. Administration of F1 alone did not significantly ($P>0.05$) affect any of these markers of oxidative stress.

Table 1. Effect of concurrent administration of most active fraction of partially purified methanol extract of *Carica papaya* seed and potassium bromate on serum urea, creatinine and uric acid of rats

	Urea (mMol/L)	Creatinine (mg/dl)	Uric acid (mg/dl)
Normal control	8.44 ±0.56	3.80 ±0.57	5.49 ±0.21
$KBrO_3$control	14.82 ±0.53[a]	7.07 ±0.25[a]	6.63 ±0.30[a]
Papaya control	8.52 ±0.33	3.59 ±0.33	5.49 ±0.19
F1 + $KBrO_3$	9.87 ±0.53[b]	4.01 ±0.64[b]	5.39 ±0.03[b]

n= mean of five sample ± SDM

[a] is significant (P<0.05) from normal control, [b] is significant (P<0.05) from $KBrO_3$ control

Table 2. Effect of concurrent administration of most active fraction of partially purified methanol extract of *Carica papaya* seed and potassium bromate on serum electrolytes

	Na^+ (mMol/L)	K^+ (mMol/L)	Cl^- (mMol/L)	HCO_3^- (mMol/L)
Normal control	139.86 ±2.01	8.97 ±0.30	103.83 ±5.02	5.45 ±0.56
$KBrO_3$control	144.76 ±2.09[a]	24.89 ±0.44[a]	143.60 ±5.11[a]	23.69 ±1.68[a]
F1 control	138 .48 ±2.34	9.19 ±0.52	103.46 ± 5.77	5.15 ±0.54
F1 + $KBrO_3$	139.87 ±1.07[b]	9.35 ±1.26[b]	103.40 ±4.32[b]	6.11 ±0.61[b]

n= mean of five sample ± SDM

[a] is significant (P<0.05) from normal control, [b] is significant (P<0.05) from $KBrO_3$ control

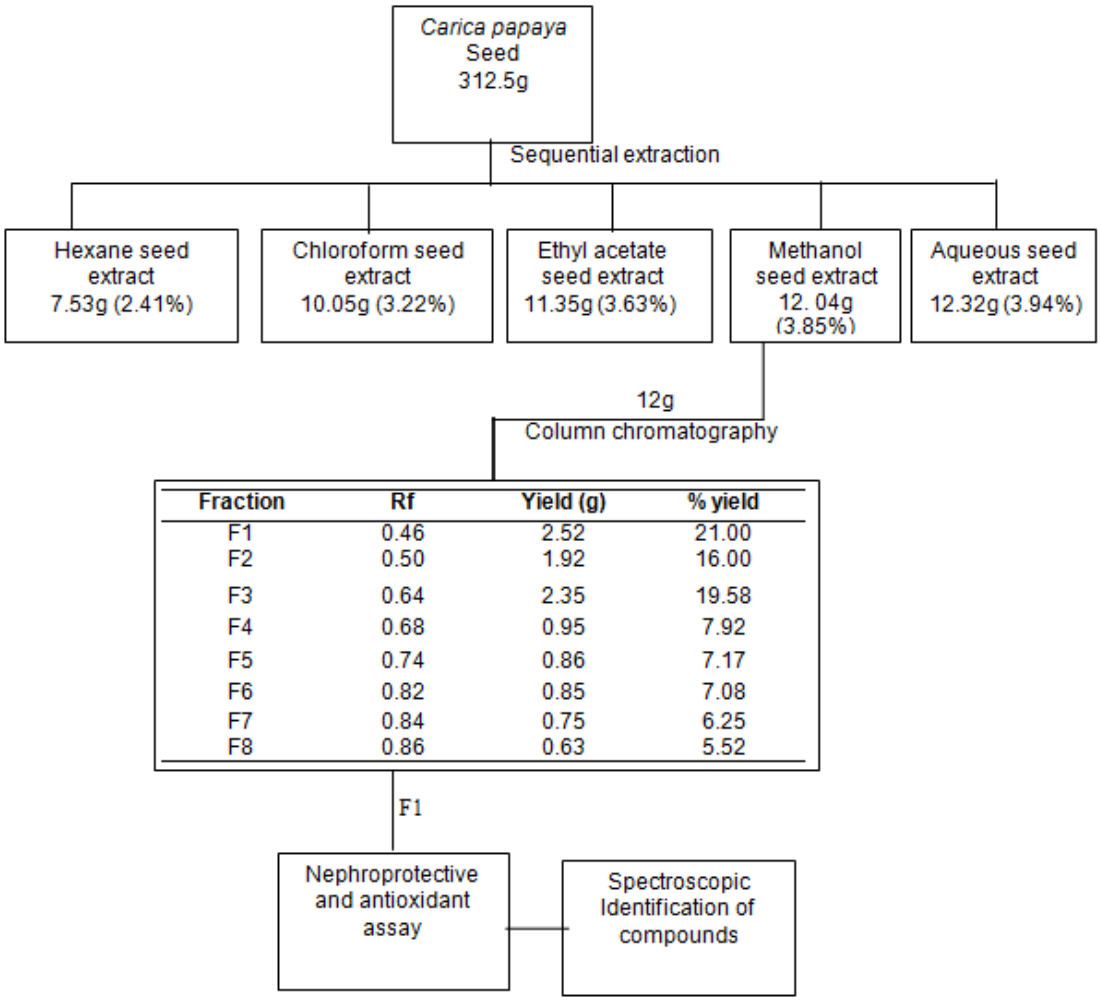

Fig. 1. Fractionation process of powdered *Carica papaya* seed

Table 3. Effect of concurrent administration of the most active fraction of partially purified methanol extract of *Carica papaya* seed and potassium bromate on some parameters of oxidative stress in homogenates of renal cortex and medulla of rats

	CAT	SOD	GPx	GSH	MDA
Normal control					
Cortex	71.76±2.48	21.16±1.70	49.49±1.11	3.16±0.57	15.41±1.00
Medulla	42.67±1.83	12.84±0.41	18.27±0.92	1.36±0.53	8.18±0.63
***Papaya* control**					
Cortex	72.16±1.24	20.64±0.28	49.74±1.24	3.40±0.48	15.47±1.46
Medulla	43.56±1.21	12.74±0.61	19.63±0.94	1.42±0.32	8.74±1.07
$KBrO_3$ control					
Cortex	44.92±1.46[a]	13.58±0.56[a]	24.89±1.41[a]	0.54±0.19[a]	32.70±0.84[a]
Medulla	22.86±1.13[a]	7.77±0.69[a]	12.45±1.34[a]	0.21±0.02[a]	23.39±1.11[a]
F 1+ $KBrO_3$					
Cortex	71.52±1.62	18.39±1.02[b]	42.23±1.65[b]	2.14±0.39	19.17±1.24[b]
Medulla	41.17±2.43	9.30±1.13[b]	16.36±1.07	1.12±0.12[b]	7.91±0.42[b]

n = mean + SD for five different preparation;
CAT = Catalase; SOD= Superoxide dismutase; GPx= glutathione peroxidise
Activities of CAT and GPx are in units/mg protein, SOD activity is in units/mg protein/min, MDA concentration is in units/mg protein, GSH concentration is in µmol/min tissue
[a] is significant (P<0.05) from normal control, [b] is significant from $KBrO_3$ control

3.2 Identification of Functional Groups and Bioactive Compounds

3.2.1 Infrared spectroscopic analysis

The functional groups were identified by the absorption frequency of the infrared waves in wave number in cm^{-1}. The infrared (IR) shows the presence of six major functional groups and the absorption frequency of each of the functional groups vary from one to another. Fig. 2 shows the IR chromatogram of F1 while the identified functional groups are shown on Table 4.

3.2.2 Gas chromatography- mass spectroscopic analysis

Analysis of F1 by GC-MS resulted in the detection of ten different compounds of which five are esters, two carboxylic acids, two aldehydes and an alkane. The major phytocomponent present in terms of relative abundance is 9-octadecenoic acid (z)- 2-hydroxyl-1- (hydroxymethyl) ethyl ester with area percentage of 95.87% whereas the remaining nine compounds existed in minute quantities. The chromatogram is shown on Fig. 3 while the list of the identified compounds with other important properties is shown on Table 5.

4. DISCUSSION

Previous literature has reported the preventive effect of crude *C. papaya* seed extract against $KBrO_3$ –induced nephrotoxicity and oxidative stress in renal tissues of rats [11] and this has justified the folkloric use of the plant in traditional practice for ameliorating poison- related renal disorders. In the present study, bioassay of extracts from different fractions of *C. papaya* seed obtained by use of different solvent of varying polarities has pointed attention towards the potent phytochemicals that could be responsible for the bioactivity of *C. papaya* seed against $KBrO_3$ –induced nephrotoxicity and oxidative stress in rat.

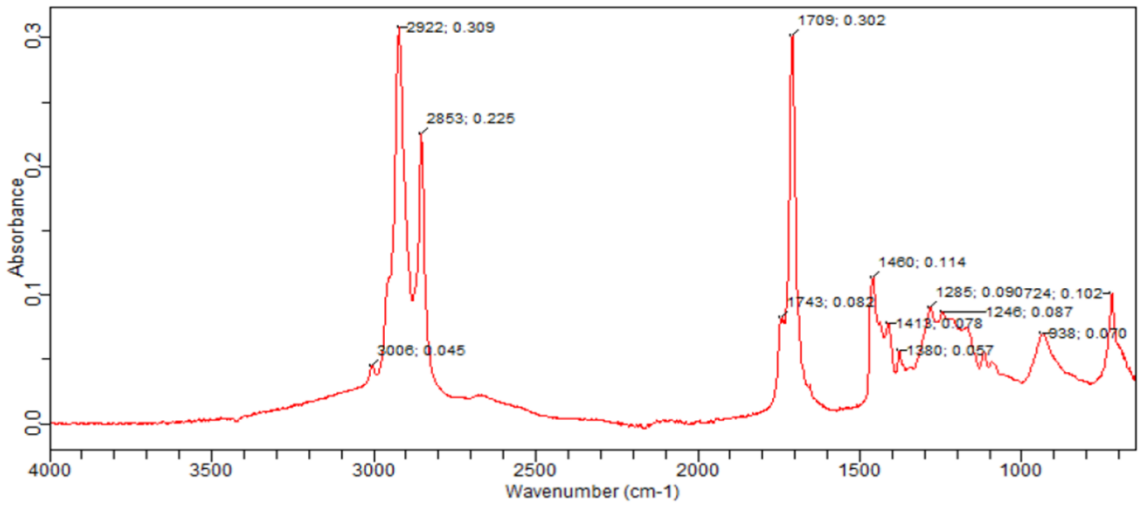

Fig. 2. Infrared chromatogram of most active fraction of methanol extract of *Carica papaya* seed

Table 4. Functional groups from FTIR spectra of the most active fraction of methanol extract of *Carica papaya* seed

Frequency (cm^{-1})	Functional group	Name of functional group
2922	C=C	Alkene (stretch)
2853	$-CH_2$ or RCHO	Alkane or aldehyde
3006	C=C	Alkene
1709	COOH or RC(=O)R’	Carboxylic acid or ketone
1743	RCOOR’	Ester
1413	$-CH_3$	Methyl (bend)

Table 5. GC-MS identified compounds in the most active fraction of methanol extract of *Carica papaya* seed and some of their properties

Peak no.	RT (minutes)	Area %	Compound	Molecular formula	Molecular weight (g/mol)	Reported antioxidant and/ or renal protective activity
1	47.270	0.11	17-octadecynoic acid	$C_{18}H_{32}O_2$	280.452	Oyekan (2005) [24]
2	50.222	0.44	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.457	Tyagi and Agarwal (2017) [25]
3	51.614	0.10	1,2-benzenedicarboxylic acid butyl 2-ethylhexyl ester	$C_{20}H_{30}O_4$	334.456	Adeyemi *et al*. (2017) [26]
4	55.278	0.26	7,11-hexadecadienal	$C_{16}H_{28}O$	236.399	No activity reported
5	55.791	0.12	9,12-octadecadienoic acid (z,z) methyl ester	$C_{18}H_{34}O_2$	280.452	Osman *et al*. (2014) [27]
6	56.011	1.50	10-octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.495	Ezekwe and chikezie (2017) [28]
7	56.817	0.16	Methyl stearate	$C_{19}H_{38}O_2$	298.511	No activity reported
8	62.019	0.83	Cycloeicosane	$C_{20}H_{40}$	280.540	No activity reported
9	67.074	0.31	9,17-octadecadienal, (z)	$C_{18}H_{32}O$	264.453	Sotiroudis *et al*. (2010) [29]
10	87.736	95.87	9-octadecenoic acid (z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{21}H_{40}O_4$	356.547	Okokon *et al*. (2017) [30]

RT= retention time

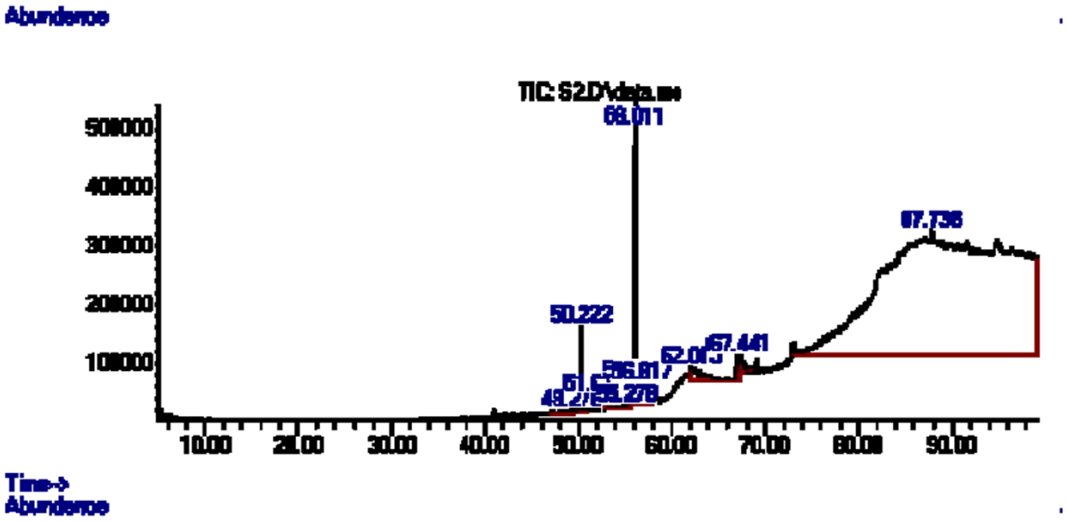

Fig. 3. Gas chromatogram of most active fraction of methanol extract of *Carica papaya* seed

In the fractionation processes of *C. papaya* seed extracts, the preventive effect of each fraction against $KBrO_3$ –induced nephrotoxicity and oxidative stress in renal tissues give a strong proof of the potency of this plant material. The methanol extract of *C. papaya* seed was selected because of its strong preventive effect against $KBrO_3$ –induced insults on renal tissues and when fractionated, it yielded eight fractions, each with reduced preventive activities with fraction F1 being the most active fraction. This suggests that there could be other phytochemicals or factors which could have acted in synergy to influence the activity of the crude extract. Previous literature has stated that likely interaction between compounds can improve its solubility and enhance its bioavailability [31]. Synergistic cooperation has been reported to be beneficial since it can influence the activity of compounds against drugs and other xenobiotics [32]. However, notwithstanding the influence of synergy among phytochemicals and its beneficial effect on bioavailability, isolation of active constituents of plant material is required to guide its characterization and study of its mechanism of action which is essential for standardization of phytomedicine [5].

Infrared spectroscopy showed that alkane, alkene, aldehyde, carboxylic acid, methyl and ester are the functional groups with likely roles in interaction of *C. papaya* seed extracts with $KBrO_3$ or the toxic intermediates generated by its metabolism in the prevention of nephrotoxicity. Interestingly, some of these functional groups namely carboxylic acid, alkane and methyl are also found in L-methionine, a conventional drug that is use in clinical practice to ameliorate acetaminophen- induced hepatic and renal injuries [33]. Thus it could be suggested that *C. papaya* seed extracts could have employed similar mechanism as L-methionine in preventing $KBrO_3$ –induced nephrotoxicity and oxidative stress in renal tissues of rats.

Identification of compounds with previous report of antioxidant activities from fraction F1 has strongly highlighted the phytochemicals that could be responsible for the bioactivity of *C. papaya* seed against $KBrO_3$ –induced renal action. 9-octadecenoic acid (z)-2- hydroxyl-1-(hydroxylmethyl) ethyl ester, the major compound among the identified phytochemicals in terms of relative percentage with 95.87% or its derivatives has been previously identified from fraction of husk extract of *Zea mays.* The workers reported that this phytochemical could possess antioxidant activity after it was found to significantly ($P<0.05$) increase the activities of SOD, CAT, GPx and GSH level and decreases the level of MDA in the kidney of alloxan-induced diabetic rats [30].

Furthermore, 17-octadecynoic acid has been strongly suspected to possess a positive effect on intra-renal blood flow in rats [24] while hexadecanoic acid methyl ester and 1,2-benzenedicarboxylic acid butyl 2-ethylhexyl ester that were previously identified from ethanol leaf extract of *Pistia stratiotes L. and Lagenaria breviflora* R. fruit respectively were reported to possess antioxidant activities among other

therapeutic significance [25,26]. 9,12-octadecadienoic acid (z,z) methyl ester was previously isolated from *Caesalpinia gilleisii* flower. The researchers stated that this phytochemical possess antioxidant activity and could prevent CCl_4 –induced increases in alanine amino transferase (ALT), aspartate aminotransferase (AST) and GSH in hepatic tissues of rats [27], 10-octadecenoic acid methyl ester was previously identified from *C. papaya* aqueous root extract where it was strongly suspected to be responsible for the reversal of the increases in serum levels of urea and creatinine, and ALP, AST and ALT in renal and hepatic tissues of diabetic rats respectively [28], 9,17-octadecadienal (z) was previously identified from *Cucumis sativus.* The investigators reported that this compound exhibited *In vitro* antioxidant activity and therefore could be useful for *In vivo* application [29].

5. CONCLUSION

This research has described a guided process for identifying compounds from *C. papaya* seed extract with bioactivity against $KBrO_3$ - induced nephrotoxicity and oxidative stress. A group of compounds which have been reported previously to possess antioxidant activities were among the compounds identified. Therefore, isolation and characterization of these compounds could identify a source of new nephroprotectant and antioxidant against potassium bromate renal action. The most active fraction F1 substantially prevented $KBrO_3$ –induced oxidative stress in kidney tissues. It was therefore hypothesized that the active components in F1 could have acted either individually or in synergy with one another to prevent $KBrO_3$- induced nephrotoxicity and oxidative stress in kidney of rat.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The ethical approval is presently being considered by the ethical committee of the College of Health Sciences, Bayero University, Kano, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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