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Evaluation of the hepato-renal functions and antimicrobial activity of fatty amido benzoic acid synthesised from *Citrullus colocynthis* seed oil

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Abstract

Rapid progression in resistance to antimicrobial agents by pathogenic organisms is a serious concern. This study aimed to synthesize fatty amido benzoic acid (FBA) from *Citrullus colocynthis* seed oil (CCO) and evaluate its safety profile as an alternative bioactive agent for combating drug-resistant pathogens. FBA was synthesised through simple chemical reaction route and examined for its antioxidant activity and antimicrobial capacity against selected drug-resistant microorganisms. Effect of FBA on hepato-renal function makers and oxidative stress was also examined using Wistar rats. Density functional theory (DFT) approach was employed to understand the action of FBA with the aid of lowest unoccupied molecular orbital (LUMO) and highest occupied molecular orbital (HOMO). Gas Chromatography (GC) revealed the most abundant fatty acid in CCO to be C18:2 (55.88%). Results from Fourier transformed infrared spectroscopy (FTIR), and proton nuclear magnetic resonance (^{1}HNMR) confirmed the synthesis of FBA with a yield of 97.10%. FBA exhibited antioxidant potential (IC_{50} of 1.96 µg mL^{-1}) as well as antimicrobial potency. Minimum inhibitory concentration (MIC) of FBA was 0.026 mg mL^{-1}. Biochemical parameters such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, hydrogen peroxide, and lipid peroxidation were significantly elevated in rats administered high dose FBA (100 mg kg^{-1}). Histology of the liver and kidney confirmed the biochemical results. Furthermore, mechanism of action of FBA could be described by quantum chemical analysis to be via nucleophilic interaction, which may be viewed electronically as donor–acceptor interaction. The study presents FBA as a promising antimicrobial agent for combating drug-resistant pathogenic organisms.

Keywords: Antimicrobial activity, Antioxidant, *Citrullus colocynthis*, Drug-resistant pathogens, Fatty acid

Introduction

Resistance from pathogenic organisms is a big concern, as most known drugs are losing efficacy against disease control and management [1]. The risk of abandoning previously efficient drugs is becoming alarming due to side effects or recurrence of the disease. It has become evident that bacteria possess the genetic ability to acquire and transmit resistance to drugs. This ability to transmit resistance is a major concern in most nations of the world. Inappropriate handling of antibiotics in the treatment of animal disease and in agricultural practice has contributed to the widespread of drug-resistant pathogenic organisms. Apart from the loss of drug efficacy, a new dimension to this problem is the re-emergence of chronic diseases, which are difficult to tackle. Overtime, pathogenic organisms are developing resistance against most known drugs used in disease control. This

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has necessitated the need to develop new drugs that can serve as a replacement for these drugs.

Most known drugs in the market were produced from synthetic chemicals. Some of which have their negative effect when they get into the environment such as soil and water. Apart from the drug-resistant problem, these synthetic drugs are becoming expensive due to the cost of production of fine chemicals required for their development. Previous studies have shown that the use of natural products or naturally sourced feedstocks in the control and management of diseases caused by pathogenic organisms have merits over drugs derived from synthetic sources [2]. A good example of such naturally sourced feedstock is the use of plant seed oil as starting material for the production of bioactive agents that can serve as an alternative for combating drug-resistant pathogenic organisms.

Plant seed oils are rich in fatty acids, which are present in varying amounts. Due to their composition and related properties, they are potential resources in the development of pharmaceutical agents [3, 4]. Moreover, they are from a renewable source, cheap, non-toxic, and environmentally friendly, which makes them better when compared with the ones sourced from synthetic chemicals. They have been reported to exhibit bioactive properties [4–6]. However, the exhibited bioactive properties can be improved by simple chemical modification, which places them at an advantage of probably having functions or properties, which are superior to those of already known pharmaceutical agents in the market. Seed oils are triglyceride, and several forms of chemical modification may be achieved by taking into account the ester bond and the possible unsaturated bonds present in the carbon chain length. Previous studies have reported various types of modification, which included; pyrolysis, amidation, transesterification, epoxidation, acetylation, hydrogenation, sulphonation, alkoxylation, and oxidation [7–9]. However, chemical modification of seed oil is a sustainable and promising route to developing an alternative to combating drug-resistant pathogenic microorganisms. Interestingly, this study aims to modify oil from the seed of *Citrullus colocynthis* as an alternative means of combating drug-resistant pathogens. This work proposes the modification of CCO via amidation using 4-amino benzoic acid in the presence of sodium methoxide as a catalyst.

Citrullus colocynthis belongs to the Cucurbitaceae plant family. It is a good source of oil, which is underutilized. Evaluating the potentials of using CCO as an alternative for treating disease-causing pathogens will be a viable way of finding useful applications for CCO. Despite the potential of natural resources being able to combat drug-resistant pathogens, it is important to evaluate the safety profile of these natural resources. Moreover, their capacity to combat disease-causing pathogens does not mean that they are not toxic to the human biological system. This has necessitated the need to screen the proposed new product (FBA) for its safety profile. The goal of the study is to synthesis FBA as an antimicrobial agent from CCO and also check whether it is toxic by evaluating its effect on rat organs. Therefore, the present study is focused on the synthesis and safety profile of FBA from CCO as an alternative bioactive agent for combating drug-resistant pathogens.

Materials and methods

Materials

Citrullus colocynthis seeds were purchased from Ede market, Osun state, Nigeria. They were ground into powder. Oil was extracted from the ground seed in a soxhlet extractor for 10 h using n-hexane [10]. Ethylacetate, n-hexane, sodium sulphate, potassium hydroxide, sulphuric acid, methanol, potassium chloride, 2,2-diphenyl-1-picrylhydrazyl, 1-chloro-2, 4-di nitrobenzene (CDNB), sodium chloride, thiobarbituric acid (TBA), epinephrine, 5′,5′- Dithiobis-2-nitrobenzoic acid (DTNB), and dimethylsulphoxide (DMSO) were purchased from Sigma Chemical Company (USA). All other chemicals used were of analytical grade and were obtained from Sigma-Aldrich, St. Louis, USA.

Fatty acid composition

CCO was analyzed for its fatty acid composition, as previously described [11]. Briefly, the oil was transesterified using 2% sulphuric acid in methanol reagent. The methyl esters obtained were extracted with ethylacetate, washed with distilled water until free of acid and dried over anhydrous sodium sulphate. Ethylacetate was removed on a rotary evaporator and finally analyzed using GC. For the GC analysis, an Agilent 6850 series GC equipped with an FID detector was used. To achieve this, a DB-225 capillary column was used; a column temperature of 160 °C was held for 2 min; this was gradually increased to 230 °C at 4 °C min^{-1} and finally maintained at 230 °C for 10 min. The detector (250 °C) and injector (230 °C) temperatures were also regulated at a split ratio of 50:1 using nitrogen as the carrier gas at a flow rate of 1.5 mL min^{-1}.

Synthesis of FBA

CCO was first transesterified using 1% KOH in methanol, which was refluxed for 3 h at 70 °C. The mixture of methyl esters obtained was extracted with ethylacetate, washed with distilled water until free of KOH, and dried over sodium sulphate. Ethylacetate was removed using a rotary evaporator. The methyl esters (0.05 mol) were reacted with 4-amino benzoic acid (0.08 mol) in

the presence of sodium methoxide (0.5% wt/wt) in a two-necked round bottom flask, which was refluxed for 3 h at 70 °C. At the end of the reaction, the mixture obtained was extracted with ethylacetate, washed with distilled water until neutral to litmus paper, and passed over sodium sulphate. The ethylacetate was removed on a rotary evaporator, which gave rise to a product yield of 97.10%. The scheme for the synthesis is shown in Fig. 1.

Characterization of CCO and FBA

CCO and FBA were evaluated for their functional groups' composition using FTIR (FTIR, Perkin Elmer, spectrum RXI 83,303, MA, USA) in the range of 400–4500 cm^{-1} while the ^{1}HNMR analysis was achieved on a 400 MHz Bruker NMR spectrophotometer in chloroform containing TMS as an internal standard.

Antioxidant activity assay

FBA was evaluated for its antioxidant capacity by determining its DPPH free radical scavenging ability spectrophotometrically as previously reported [12]. Briefly, DPPH stock solution (12.5 mg in 50 mL of methanol) was prepared and kept in the dark cupboard. Concentrations of FBA ranging from 50–100 µg mL^{-1} were prepared using methanol as a solvent in a volumetric flask. To each of these concentrates DPPH (1 mL) was added. They were incubated for 30 min at 37 °C in the dark, and the absorbance read at 517 nm. The standard (Vit C) and blank were subjected to the same conditions, and absorbance read at 517 nm. The experiment was conducted in triplicate. Antioxidant capacity of FBA was calculated as:

$$Q(\%) = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100 \quad (1)$$

Antimicrobial screening

Methicilin Resistant *Staphylococcus aureus* (MRSA) ATCC 71, *Staphylococcus aureus* ATCC 25923, *E coli* DMS 10974 and *Pseudomonas aeruginosa* ATCC 9027 were obtained from the Department of Biological

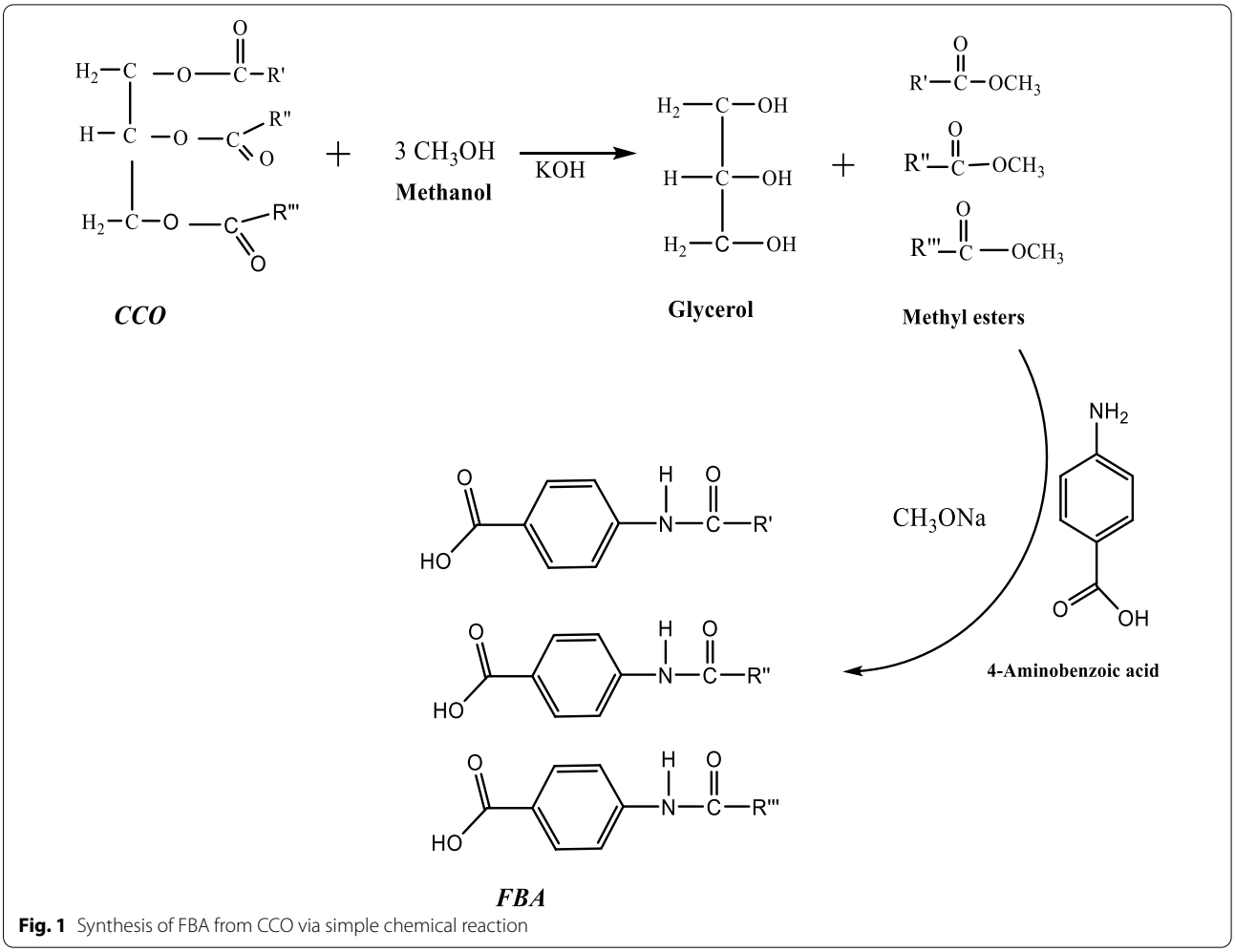

Fig. 1 Synthesis of FBA from CCO via simple chemical reaction

Sciences, Redeemer's University. They were used in the antimicrobial susceptibility-testing assay in Mueller Hinton agar. Prior to the antimicrobial susceptibility assays, the test organisms were earlier cultured on nutrient agar and incubated at 37 °C for 24 h. Cell density on their growth in peptone water was monitored in order to standardize them with the aid of a spectrophotometer at an optical density of 0.5 at 600 nm [13]. Wells (6 mm in diameter) were bored in Mueller–Hinton agar plates, filled with FBA (50 µL) at a concentration of 2.6×10^{-3} mg L^{-1}, inoculated with bacterial strains under aseptic condition and incubated at 37 °C for 24 h. Water (solvent for FBA) was the blank. The zone of inhibition was measured after the incubation.

Determination of MIC

The MIC value of FBA was established as the lowest concentration that completely inhibited the growth of the test microorganisms after 48 h of incubation at 37 °C. Microbes were transferred into the sterile nutrient broth, and with the help of hemocytometer; standard cells were adjusted to 1×10^{-6} cell mL^{-1}. The standardized culture was pipetted (1 mL) and poured unto readily prepared plate of Mueller–Hinton agar. Wells (6 mm diameter) were bored and filled with FBA (50 µL) of aqueous concentrations (ranging from2×10^{-7} to 2×10^{3} µg mL^{-1}).

Quantum chemical parameters

FBA was subjected to electronic structure modeling. All theoretical calculations were performed using the DFT electronic structure programs at B3LYP/6-31G level theory using Spartan 14.1 software [14]. This also included the distribution of frontier molecular orbitals in order to establish the reactivity and active site of FBA for the antioxidant, antimicrobial, and toxicity processes.

Animals and treatment

Healthy male rats were used for the experiments. Wistar rats weighing 150–200 g were used. The rats were randomly separated into 4 groups of 5 rats each, based on uniform average weight per group. They were housed on the following conditions: 12 h day/night cycle, temperature 25 ± 2 °C, relative humidity (70–80%), and standard pelleted diet (Ladokun Feeds, Ibadan, Nigeria). All animals received humane care following guidelines governing the handling of laboratory animals as outlined by the Redeemer's University Committee on Ethics for Scientific Research.

Animals were treated as follows for 14 days:

- Group A: Control animal fed with 0.9% saline
- Group B: Fed with FBA (10 mg kg^{-1} body weight), orally, once, daily)
- Group C: Fed with FBA (50 mg kg^{-1} body weight), orally, once, daily)
- Group D: Fed with FBA (100 mg kg^{-1} body weight), orally, once, daily)

The different doses administered to the groups represent low, medium, and high doses as previously reported [15]. As the experiment proceeded, the animals were consistently examined on a daily basis for any clinical signs and symptoms of toxicity. After the 14th day, the Wistar rats were sacrificed by cervical dislocation 24 h after the last treatment. Blood samples were collected by a cardiac puncture; the blood samples were kept in clean EDTA bottles and centrifuged at 4000 g for 10 min (Heraeus Labofuge 300, Thermo Scientific, Hampshire UK). Plasma was carefully separated and stored frozen at −20 °C until they were required for analysis. The liver and kidneys were carefully harvested and washed free of any extraneous materials. They were transferred into ice-cold 0.25 M sucrose solution, blotted with clean tissue paper and homogenized in phosphate buffer (0.1 M, pH 7.4). Homogenates were centrifuged at 10,000 g for 20 min at 4 °C to obtain the post mitochondrial fraction. Subsequently, the supernatant was collected and stored frozen at −20 °C until they were required for analysis. Biochemical indices were measured in samples using a spectrophotometer (Bibby Scientific Jenway 7305 UV, UK).

Estimation of somatic index

Since exposure to chemicals may result in bioaccumulation in the body tissues, the exposure of Wistar rats to FBA was expressed as renatosomatic index (RSI) and hepatosomatic index (HSI). The liver and kidney harvested from both test and control animals were blot-dried using an absorbent filter paper. The organosomatic index (OSI) was calculated as previously described [16]:

$$OSI = \frac{Weight\ of\ organ(g)}{Body\ weight(g)} X100 \quad (2)$$

Biochemical assays

Biochemical markers were determined by checking plasma concentrations of alanine aminotransferase, aspartate aminotransferase, albumin, total bilirubin, urea, creatinine, potassium, and sodium using diagnostic kits as reported in a previous study [17]. Catalase (CAT) was determined as described by Luke [18] while hydrogen peroxide and malondialdehyde (MDA) levels were determined based on ferrous oxidation with xylenol orange and measurement of thiobarbituric acid reactive

substances, respectively, in hepatic and renal tissues [19, 20].

Histopathology

The kidney and liver tissues of the animals were fixed in 10% formaldehyde, dehydrated in graded alcohol, and embedded in paraffin. The tissues were subsequently cut into 4–5 mm sections by a microtome, fixed on the slides, and stained with hematoxylin and eosin for light microscopic analyses.

Statistical analyses

All data were expressed as mean ± standard error of mean. Differences between the groups were determined by one-way analysis of variance (ANOVA), and post hoc testing was performed using Dunnet's multiple comparison tests (Graph Pad Prism software, Inc., San Diego, CA). Values were regarded as significantly different at $p < 0.05$.

Results

Synthesis and characterization

A golden yellow oil with a percentage yield of 54.51 was obtained, and the fatty acid composition is presented in Table 1. The most abundant fatty acid in the oil is C18:2 (55.88%), while the least is C14:0 (0.07%). The results of the fatty acid revealed the presence of long-chain fatty acids (C20–C24) in small amount in the oil. Other physicochemical characterization of CCO may be achieved by classical method of analysis. The unsaturation of the oil was found to be 78.28%, while the saturation level was 21.72%. The FTIR results of the CCO and FBA are presented in Fig. 2. The spectra shows bands corresponding to the different functional groups in CCO and FBA. The FTIR spectra of CCO and FBA revealed bands at 3010 cm^{-1}, which were attributed to the unsaturation groups (−C=C−) present in both CCO and FBA. The bands at 2930 and 2855 cm^{-1} were assigned to the vibrational frequencies of alkane, which are the methyl ($-CH_3-$) and methylene ($-CH_2-$) groups, respectively. The vibrational frequency for the ester functional group was found at 1742 cm^{-1} in CCO, but this vibrational frequency disappeared in FBA with the appearance of a new band at 1625 cm^{-1}. This new band at 1625 cm^{-1} was assigned to be the vibrational frequency of an amide functional group suggesting the synthesis of FBA. Furthermore, a new band also appeared at 3342 cm^{-1} in FBA, which was not found in CCO. This band also corroborate the synthesis of FBA, and it was considered the vibrational frequency of N–H of amide. The ^{1}HNMR spectra are presented in Fig. 3. The spectra confirmed the synthesis of FBA. It reveals presence of methyl, methylene, and vinylic protons at 0.5–0.8, 1–1.5, and 2–2.4 ppm, respectively, in both CCO and FBA. The solvent peak appeared at 7.2 ppm in both CCO and FBA. The unsaturation band which was seen in the FTIR at 3010 cm^{-1} appeared in the ^{1}HNMR spectra at 5.4 ppm in both CCO and FBA, which confirms the presence of the unsaturated functional groups and also indicates that the double bonds were intact even during and after the reaction of CCO to produce FBA. The triglyceride backbone was seen at 4–4.3 ppm only in CCO, while the ester peaks appeared at 2.8 ppm. Both protons of the triglyceride backbone and ester groups disappeared in the spectrum of FBA. This disappearance is an indication that the ester functional groups were involved in the reaction suggesting the conversion of CCO to the product (FBA). However, new peaks at 3–3.3, 7.4, and 7.5 ppm emerged in FBA, which confirms the presence of carboxyl functional groups, aromatic protons, and amide functional groups in FBA. These new peaks confirm the formation of FBA.

Table 1 Fatty acid composition of CCO

Fatty acid	Composition (%)
C14:0	0.07
C14:1	0.32
C16:0	11.18
C16:1	0.12
C18:0	9.76
C18:1	17.10
C18:2	55.88
C18:3	4.46
C20:0	0.32
C20:1	0.08
C22:0	0.10
C24:0	0.29
C24:1	0.32
Unsaturation	78.28
Saturation	21.72

Antioxidant activity assay

The antioxidant activity of FBA at different concentrations (ranging from 50–100 μg mL^{-1}) was compared with that of vitamin C, as presented in Fig. 4. However, the result revealed a higher IC_{50} for FBA (1.96 μg mL^{-1}) than vitamin C (40.20 μg mL^{-1}).

Antimicrobial screening and MIC

The antimicrobial capacity of FBA was evaluated by screening it against the growth of four organisms (MRSA ATCC 71, *Staphylococcus aureus* ATCC 25923, *E coli* DMS 10974 and *Pseudomonas aeruginosa* ATCC 9027), as shown in Table 2. The blank (water used as solvent for

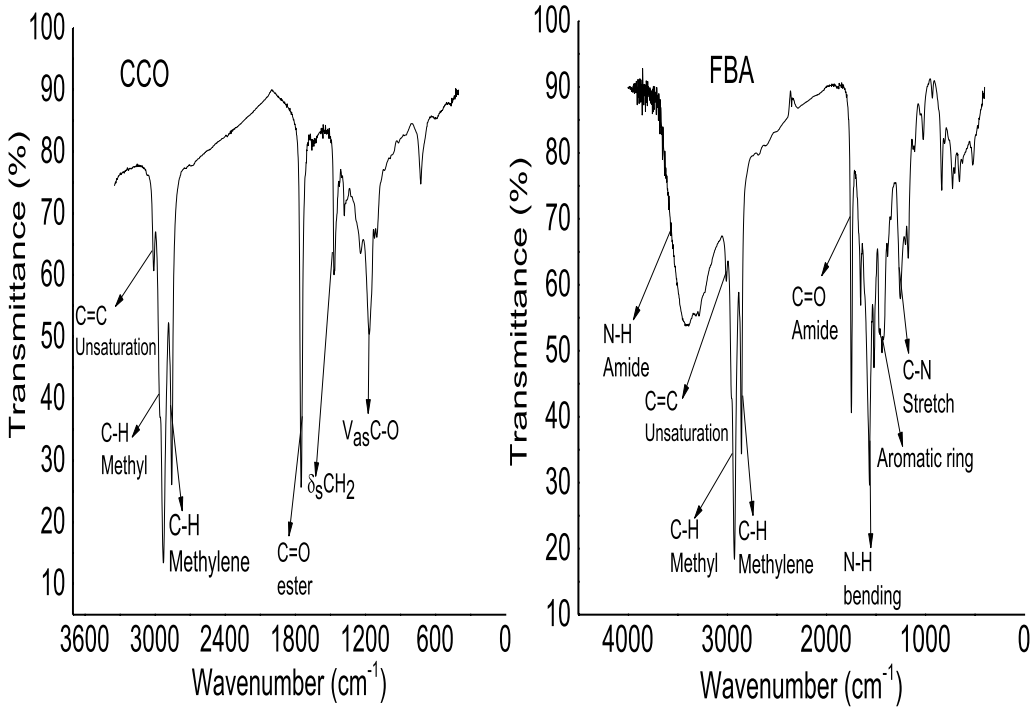

Fig. 2 FTIR spectra of CCO and FBA suggesting the presence of functional groups

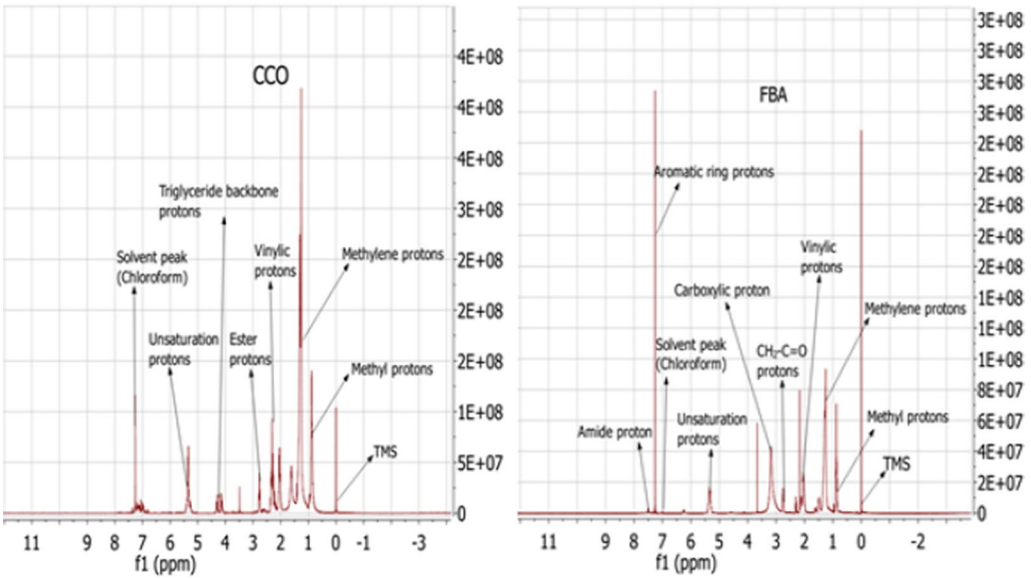

Fig. 3 ^{1}HNMR spectra of CCO and FBA confirming the chemical reaction taking place

FBA) did not show any activity against the growth of the microorganisms. Among all the organisms studied, at a concentration of 2.6×10^{-3} mg L^{-1}, FBA had the highest inhibitory activity against *Staphylococcus aureus* ATCC 25923, with a zone of inhibition of 17.67 ± 2.50 mm. The lowest inhibitory activity was recorded against *Pseudomonas aeruginosa* ATCC 9027, with a zone of inhibition of 10.33 ± 1.25 mm. The MIC of FBA was compared with other reported plant-sourced antimicrobial agents, as shown in Table 3. The lower the MIC

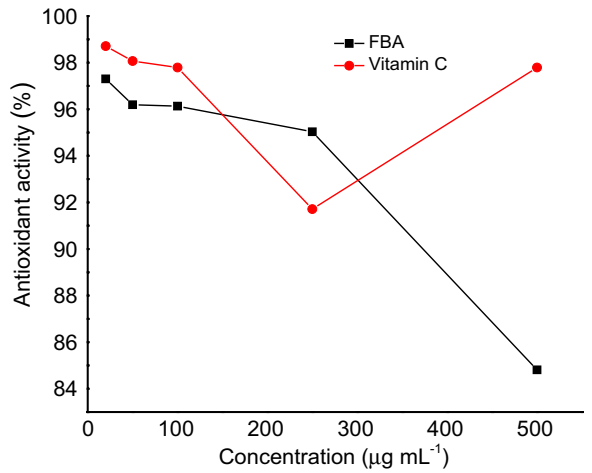

Fig. 4 Antioxidant activity of FBA and vitamin C at different concentration

Table 2 Antimicrobial screening of FBA

Organism	Inhibition (mm)
E.coli DMS 10974	12.67 ± 1.70
MRSA ATCC 71	15.00 ± 5.72
Pseudomonas aeruginosa ATCC 9027	10.33 ± 1.25
Staphylococcus aureus ATCC 25923	17.67 ± 2.50

value, the better the potency of such substance. The MIC of FBA was found to be 0.026 mg mL^{-1} for all the tested microorganisms.

Estimation of somatic index

The animals were examined throughout the feeding period and were found clinically normal and stable, with no physical defect or deterioration in locomotor performance. The results obtained for the bodyweight of the animals are presented in Table 4.

Effect of FBA on biomarkers of hepato-renal damage

In the screening of chemical compounds for pharmacological activities, it is important to test them for toxic characteristics. The acute toxicity study did not show any signs or symptoms of toxicity at the evaluated concentrations of FBA. There was no mortality recorded at the doses during the period of administration of FBA. The results obtained for the plasma clinical parameter after treatment for 14 days are presented in Table 5. There was no significant difference in AST activity between the control group and animals exposed to 10 and 50 mg kg^{-1} FBA, respectively. However, there was a significant difference in AST activity between the control group and the 100 mg kg^{-1} FBA administered rats ($p < 0.01$). AST plays an important role in aminoacid metabolism, an increase in AST value in the 100 mg kg^{-1} FBA administered rats might be indicative of a damage to the tissue. On the other hand, the ALT activity was significantly different across all treatment groups when compared with the control.

Effect of FBA on the antioxidant defense system

FBA was evaluated for its effect on the antioxidant defense system in rats. The results obtained are presented in Fig. 5. The liver catalase activity was significantly increased ($p < 0.01$) across the test groups in comparison with the control group. However, in the kidney, a significant increase was only observed at the high dose FBA administration (100 mg kg^{-1}).

Histopathology

The histopathology of the liver and kidney exposed to various doses of FBA are presented in Fig. 6. The liver and kidney of the control appeared normal, with no visible lesion while the test group showed mild congestion and periportal infiltration.

Quantum chemical parameters and mechanism of action of FBA

The exhibited antimicrobial activities of FBA may be attributed to two mechanisms as previously reported by Khameneh et al. [21], to include interference with vital components of microorganism and circumventing the conventional mechanisms of antibacterial resistance. It is obvious that by way of interference with a vital component of the microorganism, FBA may have targeted (I) cell-wall biosynthesis; (II) DNA replication and repair; (III) protein biosynthesis; (IV) cell membrane destruction; and (V) inhibition of a metabolic pathway. This mechanism was previously reported [21], as shown in Fig. 7. The electronic properties of FBA are shown in Fig. 8. This revealed the HOMO and LUMO density distribution, the ionization, and electrostatic potential. FBA exhibited some molecular properties, which are presented in Table 6. The molecular surface area was found to be 198.52 $Å^2$, and dipole moment was 2.62 debye while solvation energy was − 36.42 kJ mol^{-1}. The absolute hardness (η) was calculated as:

$$\eta = \frac{E_{LUMO} - E_{HOMO}}{2} \quad (3)$$

Table 3 Comparison of the minimum inhibitory concentration of FBA with other antimicrobial agents in literature

Sample	*E.coli*	*Staphylococcus aureus*	MRSA ATCC 71	*Pseudomonas aeruginosa*	Reference
MM	62.5	7.8–62.5	–	7.8–31.25	[31]
AC	6.24	6.24	–	–	[32]
BV	1.6	0.4		3.2	[33]
CA	12.8	1.6	–	12.8	[33]
EC	1.75	1.67	–	2.00	[34]
ZM	0.50	0.82	–	0.63	[34]
LO	13.4	–	10.2	9.4	[35]
PG	ND	–	11.8	9.4	[35]
AV	0.25	0.42	–	–	[36]
HA	0.51	0.25	–	0.12	[37]
FBA	0.026	0.026	0.026	0.026	Present work

– = Not determined

Values are in mg mL^{-1}

MM, *Moringa oleifera* methanol extract

AC, *Annona crassiflora* ethanolic extract

BV, Berberis vulgaris (Macerated Methanolic Extract)

CA, Cassia angustifolia (Macerated Methanolic Extract)

EC, Euclea crispa methanol extract

ZM, Ziziphus mucronata methanol extract

LO, Liquidambar orientalis Mill

PG, *Punica granatum* L.

AV, Aloe vera (Leaf Pulp Aqueous)

HA, Hypericum alpestre (methanol extract)

Table 4 Body weight, RSI and HSI of rats treated with FBA

Treatment group	Body weight (g)	RSI (%)	HSI (%)
Control	252.10 ± 1.29	0.65	2.92
FBA (10 mg kg^{-1})	204.20 ± 2.03**	0.60	3.87
FBA (50 mg kg^{-1})	197.90 ± 0.41**	0.57	3.10
FBA (100 mg kg^{-1})	195.00 ± 1.00**	0.53	2.31

Data expressed as mean ± SD for five rats per group. **Significantly different from control ($p < 0.01$)

RSI, Renatosomatic index

HIS, Hepatosomatic index

Table 5 Plasma clinical parameters in rats following 14 days treatment with FBA

Parameters	A	B	C	D
AST, U/l	104.8 ± 6.1	111.9 ± 12.8	104.8 ± 8.1	200.6 ± 18.7**
ALT, U/l	43.0 ± 6.1	72.1 ± 3.4*	71.2 ± 10.3*	84.7 ± 3.4**
Total Bilirubin	14.6 ± 1.4	14.6 ± 1.2	14.8 ± 1.1	17.5 ± 1.1
Albumin, g/dl	3.2 ± 0.1	1.6 ± 0.1**	3.1 ± 0.1	3.2 ± 0.1
Creatinine, µmol/l	88.4 ± 8.7	100.8 ± 8.8	115.4 ± 6.4	118.1 ± 6.9*
Urea, mmol/l	0.7 ± 0.02	1.2 ± 0.01**	1.1 ± 0.03**	1.3 ± 0.02**
Potassium, mEq/l	1.9 ± 0.2	2.5 ± 0.2*	2.8 ± 0.01**	2. 7 ± 0.1**
Sodium, mEq/l	140.7 ± 2.8	138.7 ± 3.4	140.1 ± 3.1	133.0 ± 0.1

Values are expressed as mean ± SE for five rats in each group. A, Control; B, FBA (10 mg kg^{-1}); C, FBA (50 mg kg^{-1}); D, FBA (100 mg kg^{-1}); *significantly different from control ($p < 0.05$). **Significantly different from control ($p < 0.01$)

Discussion

This observed antioxidant activity exhibited by FBA might be attributed to its ability to neutralize the free radical character of DPPH by transferring either hydrogen or electron to DPPH radical [22]; moreover, FBA contains heteroatoms with nonbonding electrons in its molecule. Interestingly, the activities of FBA against different strains of *Staphylococcus aureus* varied in this study. However, the use of plant-sourced remedy as antimicrobial agents has proved substantial [21, 23], which places the use of FBA as a potential source of antimicrobial agent for controlling the health challenges these pathogens may cause. The MIC obtained for *E.coli* (0.026 mg mL^{-1}) is lower than values previously reported [24, 25]. *E.coli* has also been associated with food poisoning [26]. Although synthetic chemicals from petrochemicals have been used in prevention and control of food poisoning disease caused by *E.coli* the repeated use of these chemicals resulted in accumulation of chemical residues in food and ultimately in human and animal, which has resulted in the emergence of microbial resistance

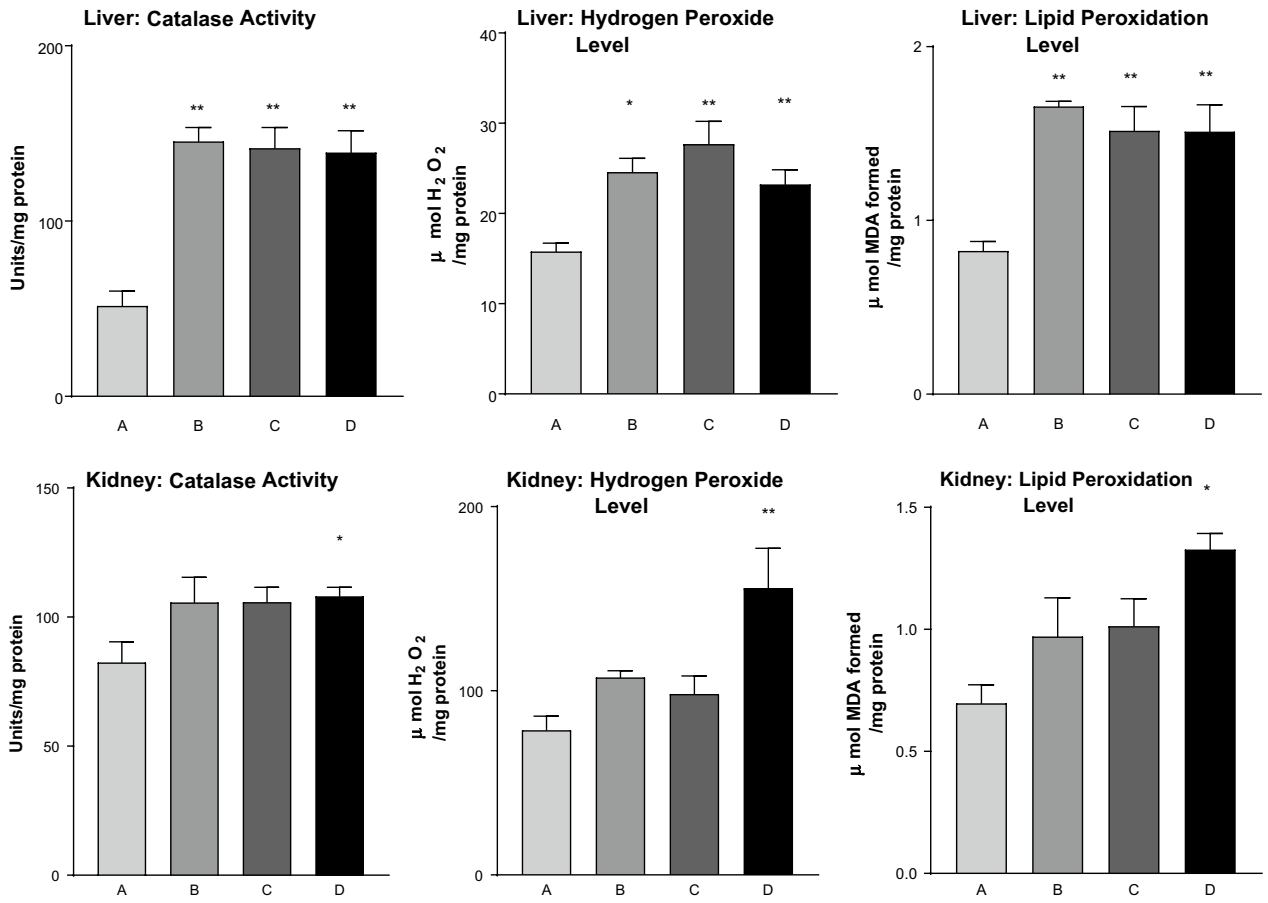

Fig. 5 Antioxidant enzyme activities and levels of lipid peroxidation following administration of FBA to rats for 14 consecutive days. Values are expressed as mean ± SE for five rats in each group. A = Control; B = FBA (10 mg kg^{-1}); C = FBA (50 mg kg^{-1}); D = FBA (100 mg kg^{-1}); *significantly different from control ($p < 0.05$). **Significantly different from control ($p < 0.01$)

and unpleasant side effect of these synthetic chemicals [21–24]. The development of plant-sourced interventions was reported as a sustainable resource to overcome this challenge [27]. The MIC of an extract is considered to be good if the value is less than 0.1 mg mL^{-1}; moderate if it is between 0.1 and 0.625 mg mL^{-1}; and weak when it is above 0.625 mg mL^{-1} [24, 28]. Currently, the MIC of FBA was 0.026 mg mL^{-1} for all the studied microorganisms, which places FBA as a good antimicrobial agent.

The OSI was calculated for RSI and HSI. The value was found higher in HSI than RSI at the study doses (10–100 mg kg^{-1}). OSI, considered as the ratio of organs to body weight, could serve as an indicator of the presence of foreign bodies [29]. A previous study [30] showed HSI as an important biomarker due to the important role of the liver in detoxification. The values obtained for AST and ALT are higher than values obtained for the control groups. Although there was a slight increase in the total bilirubin level of rats administered with FBA (100 mg kg^{-1}), there was no significant difference across all treatment groups when compared with the control group. The creatinine level increased across all test groups as the doses of FBA increased, but a significant difference ($p < 0.05$) was observed only at the dose of 100 mg kg^{-1} FBA in comparison to the control group. Additionally, there was a significant elevation in the urea levels across all the test groups when compared with the control group. The potassium level increased significantly in all the test groups when compared with the control, whereas; there was no significant difference in sodium level when the test groups were compared with the control groups. The observed high catalase activity might indicate the ability of FBA to scavenge reactive oxygen species (ROS). It is plausible to suggest that FBA has a supportive defense against ROS. The hydrogen peroxide levels increased in both hepatic and renal tissues, especially at the 100 mg kg^{-1} treatment dose when compared with the control groups. In rats administered with FBA, hepatic MDA levels were significantly elevated at all treatment doses

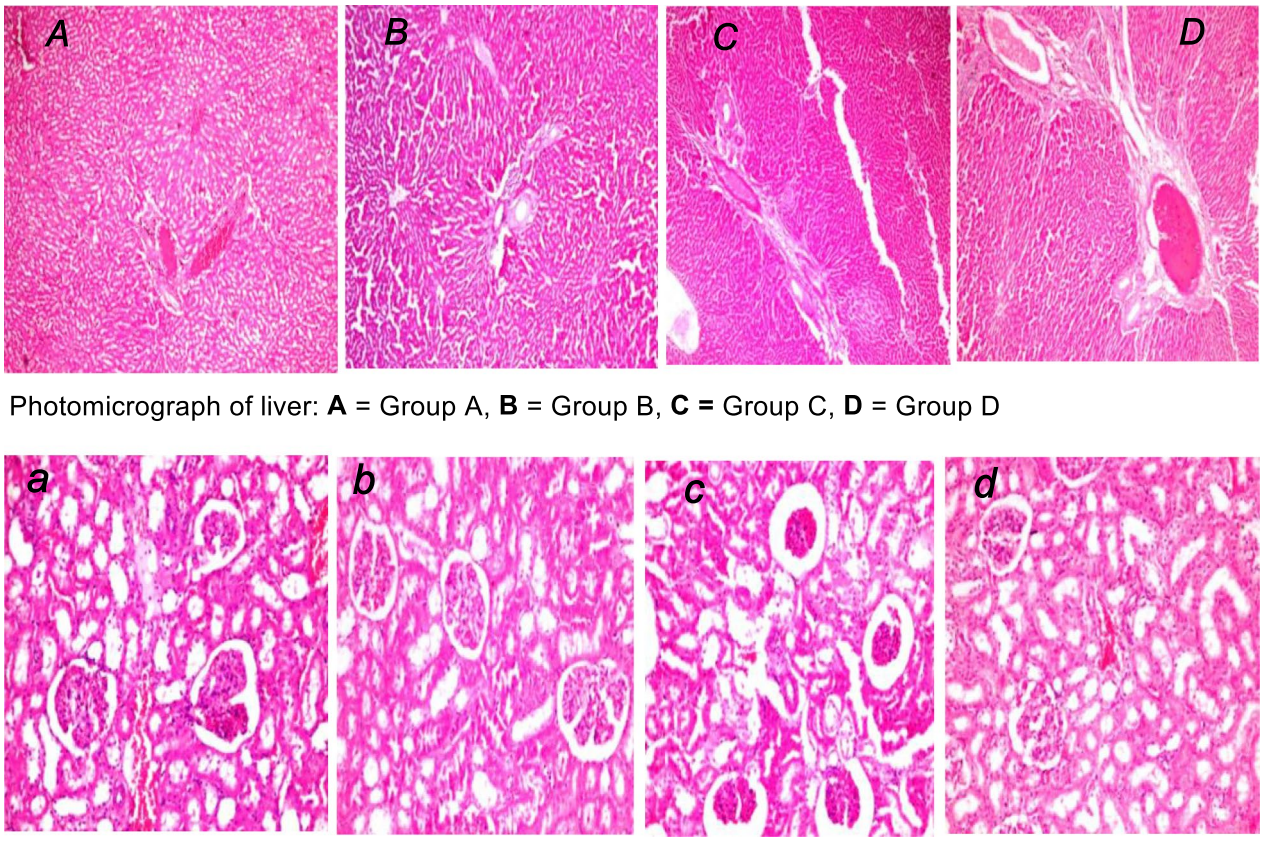

Fig. 6 Representative photomicrographs of liver and kidney of rats administered FBA

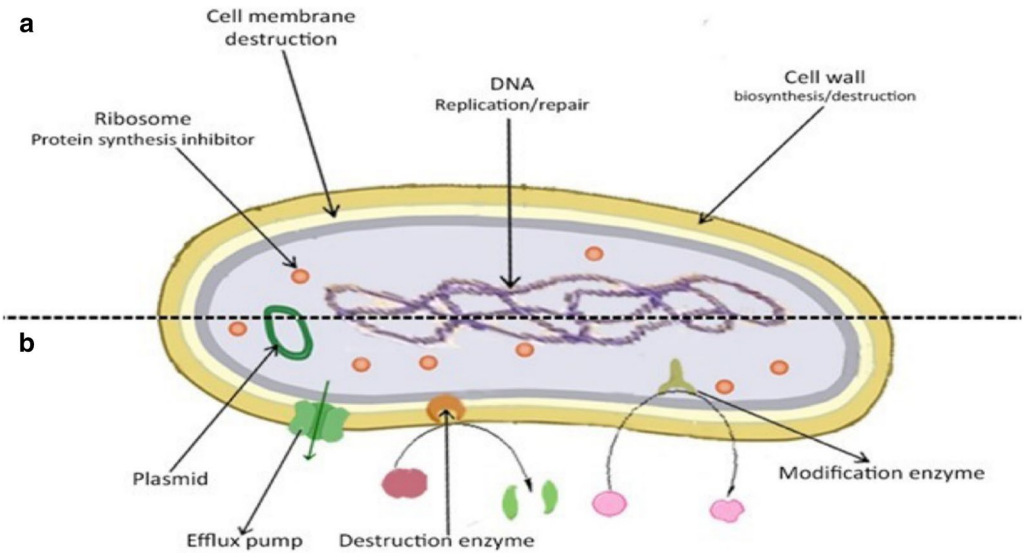

Fig. 7 Proposed mechanism of action of FBA: **a** Proven targets for antibacterial drugs; **b** Multiple antibiotic resistance mechanisms in bacteria (Khameneh et al. [21])

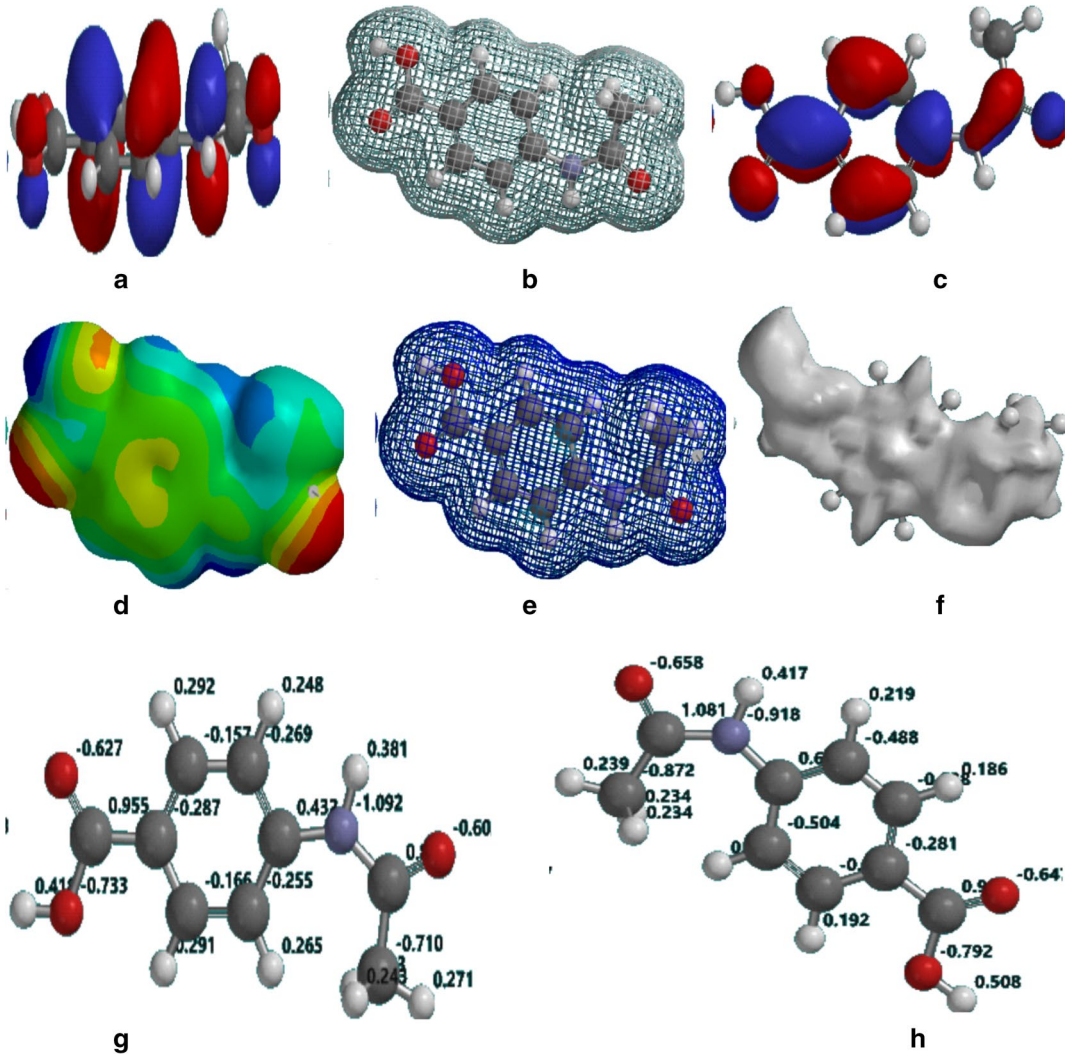

Fig. 8 Electronic properties of FBA: HOMO density distribution of FBA (**a**); Electron density of FBA (**b**), LUMO density distribution of FBA (**c**), Electrostatic potential map of FBA (**d**), Local ionization potential map of FBA (**e**), Potential of FBA (**f**), Mulliken charge (**g**), Electrostatic charge (**h**)

Table 6 Molecular properties of FBA

Quantum chemical property	FBA
Molecular surface area (Å^2)	198.52
Energy (au)	–621.65
E_{HOMO} (eV)	−8.85
E_{LUMO} (eV)	2.38
$E_{LUMO-HOMO}$ (eV)	11.23
Dipole moment (debye)	2.62
Solvation (kJ/mol)	−36.42
Volume (Å^3)	176.38
Polarizability	53.04
Group	Cs
η (eV)	5.62

when compared with the control group. Contrariwise, only the 100 mg kg^{-1} FBA treatment group elicited a significant elevation in renal MDA levels. The observed elevation in hepato-renal MDA levels might imply an overwhelming influence of oxidative stress generated by FBA, which may have affected the antioxidant defense mechanism.

The photomicrograph of liver of rats in group B (10 mg kg^{-1} FBA) revealed mild congestion by inflammatory cells, while hepatic morphology of rats exposed to 50 mg kg^{-1} FBA (group C) and 100 mg kg^{-1} FBA (group D) presented with periportal infiltration by inflammatory cells and bile duct hyperplasia, respectively. Furthermore, renal histology of rats in group B showed hemorrhagic lesion and glomerular congestion;

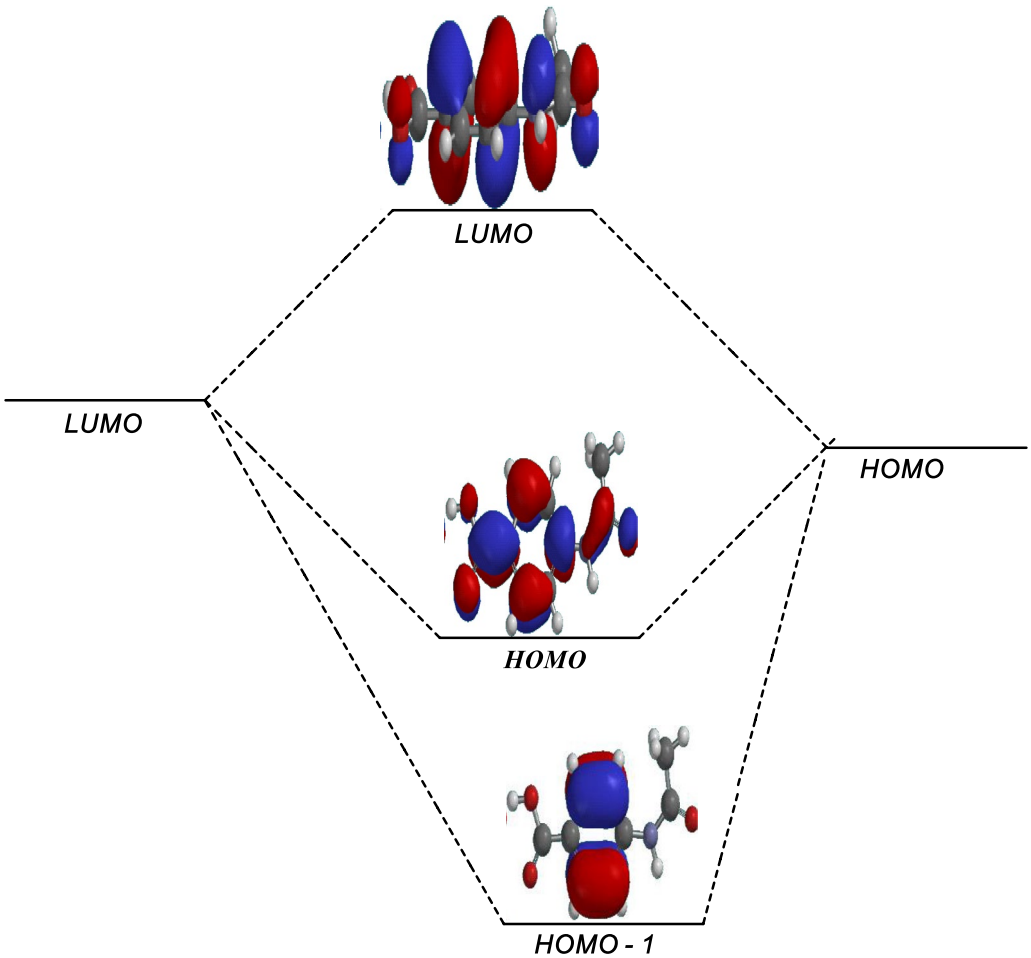

Fig. 9 Molecular orbitals of FBA indicating the transfer of nonbonding electrons for electronic interaction

group C revealed fatty infiltration of tubules while group D had disseminated congestion and hemorrhagic lesion.

As previously reported by Khameneh et al. [21] shown in Fig. 7; the antibiotic resistance mechanism in bacterial; however, FBA must have circumvented these antibiotic resistance mechanisms in the tested organisms. FBA may have altered the outer membrane permeability in the test organisms and eventually destabilized them. The structure of FBA contains heteroatoms such as nitrogen and oxygen, which exhibited negative Mulliken charges as well as negative electrostatic charges. This negative charge is an indication that FBA may have exhibited nucleophilic character when screened against the microorganism as well as when administered to the Wistar rats. This nucleophilic character explains the possible interference with vital components of the microorganisms reported by Khameneh et al. [21]. This may be attributed to the congestion by inflammatory cells, hemorrhagic lesion, and glomerular congestion observed in the photomicrograph of the liver and kidney of rats studied (groups B to D). This interaction must have resulted in a distortion of the components of the pathogens, which explains the antimicrobial properties exhibited by FBA. This nucleophilic interaction of FBA with organs of the rats accounts for the toxic effect imposed by FBA. The nucleophilic interaction may be viewed electronically as a donor–acceptor interaction. Furthermore, Fig. 9 reveals the molecular orbital of FBA, which shows electronic delocalization over the heteroatoms in FBA.

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Authors' contributions
AA, CAO and NHE conducted the experimental studies such as extraction, synthesis, characterization and the animal studies. AA and CAO conceptualized, dsigned and directed the study. They also drafted the manuscript and wrote the first draft. SSK was involved in conducting the characterization such as NMR and fatty acid composition. SSK also wrote part of the manuscript and proof read. SOF conducted the antimicrobial studies and interpretation. CBA

took part in the conceptualization and project development. All authors read and approved the final manuscript.

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Availability of data and materials
The data generated and used during the current study are available with the corresponding author on reasonable request.

Ethics approval and consent to participate
The study protocol was approved by the Redeemer's University Committee on Ethics for Scientific Research.

Competing interests
The authors have no conflicts of interest to declare.

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