



EVALUATION OF PHYTOCHEMICAL CONTENTS AND HEPATOPROTECTIVE EFFECT OF
PHYSALIS ANGULATA L. METHANOL EXTRACT-LOADED CHITOSAN NANOPARTICLES
AGAINST PARACETAMOL-INDUCED LIVER INJURY IN RATS

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ABSTRACT

Physalis angulata is a herbaceous plant whose leaves have been used for treating liver and other diseases in Nigeria's traditional medicine. This study aims to evaluate the phytochemical contents and hepatoprotective effect of the leaf extract against paracetamol-induced liver injury in Wistar rats. Phytochemical analysis was carried out following standard procedures. The bioactive compounds and mineral elements present were analyzed by GC-MS and AAS apparatus respectively. Free radical scavenging activity was determined by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays while total phenolic and flavonoid contents were determined by Folin-Ciocalteu and aluminium chloride colorimetry assays respectively. Chitosan nanoparticles were prepared by a spraying-drying technique using glutaraldehyde a cross-linking agent and characterized in terms of FTIR analysis, % yield, particle size, % drug entrapment, *in vitro* drug release, swelling index, and drug kinetics. Liver injury was induced in groups of rats weighing between 100 and 200 g using paracetamol. Liver function and biochemical parameters such as serum alkaline phosphatase (ALP), aspartate aminotransaminase (AST), alanine aminotransferase (ALT), glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), bilirubin (BIL), creatinine, thiobarbituric acid reactive substances (TBARS), gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH), albumin, globulin and total protein (TP) were determined using automated methods while body weight and liver weight were also determined with standard liver-protective agents. The preliminary phytochemical analysis of the methanol leaf extract revealed the presence of saponins, flavonoids, phenols, alkaloids, fats/oils, tannins, triterpenes and steroids while the GC-MS revealed bioactive compounds such as phytol, 2-cyclopenten-1-one-2-methyl, linoleic acid, oleic acid, stigmasterol, acetamide, and some fatty acid derivatives. The total phenolic and flavonoid contents were 880.56 ± 2.01 mg GAE/100 g and 299.18 ± 1.02 mg RE/100 g, respectively. Antioxidant effects showed DPPH activity with IC_{50} value of 3.06 ± 0.01 μ g/mL ABTS radical scavenging activity IC_{50} value of 6.12 ± 0.01 μ g/mL. The formulated extract-loaded chitosan nanoparticles (CSPA) produced 56.12% yield, 225.02 nm size, 82.42% entrapment, 66.34% swelling index, 96.12% cumulative drug release and first-order kinetics. The extract-loaded chitosan nanoparticles caused a significant decrease in the values of serum biomarkers in paracetamol-induced liver injury in rats in treated groups which were comparable to the control ($p < 0.05$). Photomicrographs of liver sections showed that the extract displayed potential degrees of protection to the liver as no histopathological damages were observed in the group treated with high doses of crude extract and extract-loaded chitosan nanoparticles. The study showed that *Physalis angulata* extract-loaded chitosan nanoparticles possess a hepatoprotective effect against paracetamol-induced liver injury in Wistar rats. The study further justified the use of *P. angulata* leaf extract to treat liver injury in traditional medicine in Nigeria.

Keywords: Chitosan nanoparticles, hepatoprotective, *Physalis angulata*, phytochemical analysis, serum biomarkers.

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INTRODUCTION

The liver is the most important visceral organ in the human body which is responsible for various metabolic activities such as the synthesis of materials, excretion of

wastes, metabolism as well as detoxification of substances like drugs [1]. This organ is often prone to diseases because of its enormous functions. There are thousands of people suffering from one liver disease or the other globally [2]. These diseases of the liver are

caused by chemical agents such as carbon tetrachloride (CCl₄), ethanol, paracetamol, thioacetamide, D-galactosamine, sodium sulphides, etc [3]. Liver damage caused by overdose of paracetamol tablet accounts for more than 13% of patients hospitalized in the world [4]. Treatments for drug-induced liver injury or damage have yielded little or no positive results. Therefore, natural products such as medicinal plants have been used since time immemorial to treat liver damage [5]. Plants such as *Silybum marianum* contain a bioactive compound called silymarin which is used globally as a hepatoprotective drug [3]. Apart from this plant, many polyherbal formulations with hepatoprotective effects as well as many plants have been reported as remedies for liver injuries[2]. In Nigeria, one of the medicinal plants used as a remedy for liver injury or damage is *Physalis angulata* L.

Physalis angulata is an erect herbaceous plant which belongs to the Family Solanaceae and is found in many West African countries such as Nigeria, Ghana, and Cote de Voire, as well as many countries of the world growing to a height of 50 cm long in the wet season [6]. The plant has been used in ethnobotany to treat many diseases such as cancer, infections, diabetes, malaria fever, anaemia, hyperlipidemia, pains and inflammations [7]. Previously phytochemical analysis of the plant revealed the presence of many metabolites such as fats/oils, carbohydrates, vitamins, minerals (like calcium, zinc, boron, etc.), triterpenes and sterols. The whole plant contains different kinds of steroidal lactones which are mainly physaline, and withanolide compounds as well as flavonoids [6].

Nanotechnology has been applied in various aspects such as in biology, pharmacy, medicine, engineering and material sciences because of their specific characteristics. This aspect of science is increasingly improving the therapeutic potentials of a drug agent in targeted drug delivery systems in a controlled fashion [8]. Nanoparticles have been designed with small particle sizes of 1-1000 nm, allowing them to navigate easily in the body with immense electronic, chemical, biological, electrical and magnetic properties [9]. Chitosan nanoparticles have tremendous advantages such as long-circulating ability in *in vivo* models bypassing blood-brain barriers and enhancing the bioavailability of drugs in tissues and organs with zero toxicity and unique pharmacokinetic features [10–14]. Because of major setbacks associated with conventional drug delivery systems such as enzymatic degradation of drugs, [9] and uneven drug distribution to both disease and healthy tissues or organs in the body, chitosan nanoparticles are now used to encapsulate drug agents for various diseases such as cancer, tumours, pains, inflammations and infections where they act as nanocarriers for these drugs due to their specificity [14]. Chitosan nanoparticles are derived

by deacetylation reaction from chitins and shells of crabs, crayfish and shrimps. Chemically, chitosan contains a linear polysaccharide chain of D-glucosamine and N-acetyl units with low or high molecular weight [15].

This current study was carried out to analyse the phytochemical contents and evaluate the hepatoprotective effect of *Physalis angulata* leaf extract-loaded chitosan nanoparticles. To the best of our knowledge, this aspect of loading the leaf extract of *P. angulata* into chitosan nanoparticles for treating paracetamol-induced liver injury in rats has not been reported before, this present study is the first.

MATERIALS AND METHODS

Reagents, chemicals, and equipment

The reagents (DPPH, ABTS, Folin-Ciocalteu, etc.) and chemicals (methanol, glutaraldehyde, sodium hydroxide, phosphate buffer saline, etc.), used were obtained from Sigma-Aldrich (St. Louse Mo, USA) and are of analytical grades. Paracetamol tablets were obtained from Emzor Pharmaceutical Ltd., Nigeria. Some of the equipment used are a magnetic stirrer (BMGS-101, BioLab, USA), UV-Vis (JASCO, USA), GC-MS 7890A (Agilent Technologies, UK), AAS apparatus (Agilent Technologies, UK), MIKRO 200/200R centrifuge (Merck, USA), perfect micropipette (range: 0-1000 µL), digital weighing balance (Hawach Scientific CO. Ltd, China), nano spray dryer B-90 HP (BUCHI, Germany), R-210 rotavapor (Buchi, Germany), electronic blender (model: BL 1500 PRO, Binatone, Nigeria) etc.

Collection and preparation of plant materials

Fresh leaves of *Physalis angulata* L. were collected in the morning hour in August 2023 from Fori, Maiduguri, Nigeria. The plant was identified and authenticated at the Department of Pharmacognosy, Faculty of Pharmacy, University of Maiduguri, Nigeria by Dr. C.A. Ukwubile. A voucher number of UMM/FPH/SON/007 was deposited in the herbarium of the Department of Pharmacognosy. The leaves were air-dried under shade for two weeks until constant weight was obtained. They were then reduced into fine powder using an electronic blender, weighed and stored in clean plastic containers for further use.

Extraction of plant materials

The powdered *P. angulata* leaf (1500 g) was extracted with 2.5 litres of absolute methanol using a cold maceration technique for 72 h [16]. The filtrate was concentrated in vacuo using a rotary evaporator at 64.7°C. A dark-green methanol leaf extract weighing 120 g (% yield: 8%) was obtained and stored in a sample bottle for further use.

Preliminary phytochemical screening of extract

The methanol leaf extract was screened for the presence or absence of alkaloids, flavonoids, tannins, saponins, triterpenes/steroids, phenols and sterols following standard methods [17 - 20].

Analysis of macro and micro mineral elements of leaf

The powdered *P. angulata* leaf was subjected to nitric acid-perchloric acid (70:30) digestion following standard procedures to determine the presence of some macro and micro elements like arsenic, chromium, lead, copper, manganese, etc., using the atomic absorption spectrophotometer (AAS) [21].

GC-MS analysis of *P. angulata* methanol leaf extract (PAME)

The bioactive compounds present in PAME were evaluated using Agilent Technologies 7890A gas chromatography (GC) coupled to a mass spectrum detector (MSD) following the methods described by Ukwubile et al. [22]. The run time was 60 min, and each compound was calculated in terms of percentage abundance, peak areas and retention times while the identification of compounds was done by comparing with existing data from the NIST library [23].

Determination of total phenolic contents (TPC) and total flavonoid contents (TFC)

The TPC was determined by the Folin-Ciocalteu (F-C) method while TFC was determined by aluminium chloride colorimetry assay as previously described [17–20; 24–26]. Briefly, for TPC, 10 g F-C was in 10 mL deionized water and mixed with 10 g of extract dissolved in 10 mL of 98.1% v/v methanol and allowed to stand for 6 min. The gallic acid standard stock was at concentrations 25 to 150 µg/mL for plotting the calibration curve. The absorbance was taken at 765 nm using the UV-vis spectrophotometer. The same procedure was adopted for TFC except that aluminum chloride and rutin were used as reagents and standard respectively while the absorbance was taken at 510 nm. All experiments were carried out in triplicates.

Preparation and characterization of *P. angulata* extract-loaded chitosan nanoparticles (CSPE)

Physalis angulata extract-loaded chitosan nanoparticles (CSPE) were prepared and characterized following the methods described by Ukwubile et al. [27]. Four formulations (CSPE1-CSPE4) were prepared and CSPE2-CSPE4 were cross-linked with glutaraldehyde at various concentrations of 500, 1000 and 1500 µg /mL respectively. The formulated microspheres were characterized in terms of % yield, FTIR, particle size, entrapment efficiency, *in vitro* drug release and

cumulative drug release at 6 h, swelling index and drug kinetics. Formulation with better-optimized parameters from the above was chosen for drug delivery [11].

Evaluation of *in vitro* antioxidant activity DPPH antioxidant assay

The DPPH radical scavenging activity of the crude extract and CSPE were determined using the method previously described by Molole et al. [28], with slight modifications. DPPH solution was prepared by dissolving 1 g of DPPH in 10 mL of 100 % (v/v) methanol (Sigma Aldrich St. Louis Mo) and then mixed with stock solution of the extract and then diluted to obtain different concentrations (100, 200, 300, 400, 500, and 600 µg/mL). The mixture was then incubated in the dark cupboard for 90 min. Ascorbic acid was used as the standard (control), and was prepared in a similar way described [29]. The absorbance for each solution was recorded at 517 nm wavelength. The scavenging radical activity was calculated from the formula below:

$$\% \text{ DPPH radical scavenging} = \frac{(\text{AbsC} - \text{AbsE}/\text{AbsC})}{100} \quad (\text{i})$$

Where AbsC denotes absorbance of the control and AbsE denotes absorbance of extracts.

ABTS antioxidant assay

In this assay, ABTS radical cation was obtained by mixing 5 mM ABTS solution (Sigma Aldrich, St. Louis Mo) in 10 mL distilled water and 1 mM potassium persulfate solution in 10 mL distilled water. The mixture was incubated in a dark cupboard for 24 h and the solution was diluted with methanol to obtain the working solution of ABTS having an initial absorbance of 0.70 ± 0.02 at 734 nm. Then, 100 mL of the crude extract, CSPE and the Trolox standard (25 to 250 µg/mL) were mixed with 3 mL of ABTS stock solution [28]. The absorbance was then measured 6 min later at 745 nm against the blank (10 mL methanol). All readings were carried out in triplicates.[30] The percentage of ABTS scavenging was then calculated from the formula below [31]:

$$\% \text{ ABTS radical scavenging} = \frac{(\text{AbsC} - \text{AbsE}/\text{AbsC})}{100} \quad (\text{ii})$$

Where AbsC denotes absorbance of the control and AbsE denotes absorbance of extracts.

Experimental animals

Sixty (60) healthy Wistar rats of both sexes weighing between 100 and 200 g were purchased from PJ Rat Farm Ltd, Jos, Nigeria. The animals were allowed to acclimatize before the experiment at room temperature in the animal house for one week with free access to food and water. Local and international ethical

guidelines for the use of these animals were strictly followed.

Acute oral toxicity study of *P. angulata* methanol leaf extract in rats

Acute oral toxicity of *P. angulata* crude leaf extract was evaluated following the OECD guidelines which specify the use of few animals [32]. Five rats consisting of three males and two females were administered a maximum dose of 2000 mg/kg body weight of leaf extract orally and individually monitored for signs of toxicity, especially in the first 4 h of 24 h and then daily for two weeks.

Evaluation of hepatoprotective effect in PCM-induced liver injury

Thirty (30) Wistar rats of both sexes weighing between 100 and 200 g were randomly grouped into six groups of five rats per group:

Group I is the normal control which received 10 mL of distilled water orally,

Group II is the paracetamol control which received 250 mg/kg PCM daily orally,

Group III received a standard 140 mg/kg of Silymarin tablet (Micronova Pharma Ind Ltd),

Group IV received 100 mg/kg CSPE orally,

Group V received 200 mg/kg crude extract orally,

Group VI received 400 mg/kg crude extract orally.

The animals in groups III to VI were administered 250 mg/kg of PCM three hours after oral administration of the standard drug, CSPE and crude extract daily for seven days [2]. At the end of 7th day of treatment, the animals were sacrificed and blood samples were collected by cardiac puncturing into EDTA bottles for analysis of serum biochemical markers such as ALT, AST, ALP, globulin, creatine, bilirubin, total protein, glutamate pyruvate transaminase (GPT), glutamic oxaloacetic transaminase (GOT), and lactate dehydrogenase (LDH). The analysis was carried out using an automated haematology analyzer and Reflotron Plus machine [33].

Histopathological examination of liver

To examine the liver for any pathological damage, liver sections from various groups dissected were subjected to hematoxylin and eosin stains (H & E) and observed under a light microscope [34].

Statistical analysis

Results obtained were expressed as means \pm SD (n=5). $P < 0.05$ was taken as statistically significant when to the control using one-way ANOVA followed by Dunnett's post hoc test. Analysis was conducted using GraphPad Prism version 9 and SPSS version 23 statistical software.

RESULTS

Phytochemical contents

The results of the phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, triterpenes/steroids, fats/oils, saponins and phenols while anthraquinones were not detected (Table 1).

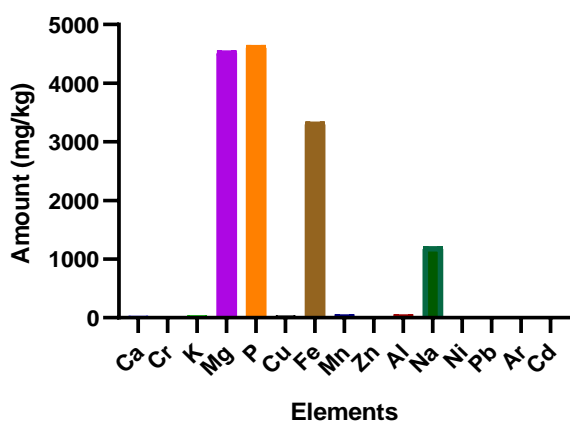
Table 1: Phytochemical contents of *P. angulata* leaf methanol extract

Constituent	Test	Inference
Alkaloids	Dragendorff's	++
	Wagner's	++
Flavonoids	Shinoda's	++
	NaOH	+
Tannins	FeCl ₃	+
	Goldbeater's	+
Triterpenes/Steroids	Liebermann's	+
Anthraquinones	Bontrager's	-
Fats/Oils	Spot	++
	Sudan III	++
Phenols	FeCl ₃	++
Saponins	Frothing	+

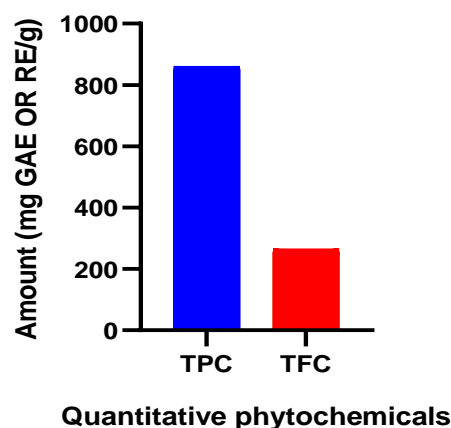
Mineral elements compositions, total phenolic content (TPC) and total flavonoid content (TFC)

The results showed that phosphorus (P), magnesium (Mg) and iron (Fe) were the highest with values of 4743.88, 4556.42 and 3437.76 mg/kg respectively. Microelements such as manganese (Mn), aluminum (Al)

and copper (Cu) were detected in smaller amounts while heavy elements such as lead (Pb), arsenic (Ar) and cadmium (Cd) were less than 0.01 mg/kg. The total phenolic content (TPC) was greater than the total flavonoid content (TFC) (Figure 1).



(a)



(b)

Figure 1: Elemental compositions (a), total phenolic content (TPC) and total flavonoid content (TFC) (b) of *P. angulata* leaf.

GC-MS composition of *P. angulata* methanol leaf extract

The GC-MS analysis of methanol leaf extract revealed the presence of thirteen (13) compounds consisting of

saturated and unsaturated fatty acids, fatty acid-derived, acetogenins, polyphenols and steroids (Table 2).

Table 2: GC-MS composition of *P. angulata* methanol leaf extract

Compounds	RT (min)	PA (%)	*Formula	m/z (g/mol)	Class
Acetamide	13.12	7.02	CH ₃ CONH ₂	59.07	COOH-amide
Oleic acid	43.16	3.55	C ₁₈ H ₃₄ O ₂	282.46	Fatty acid
Linoleic acid	44.45	12.48	C ₁₈ H ₃₆ O ₂	280.45	Fatty acid
2-cyclopenten- 1-one	30.27	3.83	C ₆ H ₈ O	96.13	Cyclic ketone
Phytol	36.22	17.91	C ₂₀ H ₄₀ O	297.20	Fatty alcohol
Hexadecanoic acid	40.52	29.81	C ₁₆ H ₃₂ O ₂	256.43	Fatty acid
Stearic acid	43.36	5.02	C ₁₈ H ₃₆ O ₂	284.5	Fatty acid
6-ethylene glycol...	45.05	5.6	C ₂₆ H ₅₄ O ₈	494.7	Polyglycol
1,1-diethoxy-ethane	2.08	0.20	C ₆ H ₁₄ O ₂	118.17	Acetals
Medroxyprogesterone	31.73	0.28	C ₂₄ H ₃₄ O ₄	386.50	Acetate ester
3β-lupa-1-20-dien...ol	31.89	0.15	C ₃₀ H ₄₈ O	424.70	Fatty alcohol
Stigmasterol	58.15	0.35	C ₂₉ H ₄₈ O	412.70	Phytosterol
Squalene	59.12	1.02	C ₃₀ H ₅₀	410.72	Hydrocarbon

Characterization of CSPE

The results in Figure 2 (a) showed that formulation code CSPE3 maintained a sustained release pattern at 8 h of *in vitro* release study when compared with CSPE2 and CSPE4. Similarly, it has the highest swelling index of

49.8% (Figure 2b), thereby efficiently encapsulating the extract. The FTIR spectra showed absorbance in the region of 3200 to 3000 wavelength cm⁻¹ typical of the amine (NH₂) group attached to CH₃ as well as

absorbance at 1720 wavelength cm^{-1} due to carbonyl of ketone (-CO-) groups.

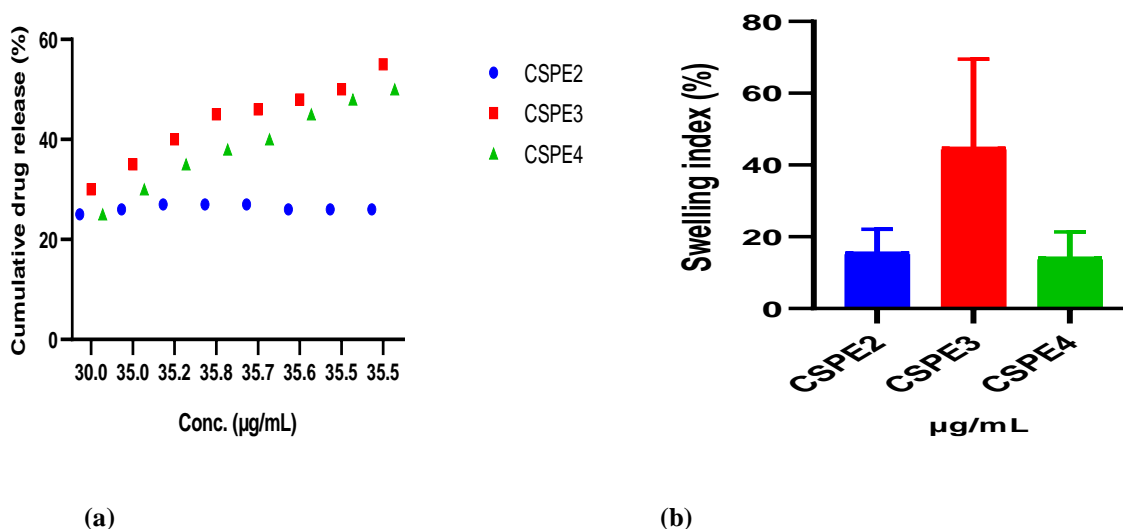


Figure 2: Drug release profile (a) and swelling index (b) of formulated CSPE at various concentrations at 8 h.

The results in Table 3 showed that CSPE3 has the best-optimized condition in terms of % yield, particle size, % entrapment and zeta potential.

Table 3: Percentage yield, particle size, % entrapment efficiency and zeta potential of CSPE

Batch code	Yield (%)	Size (nm)	% entrapment	Zeta potential (mV)
CSPE1	36.12	306.11± 2.01	22.04 ± 0.01	25.01
CSPE2	42.04	220.02 ± 1.02	28.44 ± 1.11	26.42
CSPE3	68.78	201.01 ± 1.01	88.66 ± 2.54	24.12
CSPE4	52.14	212.72 ± 2.44	72.12 ± 2.04	26.44

In vitro antioxidant activity

From the results in Figure 3 a and b, CSPE3 showed the lowest % radical scavenging activity in DPPH and ABTS antioxidation. The radical scavenging activities of the crude extract, controls and CSPE3 were concentration-dependent.

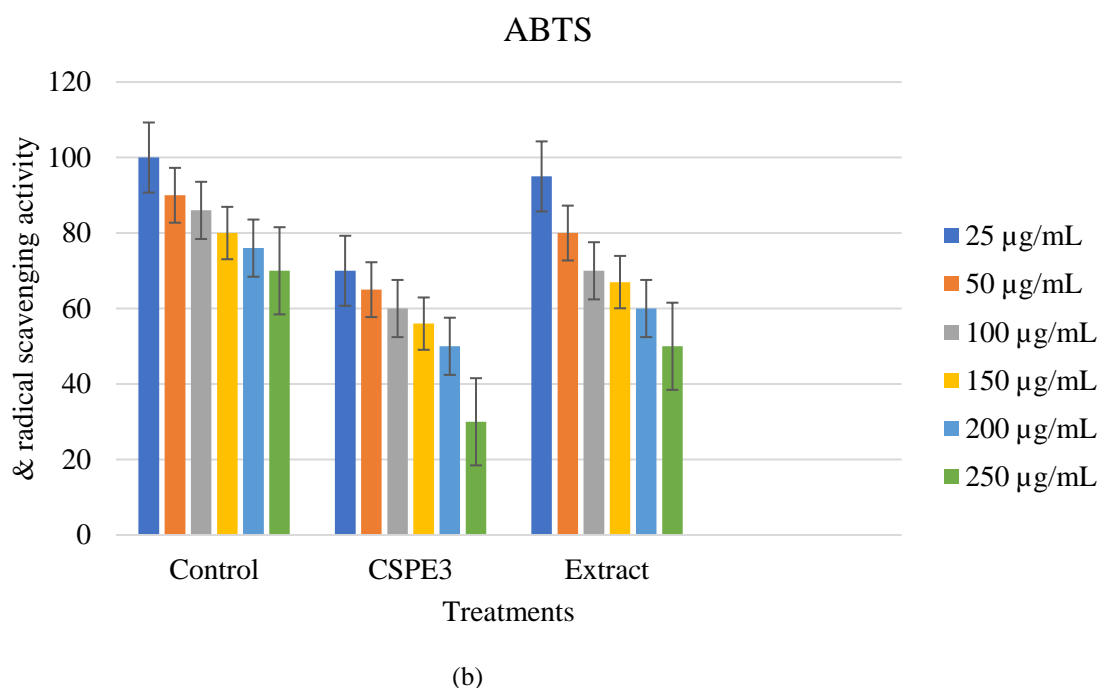
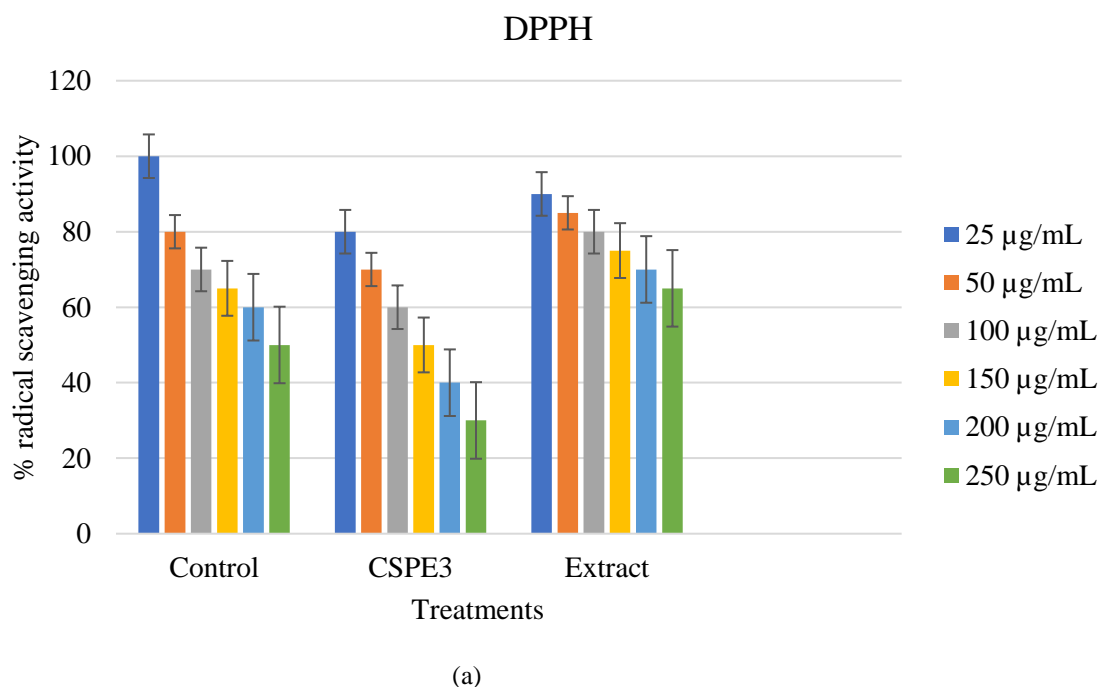


Figure 4: DPPH (a) and ABTS (b) radical scavenging activities for in vitro antioxidant activity of *P. angulata* extract-loaded chitosan nanoparticles (CSPE) at various concentrations. Results are means \pm SD (n = 3). The values of $p < 0.05$ were statistically significant (one-way ANOVA followed by Dunnett's post hoc test).

Acute oral toxicity testing

The result showed that at a maximum dose of 2000 mg/kg body of extract, no sign of toxicity was observed within the first 4 h for 24 h in the animals, thus the $LD_{50} > 2000$ mg/kg.

Hepatoprotective effects of *P. angulata* extracts against PCM-induced liver injury

The results in Table 4 showed that oral administration of 100 mg/kg of CSPE3, 200 and 400 mg/kg *P. angulata* extract (PAE) significantly ($p < 0.05$) increase the levels of liver biomarkers such as alkaline phosphatase (ALP), alanine aminotransferase (ALT), glutamic oxaloacetic transaminase (SGOT) and glutamic pyruvic transaminase (SGPT) while aspartate aminotransferase (AST), bilirubin (BIL), total proteins (TP), globulins

(GLO), albumin (ALB) creatinine, gamma-glutamyl transpeptidase (GGT) and lactate dehydrogenase (LDH) levels were significantly ($p < 0.05$) decreased in dose-dependent fashion. These increases and decreases were not out of the normal ranges except in the paracetamol control group which showed in all cases elevated values above the normal ranges. The rats treated with CSPE3 showed much hepatoprotective effect compared to the control and standard drug silymarin tablet.

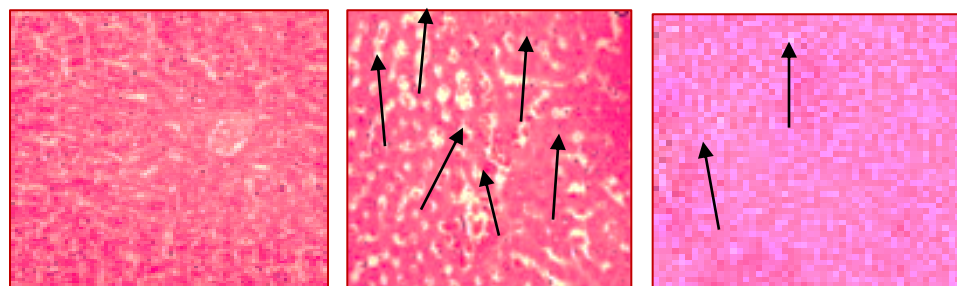
Table 4: Biochemical parameters for liver function in induced PCM-induced liver injury

Parameter	NC (mL/kg)	Dose (mg/kg) body weight			
		PCM	CSPE3	200 PAE	400 PAE
ALP (U/L)	56.50±0.01*	156.50±2.01	49.01±1.01*	67.11 ±1.04	67.88 ±1.14
AST (U/L)	10.11±0.01	50.21±1.08	8.02±0.01*	22.01±0.01	25.08±1.01
ALT (U/L)	11.02±1.01	68.04±2.04	14.12±1.01	32.23±2.02*	34.08±1.16*
BIL (mg/dL)	0.11±0.01*	5.32±0.01	0.21±0.01*	0.66±0.01*	0.68±0.01*
GLO (mg/dL)	2.63±0.01	6.44±0.01	2.08±0.01*	2.44±0.01*	2.88±0.01*
ALB (g/dL)	4.14±0.01*	8.24±1.01	4.22±1.01*	3.88±0.01	4.88±2.01
TP (g/L)	70.02±4.21*	98.45±3.24	78.04±2.05*	67.14±1.01	74.41±4.01*
CRE (umol/L)	0.88±0.01*	3.44±0.01	0.94±0.01*	0.88±0.01*	1.04±0.01*
SGPT (U/L)	44.02±2.01*	65.11±3.42	15.06±1.01*	34.31±2.01	38.56±2.11*
GGT (IU/L)	10.08±0.01*	54.22±1.01	8.02±0.01	12.06±1.01	16.24±0.01*
SGOT (U/L)	32.14±3.61	68.22±4.12	22.01±1.11	34.04±2.01	38.03±2.04
LDH (U/L)	166.04±4.02*	240.16±5.22	154.02±1.02*	174.04±3.04	178.22±1.22*

Normal ranges: ALB (3.5 to 5 g/dL), BIL (0.1 to 1.0 mg/dL), GGT (0 to 30 IU/L), LDH (122 to 222 U/L), ALP (45 to 115 U/L), AST (8 to 33 U/L), ALT (7 to 56 U/L), total protein (TP; 60 to 83 g/L), GLO (2.0 to 3.5 g/dL), creatinine (CRE; 0.7 to 1.3 mg/dL (61.9 to 114.9 µmol/L; for men and 0.6 to 1.1 mg/dL; 53 to 97.2 µmol/L; for women; women have lower creatinine than males, SGPT (7 to 56 U/L) and SGOT (8 to 45 U/L). Results are means ±SD (n=5), *Statistically significant at $p < 0.05$ (one-way ANOVA followed by Dunnett's post hoc test). NC: normal control group, PCM: paracetamol control, CSPE3: *P. angulata* extract-loaded chitosan nanoparticles, and PAE: *P. angulata* extract.

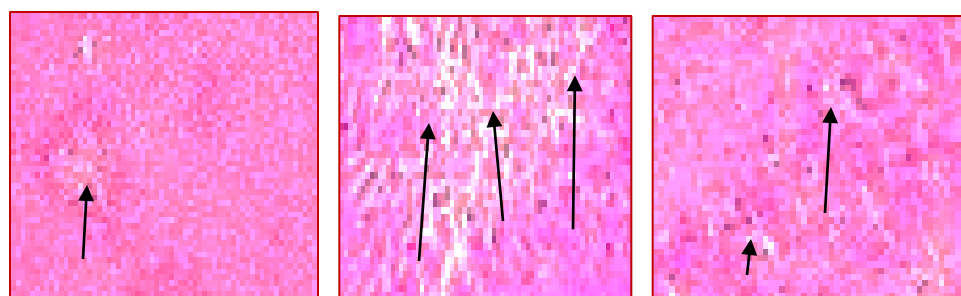
Histopathological examination of liver

From the photomicrograph of the liver section, there was hepatic architecture present in the normal control group with a clear nucleus and filled cytoplasm without observable changes or disorientation to the liver cells. However, there are visible pathological changes in the paracetamol control group in which there are necrosis, vascular congestion, steatosis, and tissue degeneration. The animals treated with CSPE3 and crude extract at a dose of 400 mg/kg showed the absence of these observed toxicities showing the ability of the extract-loaded chitosan nanoparticles to prevent liver injury (Figure 5 a-d).



(a) Normal control

(b) Paracetamol control (c) Silymarin-treated



(d) CSPE4-treated

(e) 200 mg/kg PAE

(f) 400 mg/kg PAE

Figure 5: Photomicrograph of liver sections from paracetamol-induced rats liver injury and stained with H & E stains (400x). Note: multiple arrows indicate more liver toxicities.

DISCUSSION

Previous studies have shown that herbal formulations and medicinal plant products contributed substantially toward the treatment of liver injury and diseases [2]. This practice has been sustained for decades in Africa and most developing nations of the world with low-income and poor health systems [5]. The use of herbal or medicinal plants for treating liver injuries and various diseases have yielded many successes, yet there are setbacks arising from the uneven distribution of drug agents in the body towards healthy and diseased tissues or organs [6-7]. Therefore, this present study explores a treatment alternative using drug-loaded chitosan nanoparticles for the delivery of *Physalis angulata* leaf extract to liver tissues thereby overcoming various setbacks in the conventional treatment regime.

The present study showed that *P. angulata* methanol leaf extract contains various types of secondary metabolites such as alkaloids, fats/oils, flavonoids, phenols, and sterols. These metabolites play critical roles as antioxidants and hepatoprotective agents. For instance, flavonoids and other phenols are known to exhibit various degrees of antioxidant activities in both *in vitro* and *in vivo* experiments [35]. Mineral elements such as phosphorus, magnesium, potassium and calcium are important in the healing process of wounds and diseases [35]. Magnesium has been reported to accelerate adhesion of tissues, macrophage migration, and production of collagen type I, fibroblasts and keratinocytes in wounds and injuries [36]. Similarly, the GC-MS analysis of the methanol leaf extract revealed many bioactive compounds such as linoleic acid and stigmasterol which have been reported to have strong antioxidant and hepatoprotective activities. The roles of these phytoconstituents in the current research were not different from the aforementioned.

In the current study, the high phenolic and flavonoid contents are responsible for the antioxidant

activity of the extracts. *Physalis angulata* extract-loaded chitosan nanoparticles (CSPE3) showed significant ($p < 0.05$) antioxidant activity when compared to the controls (vitamin C in for DPPH and Trolox in for ABTS assays). The DPPH scavenging radical assay is popularly used in evaluating the activity of free radicals due to the simplicity of the method. It was reported that phenolics and flavonoids are similar in antioxidant activities due to their ability to donate electrons or hydrogen radicals to unstable free radicals and reactive oxygen species (ROS) in the body [37]. Chitosan nanoparticles have been reported to specifically deliver its content in a controlled mechanism with adequate biodistribution of drugs [11]. The ability of chitosan to bypass the blood barrier conferred immense advantages over other treatments in the current study. Apart from being able to navigate the body system without being detected by the immune system, other characteristics of the CSPE3 in this study such as small particle size, % yield, % entrapment efficiency, cumulative drug release, *in vitro* release profiles and zeta potential gave CSPE3 immense advantages over the conventional drug delivery. For example, chitosan nanoparticles with small particle sizes between 100 and 300 nm have been reported as effective size for delivering most drug agents passively to tissues or organs in cancer, inflammatory, and multi-drug resistance cases [14].

Paracetamol-induced liver injury is a commonly used model for evaluating the hepatoprotective effects of drugs [38]. The elevated rise in serum levels of ALP, GPT and GOT in this study has been reported to be a result of loss of liver integrity since these enzymes are domicile in the cytoplasm finding their way into the circulatory system when there are cellular damages [38]. Our study provides a strong reason that the CSPE3 potentially inhibited the acute liver injury which was induced by a high dose of paracetamol in the animals. This is seen by the decrease in serum levels of liver functional enzymes such as ALT, GGT, LDH and AST as well as other biochemical markers such as bilirubin, total proteins, albumins,

globulins, and creatinine. The protective effects of the crude extract were dose-dependent from 200 to 400 mg/kg body weight of extract and were significantly ($p < 0.05$) different when compared to the standard drug silymarin. Similarly, the decreased levels of GPT and GOT could be due to prevention of leakages through their membrane-stabilizing effects by these enzymes [38].

From the photomicrographs of liver sections stained with H & E stains, the paracetamol caused immense necrosis, vascular congestion, and tissue degeneration. However, the CSPE3 treated groups showed potential hepatoprotective effect against paracetamol-induced liver toxicity and was significant ($p < 0.05$) when compared to the normal control group. The study further showed that the livers of rats treated with *P. angulata* methanol leaf extract (200 and 400 mg/kg body weight) did not show much variation from that of the normal control group.

The current study showed that *P. angulata* extract-loaded chitosan nanoparticles (CSPE3) possess potential antioxidant and hepatoprotective activities by stabilizing serum liver function enzymes. This was due to a high amount of phenolic and flavonoid compounds as well as certain mineral elements present in the extract. The study further showed that the use of chitosan nanoparticles to effectively deliver the leaf extract of *P. angulata* to injured liver cells in this study is promising.

CONCLUSION

Our findings showed that the *P. angulata* extract-loaded chitosan nanoparticles (CSPE3) had potential hepatoprotective effects against paracetamol-induced liver injury in Wistar rats than *P. angulata* methanol leaf extract and silymarin delivered conventionally without chitosan. The study further affirms the ethnobotanical use of *P. angulata* leaf extract as a remedy to liver injuries and disease in Nigeria.

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Conflict of interest

We have none to declare.

Author contributions

Conceptualization and draft of the manuscript (UCA); data curation, supervision, validation, and visualization (UCA, NA and MTS); formal analysis (CAU, IBB and MND); methodology (UCA, NA, NDM and MTS); review and editing (All authors). All authors read and approved the final manuscript for submission.

Ethics approval and consent to participate

The approval for the use of these animals was granted by the Research Ethical Committee of PJ Rat Farm Ltd., Nigeria, with approval number PJ/RATS-034/2023/018.

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