



Role of Chitosan-Loaded Solanine Glycoalkaloid from *Solanum scabrum* Mill. Leaf Extract as Anti-Inflammatory and *In Vitro* Anticancer Agents

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ABSTRACT

Objectives: *Solanum scabrum* Mill. commonly “African nightshade” or “huckleberry” is a plant, whose leaves are used by tribes in Nigeria and Cameroon for making the popular “Kombi” and “Njama Njama” soups, respectively. This study aimed to evaluate the anti-inflammatory and anticancer activities of the leaf crude methanol extract from *S. scabrum*.

Materials and Methods: Fractions of the plant were tested for anti-inflammatory potential and *in vitro* anticancer activity on MCF-7 and HMVII cell lines by carrageenan-induced oedema in mice, and cytotoxicity assays such as 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide, transwell migration and invasion assays, and apoptosis study by flow cytometry, respectively.

Results: Bioguided isolation yielded a white crystalline compound 3-nitro dibenzofuran (C₁₂H₇NO₃, *m/z*: 213.19 g/mol, *m.p.*: 181.49 °C). ¹H-NMR showed seven signals at δ (ppm) 2.8-4.3 consisting of two doublets and five singlets, while ¹³C-NMR revealed twelve carbons, which are majorly methyl carbons at δ (ppm) between 120 and 195. All tested samples demonstrated dose-dependent anti-inflammatory activity in carrageenan-induced mice. The isolated compound, *i.e.* solanine, and chitosan-loaded drugs showed significant inhibitory activity on the cell lines with inhibitory concentration 50 (IC₅₀) values of 8.52, 0.82, and 22.1 µg/mL, respectively on MCF-7 cell line and 4.54, 0.08, and 12.1 µg/mL, respectively, on HMVII cell line, while doxorubicin (adriamycin) positive control, had IC₅₀ values of 0.02 and 0.06 µg/mL, respectively, on MCF-7 and HMVII cancer cells. Selectivity index of solanine was the lowest in the study, hence, it lacks the ability to differentiate between cancerous and normal cell Vero E6 cell lines. Chitosan-loaded drugs quicken early apoptosis and sustained late apoptosis in cells with much improved selective indices.

Conclusion: The results obtained from this study further affirmed the use of chitosan nanoparticles as carriers for anticancer drugs.

Key words: *Solanum scabrum*, anti-inflammatory, anticancer, chitosan, solanine, glycoalkaloid

INTRODUCTION

Humans have relied on natural products such as medicinal plants for their day-to-day needs such as medicines, food, and shelter since time immemorial. Plants have played crucial roles in mitigating the deleterious effects of various diseases from different regions of the world.^{1,2} As one moves from one geographical location to the other, plant's morphological parts such as leaves, stembarks, roots, flowers, seeds,

and buds are used ethnobotanically in various forms such as anti-inflammatory, antimalarial, anticancer, antioxidant, anticonvulsant, antihelminthic, antidiabetic, antihypertensive, antiviral, and antiulcer agents. Some preparations are used as decoctions, infusions, tinctures, ointments or powders. Regardless of their mode of preparation, the main goal is to confirm a possible ethnopharmacological effect on the targeted disease. Recently, use of plants and compounds from plants as

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anti-inflammatory and anticancer agents has gained popularity, especially in countries with low access to conventional anti-inflammatory and anticancer drugs due to a lack of income and availability of quality drugs. In addition, the side effects of most anti-inflammatory and anticancer drugs have paved the way for the lack of trust in these conventional drugs in African countries such as Nigeria, Cameroon, Ghana, South Africa, Egypt, Kenya, Congo DR, Gambia, Sudan, and others.³⁻⁶ The ability of medicinal plants to illicit remarkable ethnopharmacological effects on organs, tissues, and cells is solely dependent on the secondary metabolites present in the plant. For instance, alkaloids, flavonoids, saponins, tannins, and terpenoids play various roles in the disease conditions of humans.⁷⁻⁹

Inflammatory disease is a type of illness that is usually characterized by swelling of a part of the body, organ or tissues. It usually occurs as a result of immunological response to bacterial invasion, ruptured cells or tissues, poisonous substances in the body and immune breakdown, which could be either acute or chronic.¹⁰ Treatment of inflammation is commonly carried out using non-steroidal anti-inflammatory drugs and some of these drugs have been reported to cause various degrees of side effects in the body pharmacologically, notably some proton pump inhibitors like diclofenac sodium.¹¹ Like inflammation, cancer has been one of the greatest nightmares in human health challenges before the coronavirus disease-2019 pandemic (COVID-19) in 2019.

Cancer accounts for about 40% of deaths worldwide. The major causes of cancer are not fully understood, but it is caused by mutations in oncogenes. Cancers are named according to the organ or part of the body they are found and all have semblances in how they illicit pathological effects in humans.^{12,13} To date, treatment of cancers with conventional drugs has yielded little or no results due to the negative side effects of most anticancer drugs. Current research is focused on the use of natural anticancer agents from plants that have shown potent ethnopharmacological effects on cancer cells. These phytoconstituents have been used ethnomedically to successfully treat cancers such as those of the breast, cervix, colon, scrotum, and ovary.³

Currently, in order to overcome the challenges experienced by most anticancer drugs in targeting cancer cells successfully, chemotherapeutic drugs are loaded either actively or passively in carriers such as chitosan to deliver their contents onto cancer cells, thereby inducing apoptosis in the cell.^{14,15} Chitosan nanoparticles (CSNP) are particles of size 1-1000 nm that are made by the exoskeletons of crustaceans (crabs and prawns). They are used as carriers for anticancer, antifungal, and antimicrobial drugs.^{16,17} Chitosan is a drug and protein carrier because of its numerous advantages such as biodegradability, target specificity, accessibility, little or no toxicity, easy method of preparation, ability to pass over, and evade the body's defence unnoticed.

Among the plants used to treat inflammation and cancers in Mambila (Taraba State, Nigeria) is *Solanum scabrum* Mill. (commonly called African nightshade or garden huckleberry) (Solanaceae). It is locally called "kombi" in the Hausa and

"fulfulde" languages. The leaf is used to make "kombi" and "njama" soups in Gembu (Mambila, Taraba state) and Cameroon, respectively. It grows well in countries of West Africa (Nigeria and Ghana), Central Africa Republic, and East Africa (Cameroon) at very low temperatures.³ The leaf of *S. scabrum* is an important delicacy in Nigeria and Cameroon because it is believed to possess antioxidant, anticancer, analgesic, anti-inflammatory, antispasmodic, and vasodilatory activities, while these claims are still being verified. Phytochemical screening of the leaves and roots showed that *S. scabrum* contains flavonoids, alkaloids, saponins, and tannins as well as steroidal saponins such as solanine, a glycoalkaloid derivative. It also contains numerous nutrients like proteins, iron, ascorbic acid, and vitamins.¹⁸ Despite the health and nutritional benefits derived from *S. scabrum*, there is a major concern regarding the presence of solanine glycoalkaloid in the leaf and root extracts of many nightshade families, even though it has not been confirmed in *S. scabrum*.^{19,20} Solanine is found in almost all the nightshade family (Solanaceae) and has been termed an alkaloid poison, which is used as fungicide and pesticide.²¹ In traditional medicine, the leaves and roots are also used as sedative and anticonvulsant agents and for treating asthma, acute cough, and cold.²²

In the present study, we evaluated the anti-inflammatory potential and *in vitro* anticancer activity of *S. scabrum* on breast and vaginal melanoma cell lines. We isolated solanine as a glycoalkaloid from the leaf crude methanol extract and checked its purity using thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). The effects of crude extracts, fractions, solanine, and chitosan loaded with these samples on the cell lines were also evaluated *in vitro*. The study further investigated the pH, at which chitosan-loaded drugs release more drugs *in vitro* release study. Therefore, this study was designed to evaluate the therapeutic effects of solanine glycoalkaloid in cancer treatment and chitosan-loaded drugs against cancer cell lines.

MATERIALS AND METHODS

Materials and reagents

Acetic acid, NH₄OH solution, sulphuric acid, HPLC grade, Dulbecco's minimum essential medium (DMEM), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) reagent were purchased from Sigma-Aldrich (St. Louis Mo, USA). MCF-7 human breast cancer cell, HMVII woman vaginal melanoma cancer cell, and Vero E6 cell line from an African green monkey were obtained from the ATCC (USA), dimethyl sulfoxide (DMSO), glutaraldehyde (GA), propidium iodide (PI), 0.5% tween 20 solution, and 0.1% RNase were obtained from Benrocks' Medicals Nigeria (Sigma Aldrich). Apo alert caspase-3-colorimetric assay kit was purchased from Clontech (USA), while chitosan 100 G (purity: 99+ % deacetylated) was purchased from Chemsaver (USA). Doxorubicin USP and diclofenac BP were supplied by Jude Pharmacy Ltd. (Nigeria). The remaining reagents and solvents were of analytical grade.

Collection plant material

Fresh leaves of *S. scabrum* (kombi) were collected in the early morning hours (5–6 a.m.) (temperature: 4–8 °C) from a farm land in Gembu, Sarduana Local Government Area, Taraba State, Nigeria (a border town between Nigeria and Cameroon). Identification of the plant was done by a taxonomist, Dr. Jones Ponè of the Forest Guide, Ogurugu, Nigeria, with voucher number OFG/SOL/0024. The leaves were air-dried under shade for two weeks and pulverized into fine powder using an electronic blender, weighed, and stored in a clean container for further use.

Preparation of extract

Powdered leaves of *S. scabrum* weighing 1500 g were extracted with 2.5 L of methanol using a cold maceration technique for 72 h. It was carefully filtered three times using whatman no. 9 filter papers into a clean beaker. The filtrate obtained was evaporated to dryness using a Buchi R-300 rotary evaporator (Thomas Scientific, USA) at 45 °C to produce a final yield of 50% (w/w). The dark green colored extract was then stored in a clean sample bottle and kept in a refrigerator at –4 °C for onward use.

Isolation of solanine

Exactly 80 g of powered leaves were subjected to cold maceration with 250 mL 5% acetic acid solution (v/v) in a 500 mL capacity beaker for 24 h. The extract was filtered using whatman no. 1 filter paper to remove any cellular debris present. The content was warmed to 70 °C and allowed to cool. pH was adjusted to 10 by adding 10 mL solution of concentration NH_4OH dropwise and centrifuged at 1200 rpm for 5 min. The supernatant was discarded while the precipitate was washed with NH_4OH (1%), recentrifuged, and concentrated *in vacuo* to obtain crude solanine. Presence of solanine was qualitatively confirmed by an instant production of red to violet coloration with formaldehyde and sulfuric acid solutions.²³ The crude solanine isolated was purified by boiling for 5 min in 50 mL methanol solution, filtered, and concentrated to obtain brownish solanine crystals (weight: 8.4 g). A TLC plate was used to check the purity of solanine using acetic acid:ethanol (1:3) as the solvent system by obtaining a single spot on the TLC plate.²⁴

Purity check of isolated solanine using HPLC analyses

HPLC analyses were performed on an Agilent 1290 Infinity Series HPLC system (Agilent Technologies, UK) with a G4212A diode array detector, G1316C thermostatted column compartment (TCC). Column temperature was 25 °C, a G4220A binary pump with 5 mL/min flow rates and a G4226A infinity autosampler. Solvent A mobile phase was made up of acetonitrile solution, while B was water mixed with phosphoric acid solution (0.2%). Elution was performed using a gradient elution at a flow rate of 1 mL/min. The wavelength was 270 nm, while the sample injection was 5 μL . Total runtime for the analyses was 45 min.

Bioguided partitioning of extract

The methanol extract weighing 250 g was dissolved in a 500 mL capacity separating funnel (Thomas Scientific, USA) containing mixture of distilled water and methanol (7:3) and partitioned

three times each with 500 mL of *n*-hexane (HF), ethyl acetate (EF), and *n*-butanol (BF) for bioguided anti-inflammatory and anticancer activities. Each fraction was concentrated *in vacuo* to produce respective yields of 8 g, 48.6 g, and 22.4 g fractions, and tested for their anti-inflammatory and anticancer activities *in vitro*. The fractions with most active bioactivities were each subjected to silica gel (60 x 120 mesh; 100 g) column chromatography using gradient elution fractions with the following solvent systems: HF:EF (7:3); HF:EtOAc:MeOH (5:3:2), and BF; HF:EtOAc:BF (6:3:1). 50 sub-fractions of 30 mL were collected from each fraction. Subfractions with similar profiles (R_f values) each on the TLC plates were pooled together and further subjected silica gel column to obtain additional subfractions. Subfractions with the best activity was subjected to Sephadex® G5050-10G (Merck, Germany) and eluted with EtOAc:MeOH (2:8). Characterization of bioactive compounds was performed using NMR and gas chromatography-mass spectrometry (GC-MS).

Preparation and characterization of CSNPs loaded extract

Extracts weighing 1 g each were dissolved in 400 mL acetic acid solution (1%, v/v) in a beaker and mixed with 4 g of chitosan (100% purity; 90+ % deacetylated, Chemsaver, USA), which was previously dissolved in 10 mL deionized water, and made up to 100 mL, then stirred properly for 10 min. Then, 500 μL GA (a cross-linker) was added in dropwise under constant magnetic stirring using a 10 mL sterilized syringe at 3000 rpm for 30 min on a Young-Ji HMZ 20DN magnetic stirrer (made in China). Chitosan-loaded drugs were prepared in batch codes of CE1 to CE4 for chitosan-loaded with crude leaf extract. CS1 to CS4 for chitosan-loaded with solanine and CDR1 to CDR4 for chitosan-loaded with doxorubicin as the reference anticancer drug. Batch codes CE1, CS1, and CDR1 were not crosslinked with GA for the sake of comparison. The prepared chitosan formulations were each spray-dried using a nano spraying drying apparatus (Shanghai, China) with 0.5 mm nozzle diameter, inlet and outlet temperatures of 130 °C and 65 °C, respectively, atomization air pressure of 38 mbar, liquid flow rate of 2.5 mL/min, and drying airflow of 1.5 m³/min. Characterization of drug loaded microspheres was carried out in terms of yield, morphology, particle size, % drug entrapment or encapsulation efficiency, and *in vitro* drug release.^{25–27}

Percentage yield of CSNPs

The developed CSNPs were dried using nanospray dryer (Shanghai, China). The dried microspheres were collected and weighed, while percentage yield was calculated using the formula below:

% Yield = (Final weight of dried CSNPs/initial weight of starting materials) 100.²⁵

Morphology of CSNPs

The surface morphology of CSNPs using a phenom desktop scanning electron microscope (SEM) with fully integrated energy dispersive spectroscopy and scanning electron detector (Thermo Fisher Scientific, Nigeria). Diethyl pyrocarbonate dissolved CSNPs were observed at 3000x magnification for the morphology and the extent of encapsulation.^{25–27}

Particle size of CSNPs

CSNPs were diluted in 0.1 M KCl and placed in an electrophoresis cell of field potential 15.4 v/cm connected to a Zetasizer apparatus (Malvern Zetasizer 3000 HS, UK) to determine the sizes and charges of the microspheres. Three readings were taken and means taken.²⁷

Percentage entrapment or encapsulation efficiency of CSNPs

The developed CSNPs were re-dispersed in deionized water and centrifuged at 3000 rpm for 30 min at 25 °C to separate the microspheres from the supernatant. Thereafter, CSNPs were diluted with phosphate buffer saline (PBS) (pH: 7.4) and the solution concentration was then measured using Shimadzu Ultraviolet (UV)-1900 UV-Visible (vis) Spectrophotometer (Shimadzu Europa GmbH, Duisburg, Germany) at 266 nm. The percentage entrapment was then calculated using the formula below:²⁷

Entrapment/encapsulation efficiency% = (experimental drug contents/total drug contents) 100

The prepared nanoencapsulated formulations were used in *in vitro* anticancer study.

Drug release study of CSNPs

The *in vitro* drug release study was carried out using dialysis tubes method. In this procedure, the CSNPs prepared were dissolved in 5 mL PBS of pH 7.4. Then, 500 mL-volume beakers were filled with 150 mL PBS for each formulation code. The dialysis tubes were then filled with 10 mL of formulated chitosan/drugs and both ends of the dialysis bags were tied using clean ropes. It was then placed in the beakers and subjected to stirring under a magnetic stirrer at 1500 rpm at 37 °C. In every one hour, 5 mL of the contents from the beaker was drawn and replaced with a fresh 5 mL of PBS and continued for 6 h. The amount of drug release in the study was then determined by measuring the contents using UV-vis spectrophotometer at 570 nm.^{26,27}

Experimental animals

Thirty Swiss albino mice of opposite sexes weighing between 15 and 25 g were purchased from the animal house of the Department of Pharmacology, University of Jos, Nigeria. The animals were housed in a metal cage (90 cm x 45 cm) with access to clean water and feed and were maintained at normal temperature. They were allowed to acclimatize for two weeks in the laboratory before the *in vivo* experiment. Ethical approval for the use of these animals was given by Research Ethical Committee of the University of Jos, Nigeria (UJ/FPS/ F17-00379, date: 31.07.2018).

In vivo anti-inflammatory: carrageenan-induced anti-inflammatory mouse model

The mice were grouped into five groups of six animals. Mice in group 1 served as the negative control which received 2 mL normal saline, group 2 was the positive control which received 100 mg/kg diclofenac BP, while groups 3, 4, 5, and 6 were the treatment groups which received 200, 400, 800, and 1.200 mg/kg *b.w.* doses of *S. scabrum* extracts (*i.p.*). The doses were

calculated on 30% lethal dose obtained from previous toxicity studies.²⁷ Paw edema was induced in the mice by sub-plantar injection of carrageenan (0.1 mL of 1% carrageenan solution in 0.9% normal saline). The volume of paw edema was measured at 0, 1, 2, and 3 h using a Harvard apparatus plethysmometer (Massachusetts, USA). Average paw edema volumes at various intervals were measured.²⁸ This procedure was repeated using chitosan loaded with: crude EF (CE3); EF was loaded into chitosan because it was the most bioactive fraction, solanine (CS2) and pure solanine (S). Percent inhibition of paw edema volume was thereafter calculated from the formula below:

$$\text{Inhibition\%} = (\text{Pc} - \text{Pt}/\text{Pc}) \times 100$$

where, Pc= paw edema volume of control group, Pt= paw edema volume of treated groups.

In vitro anti-inflammatory study: inhibition of protein denaturation

The *in vitro* anti-inflammatory study was performed using protein denaturation model with slight adjustment.^{29,30} Briefly, different concentrations of extracts according to the doses used in encapsulation (*i.e.*, HF, EF, BF, chitosan loaded with EF extract (CE3), chitosan loaded with solanine (CS2), and solanine (S); 500, 1000, 1500, and 2000 µg/mL, were mixed with 5% bovine serum albumin (BSA, 1 mL) incubated at 27 °C for 15 min. The control was made up of distilled water and BSA. Denaturation of proteins was achieved by placing the mixture in a water bath at 65 °C for 15 min and allowed to cool. Sample absorbance was measured at 615 nm in triplicates. Percentage inhibition of inflammation was calculated from the formula below:

$$\text{Inhibition\%} = (\text{absorbance of control} - \text{absorbance of sample}/\text{absorbance of control}) \times 100$$

In vitro anticancer studies

Culturing cell lines

Antiproliferative potentials of each extract were determined using human breast cancer (MCF-7) and woman vagina melanoma (HMVII) cell lines. HMVII cell line was maintained in DMEM supplemented with 10% (v/v) fetal bovine serum (FBS), penicillin/streptomycin (1%), and 2 mM L-glutamine, while the MCF-7 cell line (ATCC, USA) was grown in serum free (with glutamine) Roswell Park Memorial Institute medium (RPMI-1640, Biocompare) with similar treatment condition. Each cell line was seeded at 1×10^5 cells/mL regularly in a humidified atmospheric condition of 5% carbon dioxide maintained at 37 ± 2 °C.³⁰

Cytotoxicity study

Evaluation of crude extracts, solanine, and chitosan-loaded extracts/drug cytotoxicity was performed by MTT assay. Briefly, cancer cell lines were seeded at a cell density of 8.000 cells/well in 96 well plates and incubated for 24 h before transfection. After 24 h of transfection, the medium was removed and 20 µL of MTT (5 mg/mL) was added to each well and incubated for 4 h. The medium was then removed and the cells were rinsed

with PBS (pH: 7.4). The formazan crystals formed in living cells were dissolved in 100 μ L DMSO *per* well. Relative cell viability (%) was then calculated for each experiment based on absorbance at 550 nm using a microplate reader. The viability of non-treated control cells was arbitrarily defined as 100%.^{31,32}

Transwell migration assay (modified Boyden chamber assay)

In vitro cell invasion and migration ability were performed by transwell assay. In this procedure, 8.0 μ m pore size transwell filters were placed in 24 well plates coated with 50 g matrigel (Corning Inc., USA). Then, 0.2 mL of cells (1×10^8 cells/L) in serum free medium was added to the top chambers of the transwells, while 0.2 mL of RPMI-1640 medium containing FBS (10%) was added to the bottom chambers. The cells were then incubated in the transwells at 37 °C with 5% CO₂ for 24 h. The non-migratory cells on the upper surface of the filter were wiped with cotton swab, while the migrated cells on the lower surface of the filter were fixed with 98.1% (v/v) methanol (Sigma Aldrich, USA) for 30 min, and then stained with Giemsa stain for 15 min. Four fields were counted on each filter under a microscope (Olympus, England) using 400x magnification. The mean values of readings were calculated.³³

Apoptosis assay by flow cytometry

Programmed cell death (apoptosis) was evaluated using a BD FACSLytic™ flow cytometer (BD Biosciences, USA). Briefly, cancer cell lines (MCF-7 and HMVII) were each seeded at a density of 100,000 cells/well in 24 well plates (Costar, USA). The cells were then incubated at 37 °C in a 5% CO₂ incubator for 24 h. After this, the cell lines were each treated with various concentrations (2.5, 5, and 10 μ g/mL) of solanine as well as chitosan-loaded extracts (CE3, CS2, and CDr2), and were then washed in 0.1 M PBS (serum free), trypsinized, and later fixed in 90% ethanol (v/v) (JoeChem Ltd, Nigeria). Finally, the cells were stained with a prepared solution containing a mixture of 20 μ g/mL PI (Sigma Aldrich, USA), 0.5% tween 80, and 0.1% (10 mg/mL) RNase A (Biocompare, USA) and incubated at 37 °C for 30 min. The apoptotic cells were sorted out at 405 nm scan and analyzed.³⁴⁻³⁶ The untreated cells served as the control. The experiment was conducted in triplicate. The percentages of apoptotic cells were determined according to the following formula:

$$\text{Apoptotic cells\%} = (\text{Lna} + \text{Dna}/\text{Lnc} + \text{Lna} + \text{Dnc} + \text{Dna}) \times 100$$

where Lnc = live cells with normal nuclei, Lna = live cells with apoptotic nuclei, Dnc = dead cells with normal nuclei, and Dna = dead cells with apoptotic nuclei.

In vitro caspase-3-like activity assay

Cell lines were seeded at 1×10^5 /cell on coverslips and treated with equal concentrations (100 μ g/mL) of plant extracts (E, S, CE3, CS2, and CDr4). It was then incubated overnight for 24 h. To detect caspase-3-like protease activities, Apo Alert caspase-3 colorimetric assay kit (Clontech, Palo Alto, USA) was used. After treatment, cells were rinsed in PBS solution and fixed in *para*-formaldehyde (4%) for 10 min at 45 °C. Pre-chilled methanol (-20 °C) was then used to permeabilize the

cells for 30 min. Total DNA was then stained with 4,6-diamidino-2-phenylindole for 15 min. The apoptotic strand breaks and total DNA were visualized using transmission epifluorescence microscopy.³⁷⁻⁴⁰

Evaluation of cellular morphology

Chitosan-loaded extracts (formulations) and extracts were trypsinized, fixed in 5% GA, and dehydrated to remove water before observing them with the Phenom SEM (Thermofisher, USA). Cells not treated were used as control.

Statistical analysis

The data are presented as mean \pm standard deviation (SD) of three different experiments. Significance differences between treated and control groups were compared using analysis of variance (One-Way ANOVA). The values of $p < 0.05$ were taken as statistically significant using GraphPad prism version 9.1.

RESULTS

Isolation of solanine glycoalkaloid

Only brief properties of this compound were mentioned here since it has been previously isolated from some plant families.⁴¹ A brownish solid crystal of solanine weighing 8.0 g was obtained with the following data: EI-MS m/z (M^+) 867 g/mol; molecular formula: C₄₅H₇₃NO₁₅; ¹H-NMR (CDCl₃, 3.500 MHz): 73 protons with olefin groups at chemical shift 5.3 ppm, O-H groups at 0.5-5.0 ppm all peaks were in the aliphatic regions; ¹³C-NMR showed 45 carbons mainly methyl carbons, melting point; 272.5 °C and HPLC analyses showed a retention time of 19 min with sharp peak area.

Bioguided fractionation of Solanum scabrum extract

Bioguided fractionation was carried out following solvents in increasing order of their polarities using HF, EF, and BF. Fractionations of the extract yielded the following fractions: HF, EF, and BF. Each of the fractions was bioguided by anti-inflammatory and anticancer activities to determine the most active fraction. Results were expressed as a percentage inhibition of paw edema volume in mice and cell viability of MCF-7 cell line, respectively (Table 1). The most active fractions (BF; 52.41 \pm 41% inhibition in inflammation model and EF; inhibitory concentration 50 (IC₅₀): 14.28 \pm 0.12 μ g/L) were further fractionated into four subfractions (BF.1, BF.2, BF.3, and BF.4) (EF.1, EF.2, EF.3, and EF.4) for their anti-inflammatory and anticancer activities, respectively. *n*-Butanol (BF.4) and ethyl acetate subfractions (EF.2) were the most active for anti-inflammatory and anticancer activities, respectively, and were further purified in silica gel column chromatography. Purification of isolated bioactive compounds was confirmed by the presence of single spots on the TLC plate.

Identification of isolated compound from leaf

The isolated most bioactive anti-inflammatory and anticancer compound from *S. scabrum* leaf was structurally elucidated and identified based on the data obtained from ¹H, ¹³C, HSQC, and HMBC-NMR (500 MHz, Bruker) as well as MS (Agilent technologies). ¹H-NMR showed seven signals, while ¹³C-NMR

displayed twelve carbons, which are mainly methyl carbons. These data were compared with those reported in literature (NIST, 2014). The anti-inflammatory compound was identified as 3-nitrodibenzofuran (dBF3N) with the following properties: color; white crystal, m/z ; 213, and $m.p.$; 181.49 °C (Figure 1).

Preparation and characterization of chitosan loaded extracts

From the results, nano-formulated extracts yielded 12.14 to 18.25 g (CE1-CE4), 10.05 to 16.22 g (CS1- CS4), and 8.12 to 14.06 g (CDr1-CDr4). Developed microspheres with larger sizes (in μm ; $1\mu\text{m} = 1000\text{ nm}$) possessed higher percentage EE than those with smaller sizes as seen from the table below. The data

Table 1. Bioguided fractionation of extracts for their anti-inflammation and anticancer effects

Extract	Anti-inflammation (%)	MCF-7 IC_{50} ($\mu\text{g/mL}$)
HF	12.01 \pm 0.20	88.24 \pm 4.12
EF	42.11 \pm 1.20	14.28 \pm 0.12
BF	52.41 \pm 2.11	92.24 \pm 4.02
Aspirin (reference for anti-inflammation assay)	76.32 \pm 2.04	NA
Doxorubicin (reference for anticancer assay)	NA	0.80 \pm 0.01

Results are mean \pm SD (at $p < 0.05$; one-way ANOVA), NA: Not applicable, HF: *n*-hexane fraction, EF: Ethyl acetate, BF: *n*-butanol, SD: Standard deviation, IC_{50} : Inhibitory concentration 50

from the characterization of formulated CSNPs are presented in Tables 2 and 3; Figure 2 below:

Anti-inflammatory activity test of extracts

The results of anti-inflammatory effects of the extracts by carrageenan-induced paw edema and protein denaturation assays are shown in Figure 3. From the results, the highest paw edema reduction of $2.68 \pm 0.01\text{ mm}$ was obtained in the treated group, which received 1200 mg/kg EF as well as mice that received 0.5 $\mu\text{g/mL}$ CE3 and solanine (S) with $1.44 \pm 0.01\text{ mm}$, and $2.52 \pm .02\text{ mm}$ after 3 h of carrageenan induction. Mice treated with HF at 200, 400, 800, and 1200 mg/kg *b.w.* *S. scabrum* extract and CSNPs loaded solanine (CS2) does not show significant decrease in paw volume from 1 to 3 h compared to the standard drug (Figure 3a). Similar results were obtained in Figure 3b where chitosan loaded solanine and HF recorded the lowest percentage inhibition of protein denaturation when compared with those of other treatment groups ($p < 0.05$; One-Way ANOVA).

Anticancer activities of extracts

Cytotoxicity study of extracts

Chitosan-loaded doxorubicin (CDr4) showed the highest IC_{50} value of 0.02 ± 0.01 and $0.01 \pm 0.01\text{ }\mu\text{g/mL}$ against MCF-7 and HMV7 cells, respectively. These values were followed by those obtained when the cells were exposed to solanine alone (S) with IC_{50} value of 8.13 ± 0.01 and $12.01 \pm 1.10\text{ }\mu\text{g/mL}$, respectively. However, the selective index (SI) of exposing the

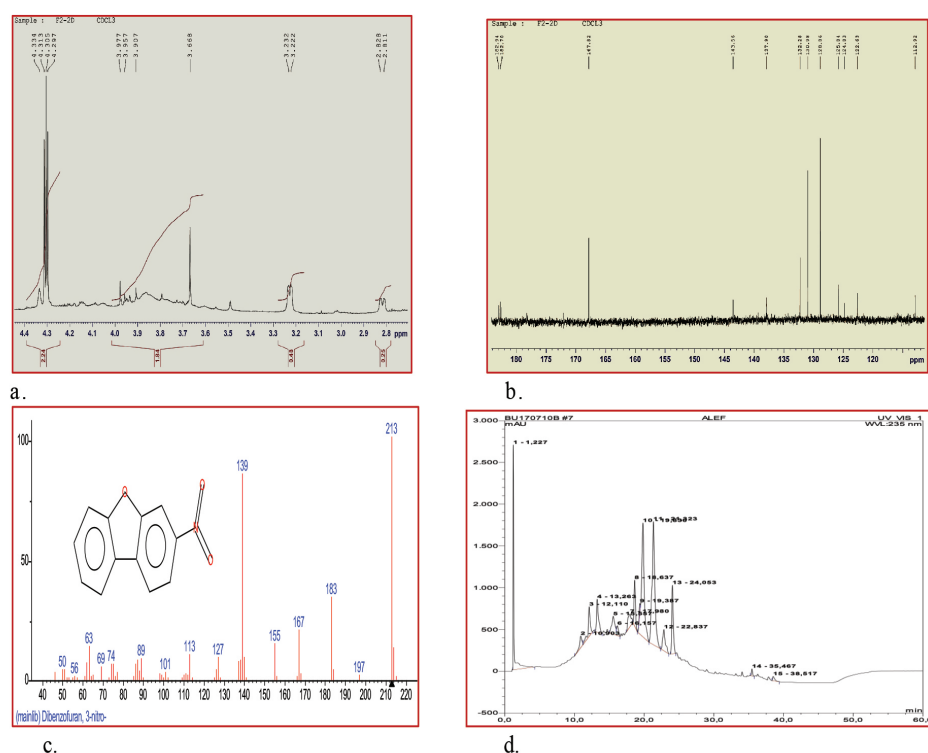


Figure 1. NMR and MS spectra and HPLC chromatogram of the isolated compound from *Solanum scabrum* leaf; a; ¹H-NMR with seven protons between 2.81-4.33 δ (ppm), b; ¹³C-NMR with twelve carbon atoms, c; MS with m/z 213 and molecular peak ion 139 at 62.89% peak area for 3-nitrodibenzofuran, d; HPLC chromatogram showed 19.11 min with isocratic elution. CDCl_3 was used as a solvent for NMR analysis. MS: Mass spectrometry, HPLC: High-performance liquid chromatography

Table 2. Preparation and characterization of chitosan NPs loaded with *Solanum scabrum* extract, solanine, and doxorubicin by spray drying technique

Batch code	GA ($\mu\text{g/mL}$)	Yield (%)	Size (μm)	% EE	Zeta* (mV)
CE1	500	12.14	0.05 ± 0.01	32.01 ± 1.2	20.22 ± 0.2
CE2	1000	12.22	0.06 ± 0.01	63.04 ± 2.1	25.14 ± 1.2
CE3	1500	18.25	0.01 ± 0.01	98.51 ± 1.3	25.66 ± 1.2
CE4	2000	18.10	0.02 ± 0.02	96.42 ± 1.2	24.15 ± 0.6
CS1	500	10.05	0.05 ± 0.01	44.24 ± 0.4	22.00 ± 0.1
CS2	1000	16.11	0.02 ± 0.01	92.12 ± 2.1	24.14 ± 0.2
CS3	1500	10.14	0.01 ± 0.02	93.22 ± 2.1	25.13 ± 0.1
CS4	2000	16.22	0.01 ± 0.10	66.12 ± 2.2	25.15 ± 0.2
CDr1	500	8.12	0.06 ± 0.01	46.08 ± 1.2	20.14 ± 0.1
CDr2	1000	8.99	0.08 ± 0.02	79.14 ± 2.1	24.00 ± 0.1
CDr3	1500	12.54	0.09 ± 0.03	92.16 ± 2.2	25.04 ± 0.1
CDr4	2000	14.06	0.02 ± 0.01	98.01 ± 2.2	24.99 ± 0.2

Results are mean \pm SD, chitosan concentration is 1.0% (w/v); drug loading is 2% (w/v). Drug released in 6 hours increases with the concentration of glutaraldehyde in all batch codes. The particle size was expressed in μm i.e., 1 μm : 1000 nm. The particle sizes range from 0.01 to 0.09 μm (i.e., 10 to 90 nm), GA: Glutaraldehyde, EE: Entrapment efficiency, *: Zeta potential, SD: Standard deviation, NPs: Nanoparticles, CE: Chitosan NPs loaded with *Solanum scabrum* extract, CS: Chitosan NPs loaded with solanine, CDr: Chitosan NPs loaded with doxorubicin

Table 3. *In vitro* drug release study of formulated chitosan NPs

Batch code	Drug release in 6 h (%)
CE1	22.01 ± 1.12
CE2	15.01 ± 0.10
CE3	$44.11 \pm 1.42^*$
CE4	18.22 ± 1.20
CS1	28.04 ± 2.12
CS2	$64.18 \pm 2.40^*$
CS3	25.10 ± 1.14
CS4	18.24 ± 1.24
CDr1	33.08 ± 2.54
CDr2	38.44 ± 0.22
CDr3	40.11 ± 1.50
CDr4	$52.10 \pm 2.01^*$

*Best optimized conditions were selected for anti-inflammatory and anticancer studies. Results are mean \pm SD (n: 3). SD: Standard deviation, NPs: Nanoparticles, CE: Chitosan NPs loaded with *Solanum scabrum* extract, CS: Chitosan NPs loaded with solanine, CDr: Chitosan NPs loaded with doxorubicin

MCF-7 breast cancer cell to solanine alone was not all that encouraging as compared with that of CDr4 after 72 h and with same concentrations (12.5, 25, 40, and 50 $\mu\text{g/mL}$) *in vitro*. From the results also, chitosan-loaded EF (CE3) established improved values in IC_{50} and SI than other treatment groups (Table 4).

Invasion and migration assay

Transwell assay helps us to know how cancer cells migrate and invade other tissues and respond to chemoattractants and move toward them. From the results in Figure 4 below, exposure of the cancer cells to chitosan-loaded solanine (CS2) does not remarkably prevent the migration and invasion of the human vaginal melanoma cells HMVII. However, the exposure of cell lines (HMVII and MCF-7) to solanine alone significantly prevented the migration and invasion of MCF-7 breast cancer cell lines. These results were comparable with that of the doxorubicin anticancer drug ($p < 0.05$; one-way ANOVA).

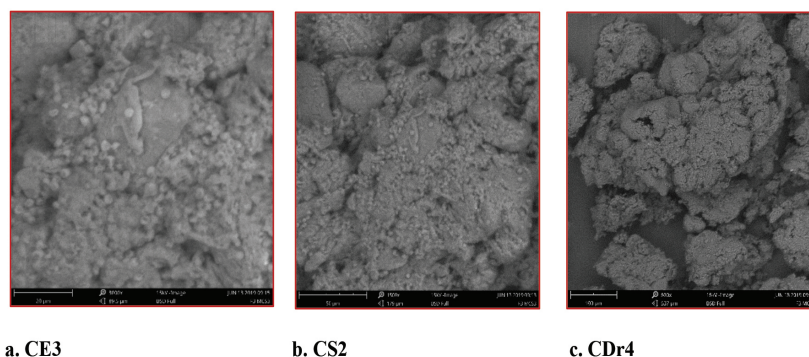


Figure 2. SEM morphology of chitosan loaded with crude *Solanum scabrum* extract (CE3), solanine (CS2), and doxorubicin (CDr4). Glutaraldehyde was used as cross-linking agent 3000x. SEM: Scanning electron microscope

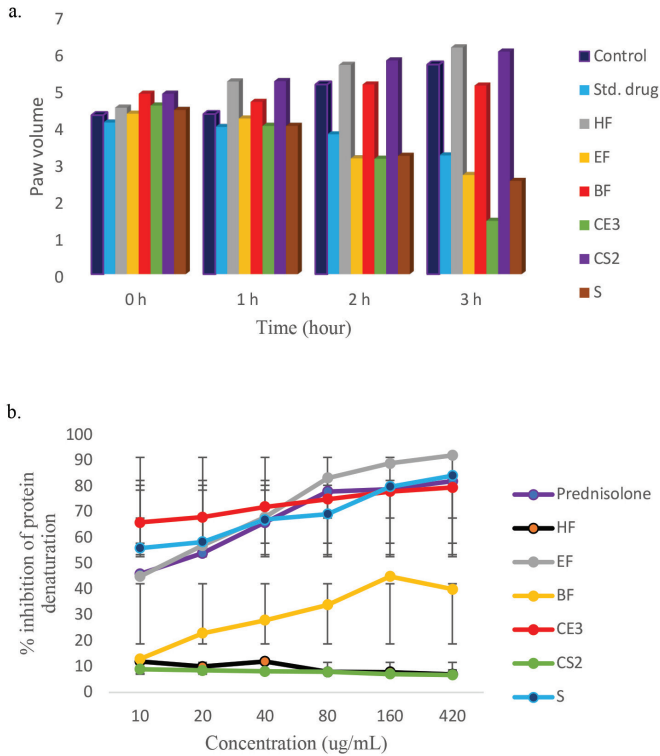


Figure 3. Anti-inflammatory activity of *Solanum scabrum* extracts and chitosan NPs loaded extracts; a; (carrageenan-induced paw oedema), b; (effects on protein denaturation). Results are means \pm SD. The values of $p < 0.05$ were statistically significant. HF: Hexane fraction, EF: Ethyl acetate fraction, BF: Butanol fraction, CE3: Chitosan-loaded crude extract batch code 3, CS2: Chitosan-loaded solanine batch code 2, S: Solanine, SD: Standard deviation, NPs: Nanoparticles

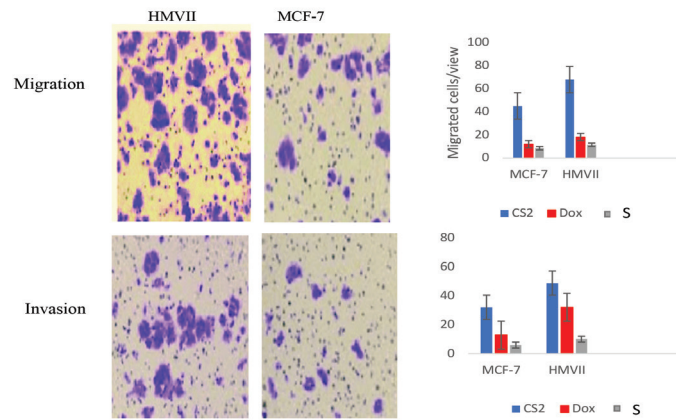


Figure 4. Transwell migration and invasion assay of solanine (S) and chitosan-loaded solanine batch code 2 (CS2) as viewed using a hemocytometer. Results are mean \pm SD for three replicate readings. Cellular migration and invasion were much more pronounced with CS2 treatment when compared to cells exposed to solanine alone. S: Solanine, CS2: Chitosan-loaded solanine batch code 2, SD: Standard deviation, Dox: Doxorubicin

Apoptosis study

From the results obtained in the figures below, exposure of the cancer cells to CS2 does not result in early apoptosis of the cells, however, cellular integrity was lost as seen from the flow cytometry images. Early apoptosis was induced on exposure of cells to solanine glycoalkaloid after 72 h with shrinking nuclei after staining with PI. Percentage apoptosis in cells was high with increased concentration of solanine (S) in the early and late stages of apoptosis as well as increased caspase-3-activity in MCF-7 breast cancer cell line (Figure 5 a-c). This result was comparable to that of doxorubicin as reference drug.

Table 4. Inhibition of MCF-7 and HMVII cancer cells growth by crude fractions and chitosan NPs-loaded extracts after 72 h incubation by MTT assay

Extracts	IC ₅₀ (μg/mL)		CC ₅₀ (μg/mL)	
	MCF-7	HMVII	VeroE6 (normal cell)	Selectivity index (MCF-7)
HF	234.17 \pm 6.12	128.44 \pm 4.20	132.11 \pm 1.24	0.56
EF	10.10 \pm 0.01	18.32 \pm 1.21	96.34 \pm 2.11	9.54
BF	22.10 \pm 0.01	88.21 \pm 2.52	112.24 \pm 1.42	5.08
S	8.13 \pm 0.01	12.01 \pm 1.10	206.94 \pm 2.21	25.45
E	21.01 \pm 0.21	24.00 \pm 1.22	88.94 \pm 0.18	4.23
CE3	10.04 \pm 0.01	12.44 \pm 1.14	128.14 \pm 4.12	12.76
CS2	96.22 \pm 4.12	102.10 \pm 6.12	220.45 \pm 4.22	2.29
Dox	0.8 \pm 0.01	0.24 \pm 0.01	22.02 \pm 1.01	27.53
CDr4	0.02 \pm 0.01	0.01 \pm 0.01	56.36 \pm 0.14	>100

Results are mean \pm SD (n: 3). MCF-7, HMVII, and VeroE6 cells (1×10^6 /cells) were treated with each of the extract alone, CE3, CS2, and CDr4 loaded chitosan NPs as well as doxorubicin alone for 72 h. Cancer cells were pre-treated 30 min separately for each sample prior to chitosan-loaded drugs treatment. Each experiment was performed in triplicate. The concentration of doxorubicin was 0.5 mg/mL in all the experiments involving doxorubicin. The selectivity index of these treatments against the MCF-7 was determined from the normal cell VeroE6. HF: Hexane fraction, EF: Ethyl acetate fraction, BF: Butanol fraction, S: Solanine, E: *Solanum scabrum* leaf methanol extract, CE3: Chitosan-loaded extract batch code 3, CS2: Chitosan-loaded solanine batch code 2, Dox: Doxorubicin, CDr4: Chitosan-loaded doxorubicin batch code 4, SD: Standard deviation, NPs: Nanoparticles, MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide

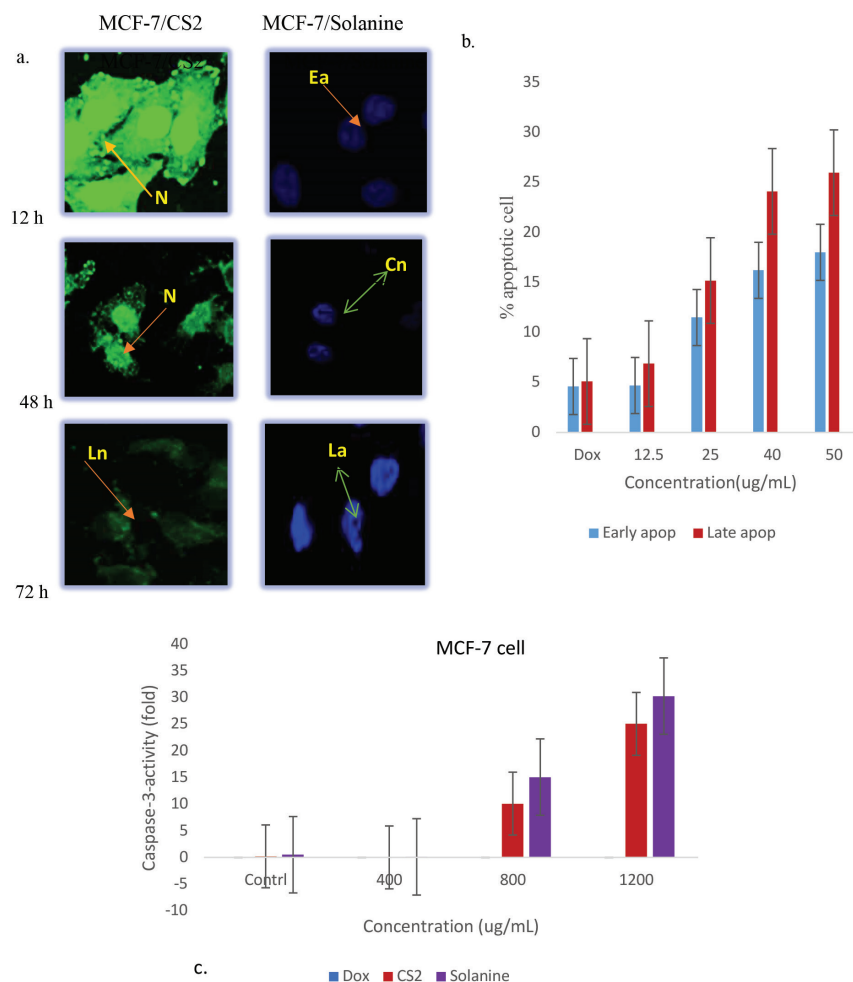


Figure 5. Fluorescence images of MCF-7 cancer cells after treatment with CS2 and solanine (S) with various concentrations (12.5, 25, 40, and 50 $\mu\text{g/mL}$) at 12, 48 and 72 hours. Apoptotic cellular death and necrosis were detected by staining with propidium iodide and analysed with flow cytometry. Results are mean \pm SD for three consecutive readings. $p < 0.05$ was taken as a significance different. Ea: Early apoptosis, La: Late apoptosis, Cn: Clear nuclear, N: Necrosis of cells, Ln: Late necrosis, S: Solanine, SD: Standard deviation

Similarly, chitosan-loaded extract (CE3) and solanine (CS2), do not induce higher percentage of cellular apoptosis in human vaginal melanoma cell HMVII when compared to that in breast cancer cell MCF-7. The result further showed that solanine induced the highest percentage of cellular apoptosis in all the cell lines followed by chitosan loaded doxorubicin (CDr4) after 72 h (Table 5). These values obtained were statistically significantly different from the control ($p < 0.05$; one-way ANOVA).

DISCUSSION

In traditional medicine, plant parts such as leaves are reduced into fine powders using mechanical means to improve their contact with solvents and promote the penetration of extracting solvents into the tissues. Extraction, which normally follows pulverization, is usually carried out by a cold maceration technique, where the powdered leaves are soaked in an appropriate volume of methanol to obtain the active phytoconstituents from the leaves. In this study, the use of

this extraction method also helps to prevent the loss of active metabolites due to heat. This is because active constituents (secondary metabolites) in plants are readily destroyed on exposure to heat,⁵ hence, the main reason why the leaves of *S. scabrum* we collected were air-dried under shade. Moreover, drying of medicinal plant parts helps to reduce the moisture content thereby preventing microbial and enzymatic action, hence, extending the shelf life of the part.⁴¹

Nanotechnology as an aspect of medical sciences involves the engineering of functional systems at the molecular scale. Nanomedicine is an emerging aspect of nanotechnology where NPs are employed to prevent, diagnose, and treat diseases. Because of their biocompatibility, specificity, non-toxicity, and biodegradability, NPs have been used to deliver various drugs to a specific sites in the body.⁴² In our current study, CSNP was used to deliver various extracts and doxorubicines to cancer cells. Chitosan is naturally obtained by chitin deacetylation. It is a positively charged nanocarrier. Because of its ability to adhere to cells and readily absorbed in the body, it is the most

Table 5. Percentage apoptosis induced by exposure of MCF-7 and HMVII cancer cell lines to CE3, CS2, CDr4, solanine, and doxorubicin

Cancer cells	Sample	Concentration (µg/mL)			
		12.5	25	40	50
MCF-7	CE3	25.12 ± 0.01*	28.02 ± 1.01	28.98 ± 1.20	30.41 ± 2.01*
	CS2	5.10 ± 0.01*	5.88 ± 0.01*	6.15 ± 0.01	4.02 ± 0.01*
	CDr4	76.87 ± 2.11	78.82 ± 2.56	88.14 ± 4.22	88.14 ± 2.88*
	Solanine	68.24 ± 2.41*	72.12 ± 4.01	78.99 ± 3.24*	84.44 ± 4.10*
	Dox	44.22 ± 2.08	58.38 ± 2.11	58.88 ± 2.21	62.10 ± 2.24
HMVII	CE3	5.12 ± 0.01*	25.04 ± 2.11*	28.10 ± 1.01	36.02 ± 2.01*
	CS2	5.44 ± 0.01*	5.88 ± 0.01	6.21 ± 0.01*	5.02 ± 0.01*
	CDr4	28.10 ± 2.01	35.82 ± 2.01*	38.40 ± 2.01	45.15 ± 2.03
	Solanine	36.22 ± 1.01*	48.26 ± 2.01	56.44 ± 2.44	56.88 ± 2.10*
	Dox	15.18 ± 1.11*	28.02 ± 1.02	28.42 ± 1.01	36.56 ± 1.11*

Results are mean ± SD, *Significance different at $p < 0.05$ (one-way ANOVA) for n: 3. CE3: Chitosan-loaded crude extract batch code 3 from ethyl acetate fraction, CS2: Chitosan-loaded solanine batch code 2, CDr4: Chitosan-loaded doxorubicin batch code 4, Dox: Doxorubicin

favorable nanocarrier for targeted drug delivery. It also has the characteristics of always being attracted to negatively charged cells and this is the feature that makes CSNPs a candidate of choice for the treatment of solid tumors.^{43,44} Apart from the aforementioned, CSNPs have also shown the capacity to increase the permeability of cell membranes in both *in vitro* and *in vivo* studies, stabilizing agent owing to its ability to form film, ability to be modified chemically and its low cost.⁴⁵⁻⁴⁷ From the results obtained in this study, drugs loaded into CSNPs exhibited more biological activities than when the drugs were used directly on the organs or tissues.

In this study, *S. scabrum* extracts were evaluated for their anti-inflammatory and anticancer effects on human vaginal melanoma (HMVII) and breast cancer cell lines (MCF-7) *in vitro*. In our study, bioguided fractionation of *S. scabrum* extracts indicated that BF and EF exhibited high anti-inflammatory activity *in vitro*. No anti-inflammatory and anticancer activities were observed in the HF. EF was the most sensitive and biologically active of all the fractions in the MCF-7 cell line at the concentration investigated (Table 1). Further subjection of EF to chromatography isolation gave rise to 3-nitrobenzofuran (dBF3N) as characterized by GC-MS and NMR spectroscopy, which is the bioactive compound from the fraction, and it is one of the active compounds responsible for the observed biological activities of the fraction in this study. Similarly, in a previous study, solanine alkaloids were isolated from *S. scabrum*, where a broad range of glycoalkaloids of solasodine were reported in *S. scabrum* berries by using HPLC-UV/VIS-MS or MS/MS methods.⁴⁸ This study was the first time that it is being isolated from the leaf extract.

In this present study, cross-linking of CSNPs with GA slightly affected the yield, size (expressed in µm), zeta potential, and percentage entrapment from 500 to 2000 µg/mL (Table 2, Figure 2), as was also reported that the use of GA as a cross-

linking agent resulted in increase in size of NPs with narrow polydispersity index and highest zeta potential.⁴⁹ The presence of some aggregates in the morphology of the prepared CSNPs as displayed by the SEM (Figure 2) do not mean that it was not uniform with the drugs but they represent medium sized CSNPs which ranges from 0.01 to 0.09 µm (*i.e.*, 10 to 90 nm). This is because NPs range for 1-100 nm in size.⁴⁹ From the present study, as the conditions of prepared CSNPs were optimized, the particle size, encapsulation efficiency, and yield increased linearly with the concentration of GA (Table 2). Size and amount of drug entrapped or encapsulated by NPs are very crucial in drug delivery as these will affect the amount of drug release.^{26,27} In this current study, the small and medium sizes of formulated CSNPs greatly assisted in effective delivery of these encapsulated drugs. This is because the smaller the size of a NP, the more it is unnoticed by the body's defence mechanisms until it reaches the targeted site to deliver its contents in controlled release.²⁷

Similarly, concentration of the cross-linking agent GA plays crucial roles in optimization conditions of nanosized materials as seen in the study (Table 3). Anti-inflammatory agents are substances that are capable of reducing inflammations such as redness of eyes, swellings, and pains in the body. The mechanism of these agents is by blocking some pain receptors in the body, which can cause inflammation. In this current study, CSNP-loaded solanine resulted in a percentage increase in paw volume, whereas the animals treated with solanine alone showed a significant reduction in paw volume within 3 h (Figure 3a, b). Apart from chitosan loaded with solanine, other chitosan-loaded drugs showed similar reduction in paw volume, which is a sign of anti-inflammation, thereby reducing swelling. Similarly, mice exposed to solanine glycoalkaloid produced the best denaturation of proteins than CS2, HF, and BF. The implication of this may be that chitosan must have

exhibited activity reducing effects on solanine glycoalkaloid. The reasons for such reduction were not fully understood in the current study, however, it can be linked to the presence of nitrogen atom in the compound since chitosan also possessed some nitrogen atoms.⁵⁰ Solanine-treated carrageenan-induced inflammation, EF and CE3 exhibited the highest percentage protein denaturation (Figure 3b). These results were compared with that of the control drug prednisolone ($p < 0.05$; one-way ANOVA). The result further showed that CSNP drug delivery act synergistically with other drugs and extracts except solanine. The presence of aglycone moiety in solanine must have been responsible for the observed characteristics since solanine like other steroidal glycoalkaloids were reported to possess anti-inflammatory, anticarcinogenic, and antimicrobial activities.⁵⁰ It has been reported that most anti-inflammatory agents exert their pharmacological potentials by inhibiting the enzyme cyclooxygenase, which produces the hormone prostaglandins, from inducing cytokines, which are usually released during inflammation.⁵¹ Chitosan-loaded drugs and extracts must have exerted their action similarly but the release pattern was sustained release.

An ideal anticancer agent must be able to inhibit the growth of cancer cells, prevent metastasis, and induce cellular apoptosis.⁵² In this study, nanoencapsulated drug and extracts showed some levels of cytotoxicity against cancer cells (Table 4). For instance, chitosan-loaded doxorubicin (CDr4) has the highest cytotoxic effects on breast cancer cell MCF-7 and human vaginal melanoma cell HMVII with IC_{50} values of $0.02 \pm 0.01 \mu\text{g/mL}$ and $0.01 \pm 0.01 \mu\text{g/mL}$, respectively and with a SI of greater than 100 against MCF-7 cell line. Likewise, cancer cells exposed to CE3 treatment had IC_{50} values of $10.04 \pm 0.01 \mu\text{g/mL}$ and $12.44 \pm 1.12 \mu\text{g/mL}$ against MCF-7 and HMVII cell lines, respectively. In all treatments, there were greater synergistic effects among these drugs and CSNPs. However, exposure of cancer cells to solanine steroidal glycoalkaloid showed the highest cytotoxic effects on MCF-7 and HMVII cancer cell lines with IC_{50} values of $8.13 \pm 0.01 \mu\text{g/mL}$ (SI; 25.45) and $12.01 \pm 0.01 \mu\text{g/mL}$, respectively. For effective cancer therapy, drug agents must be able to initiate cellular apoptosis in cancer cells, resulting in the death of cancer cells.^{35,40} The present study revealed that chitosan loaded with solanine does not result in elevated percentage apoptosis induction in all cancer cell lines unlike other treatments where there was significant rise in percentage apoptosis induction (Table 5). From the study, cancer cells treated with chitosan-loaded doxorubicin anticancer drug showed the highest percentage of apoptosis induction. It was followed by cancer cells treated with solanine unloaded into CSNPs. The significant reduction in apoptotic effect of CS2 was not fully understood in this current study but may be due to the presence of similar nitrogen atoms in solanine and CSNPs that work in anti-synergism with each other. However, this mechanism needs to be further studied for a conclusive report. Also, in the transwell migration assay, migration and invasion were greatly reduced in MCF-7 cancer cells exposed to solanine alone than chitosan-loaded solanine glycoalkaloid (CS2) at various concentrations (Figure 4). Our

study further revealed that MCF-7 breast cancer cells exposed to solanine alone induced both early and late apoptosis with minimal clear nuclei in concentration dependent fashion as seen from the fluorescence images after 72 h (Figure 5). Chitosan loaded solanine glycoalkaloid (CS2) does not induce significant apoptosis. Moreover, exposure of MCF-7 breast cancer cells to solanine alone results in an increase in caspase-3 activity from 400 to 1200 $\mu\text{g/mL}$ concentrations.

It was suggested from previous studies that the toxicity mechanism of solanine was due to chemical interactions within mitochondrial complexes. This is because exposure of cancer cells to solanine alone opens K^+ channel of mitochondria thereby setting up membrane potential difference, which will in turn force Ca^{2+} to be transported down the concentration gradient into the mitochondria complexes. This elevated calcium ion concentration created cellular damage and apoptosis of cells.⁵³ This claim was consistent with the role of solanine in the current study. It has been reported that caspase-3 is important for proper development of the brain as well as necessary for other apoptotic processes in cancer cells.^{54,55} Caspase-3 is implicated in apoptosis and is very crucial for the condensation of chromatin as well as fragmentation of DNA in many cells, especially cancer cells. This elevated level of caspase-3 activity witnessed in this study from MCF-7 breast cancer cells exposed to solanine alone was responsible for the observed high percentage of apoptosis obtained from this study (Table 4). Furthermore, this study affirmed that solanine isolated from *S. scabrum* leaf extract exerted cytotoxic effects on MCF-7 and HMVII cancer cells by inducing early- and late-stage apoptosis as well as necrosis.

CONCLUSION

Our studies showed that CSNPs loaded with *S. scabrum* extracts possessed anti-inflammatory in carrageenan-induced paw edema and anticancer activity against MCF-7 breast cancer and HMVII human vaginal melanoma cell lines. However, the study also revealed that the encapsulation of solanine into CSNPs produced lower activities than when solanine was used alone. It disclosed that the use of solanine alone reduced percentage paw volume, decreased migration and invasion, increased apoptosis, and elevated caspase-3 activity. Finally, due to the reported toxicity of solanine, the concentration was reduced in this study. It is suggested therefore that the lack of synergistic effects between solanine and CSNPs should be further investigated.

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Ethics

Ethics Committee Approval: The approval was issued by the University of Jos Research and Ethical Committee with the approval number of UJ/FPS/F17-00379, date: 31.07.2018.

Informed Consent: Not applicable.

Peer review: Externally peer-reviewed.

Authorship Contributions

Concept: C.A.U., Design: C.A.U., E.O.I., Data Collection or Processing: C.A.U., E.O.I., A.C.F., Analysis or Interpretation: C.A.U., E.O.I., Literature Search: C.A.U., E.O.I., A.C.F., Writing: C.A.U., E.O.I.

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