

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

Short Title: Anti-Inflammatory and Anticancer Activity of Chitosan-Solanine

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ABSTRACT

INTRODUCTION: *Solanum scabrum* Mill. commonly 'African nightshade' or 'garden huckle berry' 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 was aimed at evaluating the anti-inflammatory and anti-cancer activities.

METHODS: Fractions were tested for anti-inflammatory potential and *in vitro* anti-cancer activity on MCF-7 and HMVII cell lines by carrageenan-induced edema in mice, and cytotoxicity assays such as MTT, transwell migration and invasion assays, apoptosis study by flow cytometry respectively.

RESULTS: Bio-guided isolation yielded a white crystalline compound 3-nitrodibenzofuran (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 the ¹³C showed twelve carbons which are majorly methyl carbons at δ (ppm) between 120-195. All tested samples showed a dose-dependent anti-inflammatory activity in carrageenan induced mice. The isolated compound, solanine and chitosan-loaded drugs showed significant inhibitory activity on the cell lines with an 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 an IC₅₀ values of 0.02 and 0.06 µg/mL on MCF-7 and HMVII cancer cells respectively. The selective index of solanine was the lowest from the study, hence, it lacks the ability to differentiate between cancerous and normal cell Vero E6 cell line. Chitosan-loaded drugs quicken early apoptosis and sustained late apoptosis in cells from the study, with much improved selective indices.

CONCLUSION: The results obtained from this study further affirmed the use of chitosan NPs as carrier for anticancer drugs.

Keywords: *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 like medicines, foods, shelters, etc., since time immemorial. Plants have played crucial roles in mitigating against the deleterious effects of various diseases from different regions of the world.^{1,2} As one moves from one geographical location to the other, plants morphological parts such as leaves, stembarks, roots, flowers, seeds and buds are used ethnobotanically in various forms such as anti-inflammatory, anti-malarial, anti-cancer, antioxidant, anti-convulsant,

anti-helminthic, antidiabetic, anti-hypertensive, anti-viral and anti-ulcer agents. Some of the preparations are used as decoctions, infusions, tinctures, ointments or powders. Regardless of their mode of preparation, the main goal is to achieve an ethnopharmacological effect on the targeted disease. Recently, the use plants and compounds from plants as anti-inflammatory and anti-cancer agents have gained popularity, especially in countries with low access to conventional anti-inflammatory and anti-cancer drugs due to lack of income and availability of quality drugs. In addition, the side effects of most anti-inflammatory and anti-cancer drugs have paved way for the lack of trust on these conventional drugs in Africa countries such as Nigeria, Cameroon, Ghana, South-Africa, Egypt, Kenya, Congo DR, Gambia, Sudan, among others.³⁻⁶ The ability of medicinal plants to illicit remarkable ethnopharmacological effect on organs, tissues and cell is solely dependent on the secondary metabolites present in the plant. For instance, alkaloids, flavonoids, saponins, tannins and terpenoids, have been reported to play various roles in disease situations of humans.⁷⁻⁹

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

Cancer accounts for about 40 % deaths in the world. The major causes of cancer are not fully understood, but it is believed that they are caused by mutations of genes called oncogenes. Cancers are named according to the organ or part of the body they are found, and they all have semblances in the way they illicit pathological effects in the humans.^{12,13} Till date, treatment of cancers with conventional drugs have yielded little of no results at all due to the negative side effects of most anticancer drugs. Current research is focused on the use natural anti-cancer agents from plants that have shown potent ethnopharmacological effects on cancer cells. These phytoconstituents have been used ethno-medically to treat successfully cancers like those of 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 carries such as chitosan to deliver their contents onto cancer cells, thereby inducing apoptosis in the cell.^{14,15} Chitosan nanoparticles are particles of size 1-1000 nm that are made the exoskeletons of crustaceans (crabs and prawns). They are used as carries for anticancer, antifungal, and anti-microbial 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 and ability to pass overcome evade the body's defence un-noticed.

Among the plants used to treat inflammation and cancers in Mambila (Taraba State, Nigeria) is *Solanum scabrum* Mill (commonly called African nightshade or garden huckle berry). It is locally called 'Kombi' in Hausa and 'Fulfulde' languages. The leaf is used to make 'kombi' and 'njama 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 anti-oxidant, anticancer, analgesic, anti-inflammatory, anti-spasmodic, and vasodilatory activities (these claims are still being been 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). 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 on 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 as well as for treating asthma, acute cough and cold.²²

In this present study, we evaluated the anti-inflammatory potential and *in vitro* anti-cancer activity of *S. scabrum* on breast and vaginal melanoma cell lines. We isolated solanine glycoalkaloid from the leaf crude methanol extract, and checked its purity using TLC and HPLC. The effects of the 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 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), DMSO, glutaraldehyde, propidium iodide (PI), 0.5% tween 20 solution, and 0.1% RNase were obtained from Benrocks' Medicals Nigeria (retail agent Sigma Aldrich). Apo alert caspase-3-colorimetric assay kit was purchased from Clontech (USA) while, chitosan 100G (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 grades.

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). The identification of the plant was done by a Taxonomist Dr. Jones Ponè of the Forest Guide, Ogurugu, Nigeria, with a voucher number OFG/SOL/0024. The leaves were air-dried under shade for two weeks and pulverized into fine powder using an electronic blender (made in China), weighed and stored in a clean container for further use.

Preparation of extract

The powdered leaves of *S. scabrum* weighing 1500 g were extracted with 2.5 L of methanol using cold maceration technique for 72 h. It was carefully filtered thrice 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 coloured extract was then stored in a clean sample bottle and kept in a refrigerator at -4 °C for onward use.

Isolation of solanine glycoalkaloid

Exactly 80 g of powdered leaves were subjected to cold macerated with 250 mL 5 % acetic acid solution (v/v) in a 500 mL capacity beaker for 24 h. The extract was filtered using a Whatman No. 1 filter paper to remove any cellular debris present. The content was warmed to 70°C and allowed to cool. The pH was adjusted to 10 by adding 10 mL solution of conc. NH₄OH in dropwise, and centrifuged at 1200 rpm for 5 min. The supernatant was discarded while the precipitate was washed with 1 % NH₄OH, re-centrifuged and concentrated *in vacuo* to obtain crude solanine. Presence of solanine alkaloid was qualitatively confirmed by an instant production of red to violet colouration with formaldehyde and sulphuric 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). TLC plate was used to check for the purity of the solanine glycoalkaloid 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

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

Bio-guided 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 respectively), and partitioned three times each with 500 mL of n-hexane (HF), ethyl acetate (EF) and n-butanol (BF) bio-guided by anti-inflammatory and anti-cancer 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 anti-inflammatory and anti-cancer activities *in vitro*. The fractions with most active bio-activities were each subjected to silica gel (60 x 120 mesh; 100 g) column chromatography using gradient elution fractions with the following solvent systems: HF; hexane: ethyl acetate (7:3), EF; hexane: EtOAc: MeOH (5:3:2), and BF; hexane: EtOAc: butanol (6:3:1). 50 sub-fractions of 30 mL were collected from each fraction. Sub-fractions with similar profiles (R_f values) each on the TLC plates were pooled together, and further subjected silica gel column to obtain additional sub-fractions. Sub-fractions with the most 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 GC-MS.

Preparation and characterization of chitosan NPs-loaded extracts

Extracts weighing 1 g each was dissolved in 400 mL 1 % acetic acid solution (v/v) in a beaker was 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, 500µL glutaraldehyde (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 glycoalkaloid, and CDr1 to CDr4 for chitosan-loaded with doxorubicin anti-cancer drug. Batch codes CE1, CS1 and CDr1 were not cross-linked 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 chitosan NPs

The formulated chitosan NPs were dried using a nano spray dryer (Shanghai, China). The dried microspheres were collected and weighed, while the % yield was calculated using the formula below:

$$\% \text{ Yield} = (\text{Final weight of dried chitosan NPs} / \text{Initial weight of starting materials}) \times 100. \text{ }^{25}$$

Morphology of chitosan NPs

The surface morphology of chitosan NPs using a phenom desktop scanning electron microscope with fully integrated EDS and SE detector (Thermo Fisher Scientific, NG). Diethyl pyrocarbonate dissolved chitosan NPs was observed at 3000x magnification for the morphology, and the extent of encapsulation.²⁵⁻²⁷

Particle size of chitosan NPs

The chitosan NPs were diluted in 0.1M KCl and placed in an electrophoresis cell of field potential 15.4 v/cm connected to Zeta sizer apparatus (Malvern Zeta sizer 3000HS, UK) to determine the sizes and charges of the microsphere. Three readings were taken and means taken.²⁷

Percentage entrapment or encapsulation efficiency of chitosan NPs

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

$$\% \text{ entrapment/encapsulation efficiency} = (\text{experimental drug contents} / \text{total drug contents}) \times 100.$$

The prepared nanoencapsulated formulations were used for *in vitro* anti-cancer study.

Drug release study of chitosan NPs

The *in vitro* drug release study was carried out using dialysis tubes method. In this procedure, the chitosan NPs prepared were dissolved in 5 mL phosphate buffer saline (PBS) of pH 7.4. Then 500 mL 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 tied using clean ropes. It was then placed in the beakers and subjected to stirring under magnetic stirrer at 1500 rpm at 37 °C. In every one hour, 5 mL of the contents from the beaker is drawn and replaced with a fresh 5 mL PBS, and continued for 6 h. The amount of drug release in the study was then determined by measuring the contents using the 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 45cm) with access to clean water and feed and were maintained at normal temperature. They were allowed to acclimatized for two weeks in the laboratory prior to *in vivo* experiment. Ethical approval for the use of these animals was given by the Research Ethical Committee of the University of Jos, Nigeria.

In vivo anti-inflammatory: carrageenan induced anti-inflammatory mice 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 hours 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 ethyl acetate fraction (CE3); the ethyl acetate fraction was loaded into the chitosan because it was the most bioactive fraction, solanine (CS2) and pure solanine (S). The % inhibition of paw edema volume was thereafter calculated from the formula below:

$$\% \text{ inhibition} = (P_c - P_t/P_c) \times 100$$

where, P_c = paw edema volume of control group, P_t = 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., hexane fraction (HF), ethyl fraction (EF), butanol fraction (BF), chitosan loaded with ethyl acetate extract (CE3), chitosan loaded with solanine (CS2), and solanine glycoalkaloid (S); 500, 1000, 1500 and 2000 $\mu\text{g/mL}$) were mixed with 1 mL 5 % bovine serum albumin (BSA) 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 water bath at 65 °C for 15 min, and allowed to cool. Sample absorbance was measured at 615 nm in triplicates. The 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 anti-cancer studies

Culturing of cell lines

The anti-proliferative potentials of each extract were determined using human breast cancer (MCF-7) and woman vagina melanoma (HMVII) cell lines. The HMVII cell line was maintained in Dulbecco's minimum essential medium (DMEM) supplemented with 10 % (v/v) foetal bovine serum (FBS), 1% penicillin / streptomycin and 2 mL-glutamine, while the MCF-7 cell line (ATCC, USA) was grown in a 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 °C ± 2 °C.³⁰

Cytotoxicity study

Evaluation of crude extracts, solanine glycoalkaloid and chitosan-loaded extracts/drug cytotoxicity were 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 prior to 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 the living cells were dissolved in 100 μL DMSO per well. Relative cell viability (%) was then calculated for each experiment based on absorbance at 550 nm using a micro plate 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 performed by transwell assay. In this procedure, 8.0 μm pore size transwell filters were placed in 24-well plates coated with 50 μg 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 10 % FBS was added to the bottom chambers. The cells were then incubated in the transwells at 37 °C with 5 % CO_2 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 the microscope (Olympus, England) using 400x magnification. The mean values of the readings were calculated.³³

Apoptosis assay by flow cytometry

Programmed cell death (apoptosis) was evaluated using a BD FACSLyric™ flow cytometer (BD Biosciences, USA). Briefly, cancer cell lines (MCF-7 and HMVII) were each seeded at the density of 100, 000 cells/well in 24-well plates (Costar, USA). The cells were then incubated at 37 °C in a 5 % carbon dioxide incubator for 24 h. After this, the cell lines were each treated with various concentrations (2.5, 5, and 10 $\mu\text{g/mL}$) of solanine alkaloid as well as chitosan-loaded extracts (CE3, CS2, and CDr2), and were then washed in 0.1M PBS (serum free), trypsinized, and later fixed in 90 % ethanol (v/v) (JoeChem Ltd, Nigeria). Finally, the cells were then stained with a prepared solution containing mixture of 20 $\mu\text{g/mL}$ propidium iodide (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 triplicates. 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 coverslip, and treated with equal concentrations (100 $\mu\text{g}/\text{mL}$) of plant extracts (E, S, CE3, CS2, and CDr4). It was then incubated overnight for 24 h. To detect caspase-3-like protease activities, the Apo Alert caspase-3 colorimetric assay kit (Clontech, Palo Alto, USA) was used. After treatment, cells were rinsed in PBS solution, and fixed in 4 % para-formaldehyde for 10 min at 45 °C. Pre-chilled methanol (-20 °C) was then used to permeabilized the cells for 30 min. The total DNA was then stained with 4, 6-diamidino-2-phenylindole (DAPI) 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 scanning electron microscope (Thermofisher, USA). Cells not treated were used as the control.

Statistical analysis

The data are presented as mean \pm SD of three different experiments. Significance different between treated and control groups was compared using the analysis of variance (one-way ANOVA). The values of $p < 0.05$ were taken as statistically significance 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 (Milner et al., 2011). 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: $C_{45}H_{73}NO_{15}$; $^1\text{H-NMR}$ ($\text{CDCl}_3, 500\text{MHz}$): 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; $^{13}\text{C-NMR}$ 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.

Bio-guided fractionation of Solanum scabrum extract

The bio-guided fractionation was carried out following solvents in increasing order of their polarities using n-hexane, ethyl acetate, and n-butanol. Fractionations of the extract yielded the following fractions: n-hexane fraction (HF), ethyl acetate (EF) and n-butanol (BF). Each of the fractions were bio-guided by anti-inflammatory and anti-cancer activities in order to determine the most active fraction. Results were expressed as 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 ± 41 % inhibition inflammatory and EF; $\text{IC}_{50} = 14.28 \pm 0.12$ $\mu\text{g}/\text{L}$) were further fractionated into four sub-fractions (BF.1, BF.2, BF.3, and BF.4) (EF.1, EF.2, EF.3 and EF.4) for anti-inflammatory and anti-cancer activities respectively. The n-butanol (BF.4) and ethyl acetate sub-fractions (EF.2) were the most active for anti-inflammatory and anti-cancer activities respectively, and were further purified in silica gel column chromatography. Purification of isolated bioactive compounds were confirmed by presence of single spots on TLC plate.

Identification of isolated compound from leaf

The isolated most bioactive anti-inflammatory and anti-cancer compound from *S. scabrum* leaf was structurally elucidated and identified based on the data obtained from $^1\text{H}, ^{13}\text{C}, \text{HSQC}$ and HMBC-NMR (500MHz, Bruker) as well as MS (Agilent technologies). The $^1\text{H-NMR}$ showed seven signals while the $^{13}\text{C-NMR}$ showed the presence of 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: colour; 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). Formulated microspheres with larger sizes (in μm ; $1\mu\text{m} = 1000\text{ nm}$) possessed higher % EE than those with smaller sizes as seen from the table below. The data from the characterization of formulated chitosan NPs were presented in the Tables 2 & 3; Fig.2 below:

3.4. Anti-inflammatory activity test of extracts

The results of anti-inflammatory effects of the extracts by carrageenan-induced paw edema and protein denaturation assays were shown in Figure 3. From the results the highest paw edema reduction of 2.68 ± 0.01 mm was obtained in the treated group which received 1200 mg/kg ethyl acetate fraction (EF) as well as mice that received 0.5 µg/mL CE3 and solanine (S) with 1.44 ± 0.01 mm and $2.52 \pm .02$ mm after 3 h of carrageenan induction. Mice treated with hexane fraction (HF) at 200, 400, 800 and 1200 mg/kg b.w. *Solanum scabrum* extract and chitosan NPs-loaded solanine (CS2) does not show significant decrease in paw volume from 1 to 3 h compared to the standard drug (Fig. 3a). Similar results were obtained in Fig. 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 ± 0.01 µg/mL against MCF-7 and HmVII cells respectively. These values were followed by that obtained when the cells were exposed to solanine alone (S) with IC_{50} value of 8.13 ± 0.01 and 12.01 ± 1.10 µg/mL respectively. However, the selective index of exposing the 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 µg/mL) *in vitro*. From the results also, chitosan-loaded ethyl acetate fraction (CE3) showed improved values in IC_{50} and selective index than other treatment groups (Table 4).

Invasion and migration assay

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

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, there were lost of cellular integrity as seen from the flow cytometry images. Early apoptosis were induced on exposure of cells to solanine glycoalkaloid after 72 h with shrinking nuclei after staining with propidium iodide (PI). Percentage apoptosis in cells were high with increased concentration of solanine (S) in early and late stages of apoptosis as well as increased caspase-3-activity in MCF-7 breast cancer cell line (Fig. 5a-c). This result was comparable to that of the doxorubicin standard drug.

Similarly, chitosan-loaded: extract (CE3) and solanine (CS2), does not induced higher percentage cellular apoptosis in human vaginal melanoma cell HmVII when compared to that the 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 part such as leaves are reduced into fine powders using mechanical means so as to improve its contact with solvents, and promote the penetration of extracting solvents into the tissues. Extraction which normally follows pulverization is usually carried by cold maceration technique where the powdered leaves are soaked in appropriate volume of methanol to obtain the active phytoconstituents from the leaves. In this study, the use of this method of extraction also helps to prevent loss of active metabolites due to heat. This is because, plants active constituents (secondary metabolites) 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 the an emerging aspect of nanotechnology where nanoparticles are being employed to prevent, diagnose and treat diseases. Because of their biocompatibility, specificity, non-toxicity and

biodegradability, nanoparticles have been used to deliver various drugs to specific site in the body.⁴² In our current study, chitosan nanoparticle (CSNP) was used to deliver various extracts and doxorubicine to cancer cells. Chitosan is naturally obtained chitin deacetylation. It is a positively charged nanocarrier. Because of its ability to adhere to cells as well as being readily absorbed in the body, it is the most 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 chitosan NPs a candidate of choice for the treatment of solid tumors.^{43,44} Apart from the aforementioned, chitosan NPs 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 chitosan NPs showed more biological activities than when the drugs were used directly on the organs or tissues.

In this current study, the effect of *Solanum scabrum* extracts were evaluated for anti-inflammatory activity and anticancer effects on human vaginal melanoma (HMVII) and breast cancer cell line (MCF-7) *in vitro*. From our study, bioguided fractionation of *S. scabrum* extracts showed that butanol fraction (BF) and ethyl acetate fraction (EF) exhibited high anti-inflammatory activity *in vitro*. No anti-inflammatory and anticancer activities were observed in n-hexane fraction (HF). EF was the most sensitive and biologically active of all the fractions on 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 the *Solanum scabrum*, where a broad range of glycoalkaloids of solasodine were reported in *Solanum scabrum* berries by using HPLC-UV/Vis-MS or MS/MS methods.⁴⁸ This present study was the first time it is being isolated from the leaf extract.

In this present study, cross-linking of chitosan NPs with glutaraldehyde (GA) slightly affected the yield, size (expressed in μm), zeta potential and % entrapment from 500-2000 $\mu\text{g/mL}$ (Table 2; Fig.2), as was also reported that the use of GA as a cross-linking agent resulted in increase in size of nanoparticles with narrow polydispersity index and highest zeta potential.⁴⁹ The presence of some aggregates in the morphology of the prepared chitosan nanoparticles as shown by the SEM (Fig. 2) do not mean that it was not uniform with the drugs but they represent medium sized chitosan NPs which ranges from 0.01 to 0.09 μm (i.e., 10 to 90 nm). This is because nanoparticles range for 1-100 nm in size.⁴⁹ From the present study, as the conditions of prepared chitosan NPs were optimized, the particle size, encapsulation efficiency and yield linearly increased with concentration of GA (Table 2). Size and amount of drug entrapped or encapsulated by nanoparticles are very crucial in drug delivery as these will affect amount of drug release.^{26,27} In this current study, the small and medium sizes of the formulated chitosan NPs greatly assisted in effective delivery of these encapsulated drugs. This is because, the smaller the size of a nanoparticle the more it is un-noticed 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 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, chitosan NPs loaded solanine results in percentage increase in paw volume while the animals that were treated with solanine alone showed significant reduction in paw volume within 3 h (Fig.3 a & b). Apart from chitosan loaded with solanine, other chitosan-loaded drugs showed similar reduction in paw volume which is sign of anti-inflammation thereby, reducing swellings. 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 showed the highest percentage protein denaturation (Fig. 3b). These results were compared with that of the control drug prednisolone ($p < 0.05$; one-way ANOVA). The result further showed that chitosan NPs 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 produce the hormone prostaglandins (PGs) from inducing cytokines which are usually released during inflammation.⁵¹ Chitosan-loaded drugs and extracts must have exerted its action in a similar way but the release pattern was sustain-release.

A good anticancer agent must be able of inhibiting 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 selective index (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 the treatments, there were greater synergistic effects among these drugs and chitosan NPs. However, the 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 the cancer cell lines unlike other treatments where there was significant rise in percentage apoptosis induction (Table 5). From the study, cancer cells treated chitosan loaded doxorubicin anticancer drug showed the highest percentage apoptosis induction. It was followed by cancer cells treated with solanine not loaded into chitosan NPs. 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 which exist in solanine and chitosan NPs that work in anti-synergism with each other. However, this mechanism needs to be further studied for a conclusive report. Also, in transwell migration assay, migration and invasion were greatly reduced in MCF-7 cancer cell exposed to solanine alone than chitosan-loaded solanine glycoalkaloid (CS2) at various concentrations (Fig. 4). Our study further revealed that MCF-7 breast cancer cell 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 (Fig. 5). Chitosan-loaded solanine glycoalkaloid (CS2) does not induce significant apoptosis. Moreover, exposure of MCF-7 breast cancer cell to solanine alone results in increase in caspase-3 activity from 400-1200 $\mu\text{g/mL}$ concentrations.

It was suggested from previous studies that the toxicity mechanism of solanine glycoalkaloid was due to the chemical interaction within the mitochondrial complexes. This is because, exposure of cancer cells to solanine alone opens the K^+ channel of the 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 the cells.⁵³ This claim was not different from the role of solanine in this current study. It has been reported that caspase-3 is important for proper development of brain as well as necessary for other apoptotic processes in cancer cells.⁵⁴⁻⁵⁶ 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 levels in caspase-3 activity witnessed in this present study from MCF-7 breast cancer cell 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 exert 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 chitosan NPs loaded with *Solanum 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 encapsulation of solanine glycoalkaloid into chitosan NPs produced low activities than when solanine was used alone. It showed that use of solanine alone reduced % paw volume, decreased migration and invasion, increased apoptosis as well as elevated caspase-3 activity. Finally, due to the reported toxicity of solanine, the concentration was reduced in this study. It is suggested therefore that lack of synergistic effects between solanine and chitosan NPs 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 approval number of UJ/FPS/F17-00379.

Informed Consent: Not applicable.

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Authorship Contributions

CAU and EOI: Designed the study and supervised the work. CAU, EOI and ACF: Performed the laboratory experiments and prepare the manuscript. CAU and EOI: Edited the manuscript. All authors read and approved the final manuscript for submission.

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Table 1. Activity guided fractionation of extracts for anti-inflammation and anticancer.

Extract	Anti-inflammation (%)	MCF-7 IC ₅₀ (µg/mL)
HF	12.01 ± 0.20	88.24 ± 4.12
EF	42.11 ± 1.20	14.28 ± 0.12
BF	52.41 ± 2.11	92.24 ± 4.02
Aspirin	76.32 ± 2.04	Na
Doxorubicin	Na	0.80 ± 0.01

Results are mean ± SD (at p < 0.05; one-way ANOVA), Na (not applicable).

n-hexane fraction (HF), ethyl acetate (EF) and n-butanol (BF).

Table 2. Preparation and characterization of Chitosan NPs loaded with *Solanum scabrum* extract (CE), solanine (CS) and doxorubicin (CDr) by spray drying technique.

Batch code	GA (µg/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 ± SD; GA (glutaraldehyde); % EE (percentage entrapment efficiency); ζ (zeta potential). 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).

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 ± 1.42*
CE4	18.22 ± 1.20
CS1	28.04 ± 2.12
CS2	64.18 ± 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 ± 2.01*

* Best optimized conditions were selected for anti-inflammatory and anticancer studies. Results are mean ± SD (n = 3).

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

Results are mean±SD for three readings. HF (hexane fraction), EF (ethyl acetate fraction), BF (butanol fraction), S (solanine glycoalkaloid), 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). MCF-7, HMVII and VeroE6 cells ($1 \times 10^8/\text{cells}$) 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 selective index of these treatments against the MCF-7 was determined from the normal cell VeroE6.

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

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

Results are mean \pm 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 standard anticancer drug)

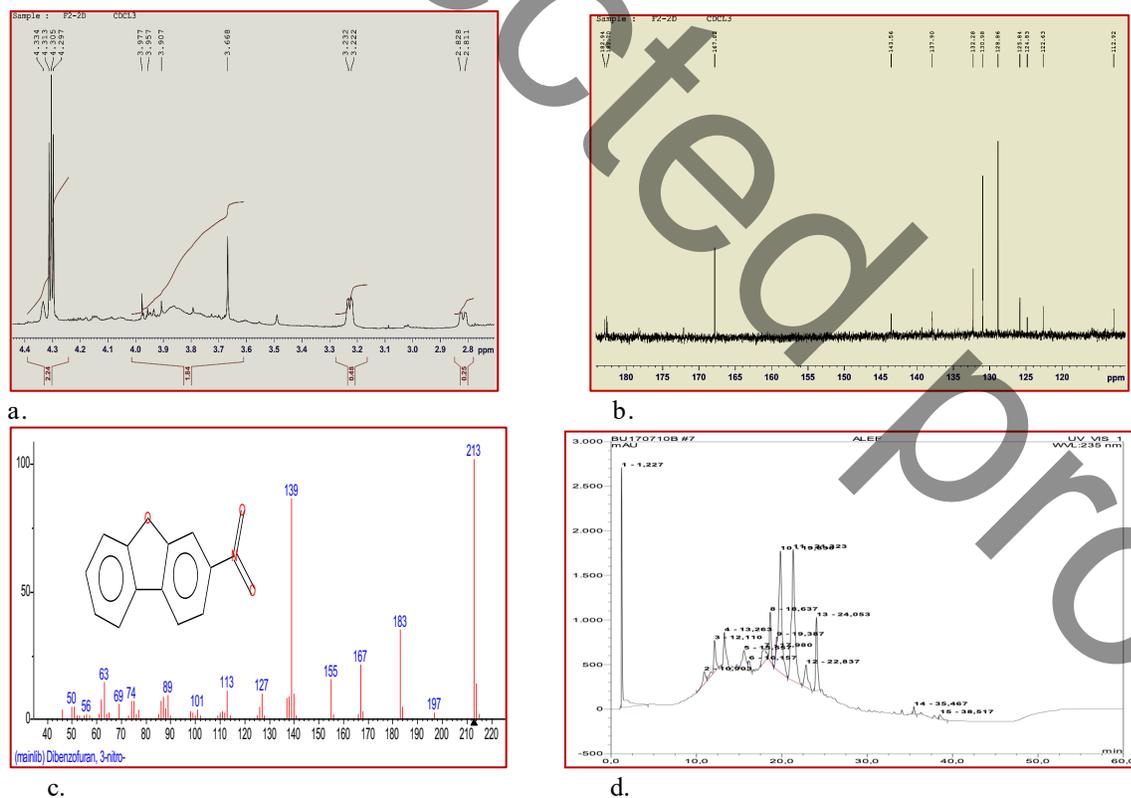
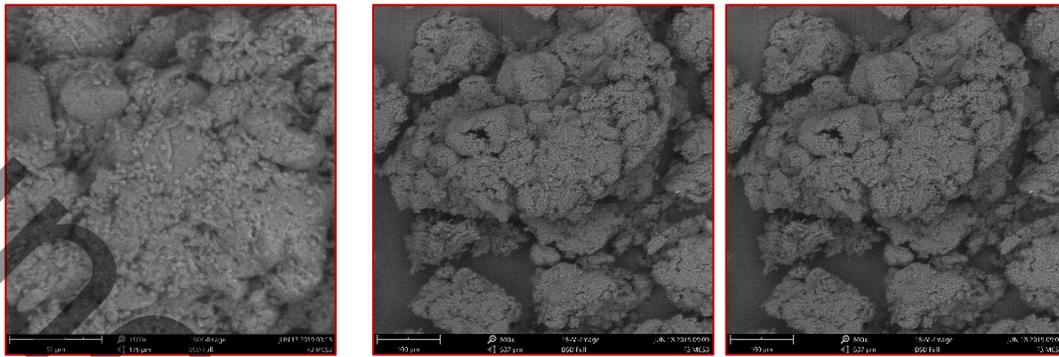
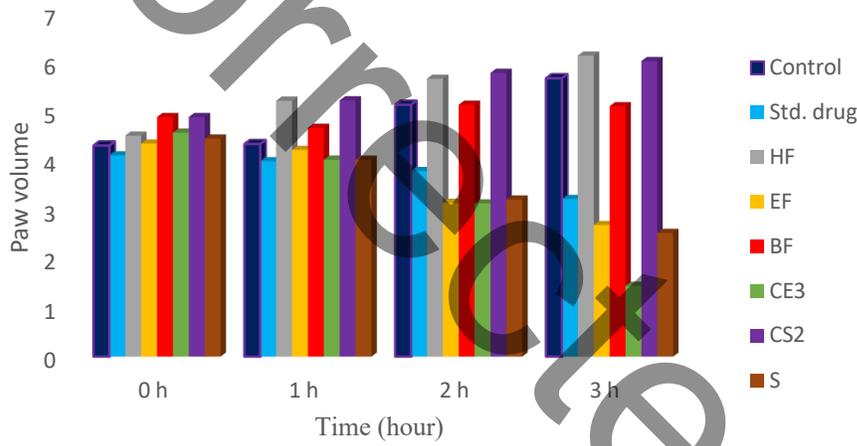


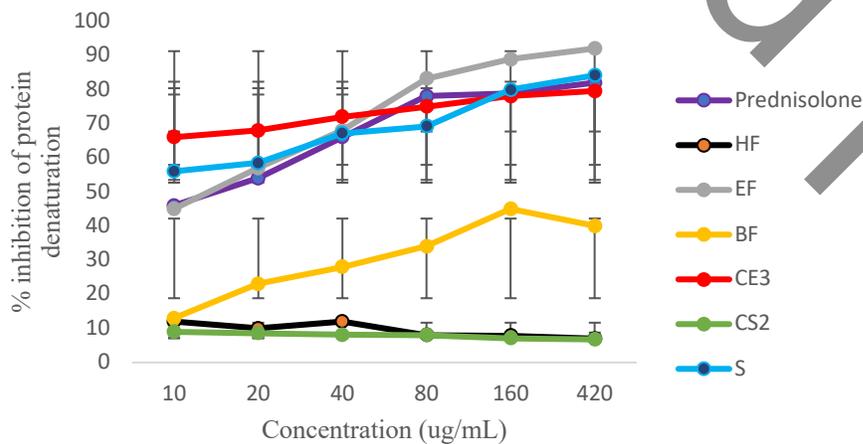
Figure 1. NMR, MS and HPLC spectra of isolated compound from *S. scabrum* leaf; a) $^1\text{H-NMR}$ with seven protons between 2.81– 4.33 δ (ppm), b) $^{13}\text{C-NMR}$ with twelve carbon atoms, c) MS with m/z 213 and molecular peak ion 139 at 62.89 % peak area for 3-nitrodibenzofuran, d) HPLC spectrum showed 19.11 min with isocratic elution. CDCl_3 was used as solvent for NMR analysis.



a. CE3 **b. CS2** **c. CDr4**
Figure 2. SEM morphology of chitosan loaded with crude *S. scabrum* extract (CE3), solanine (CS2), doxorubicin (CDr4). Glutaraldehyde was used as cross-linking agent. 3000x.



a.



b.

Figure 3. Anti-inflammatory activity of *S. scabrum* extracts and chitosan NPs loaded extracts; a (carrageenan-induced paw edema), b (effects on protein denaturation). 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 glycoalkaloid. Results are means \pm SD. The values of $p < 0.05$ were statistically significant.

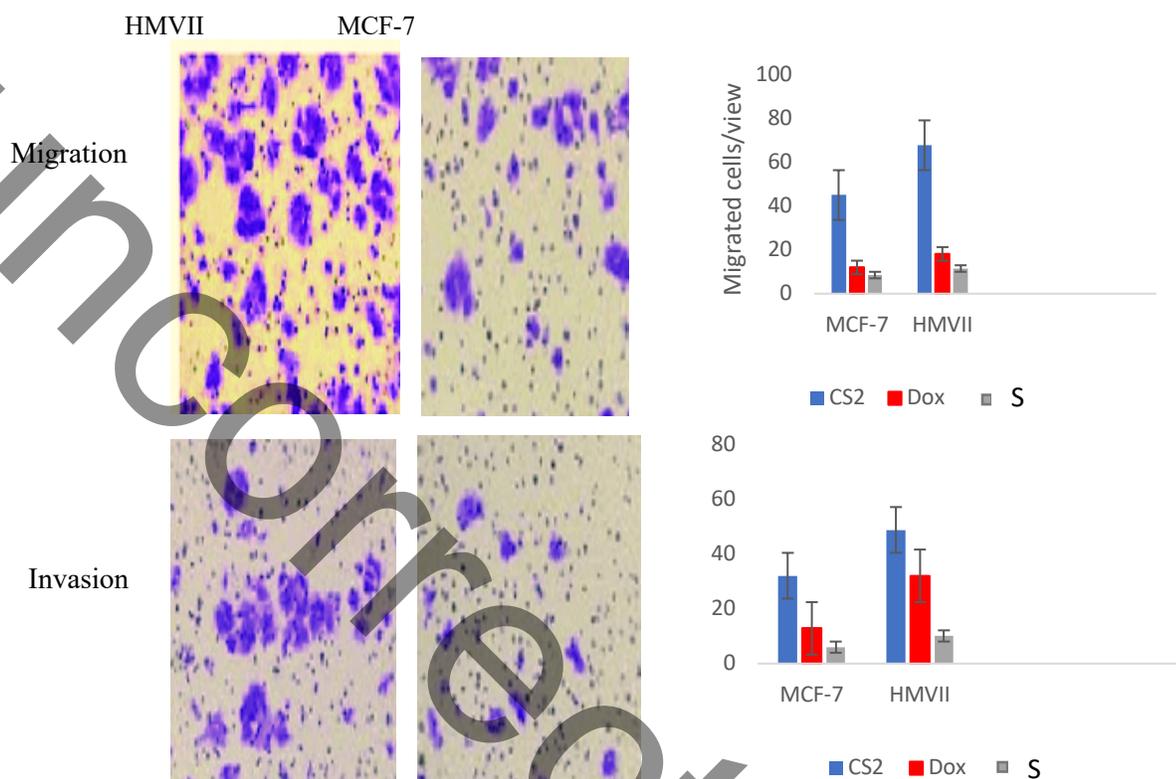


Figure 4. Transwell migration and invasion assay of solanine (S) and chitosan-loaded solanine (CS2) as viewed using hemocytometer. Dox (doxorubicin first-line anticancer drug). Results are mean \pm SD for three replicate readings. Cellular migration and invasion were much pronounced with CS2 treatment when compared to cells exposed to solanine alone.

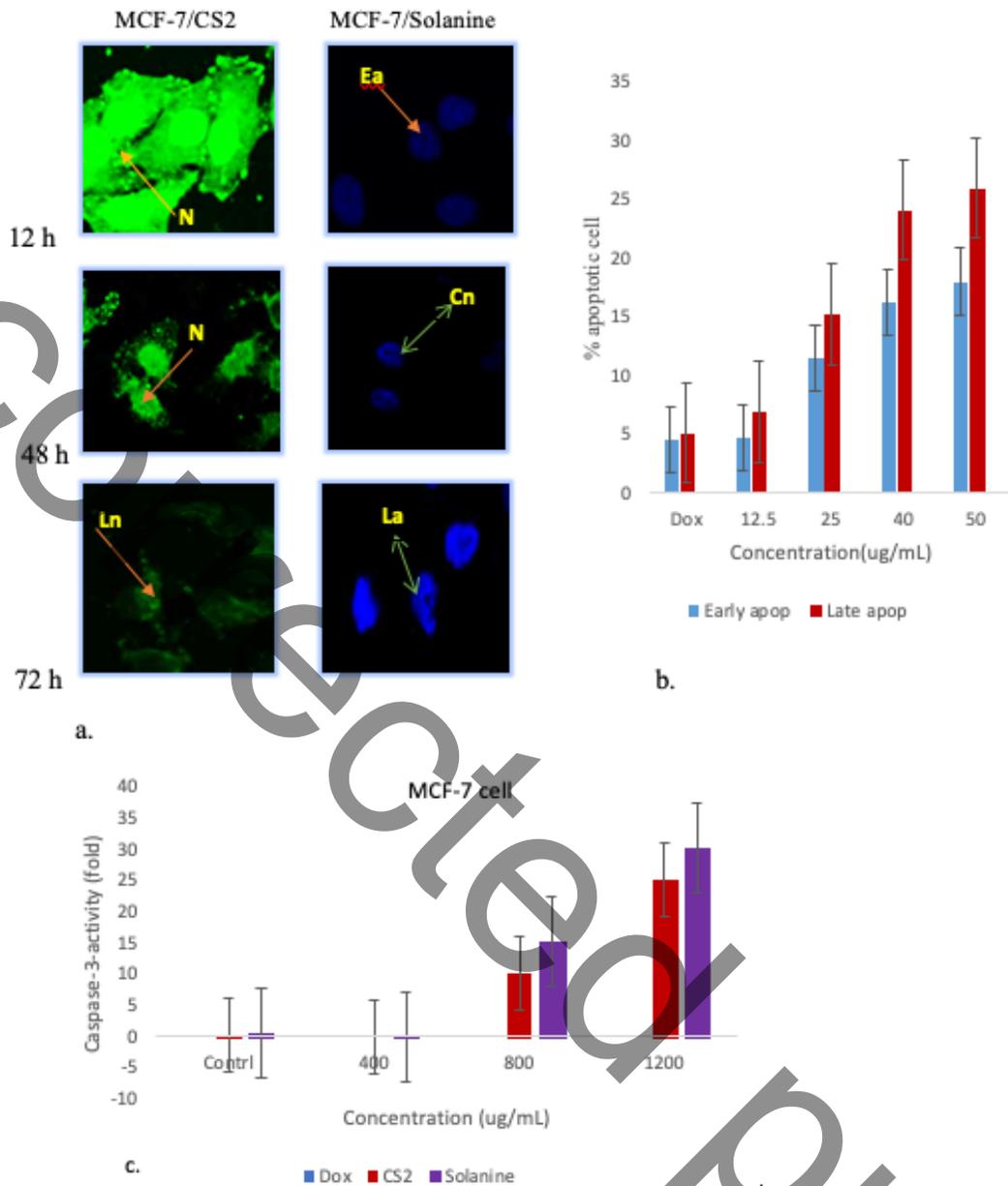


Figure 5: Fluorescence images of MCF-7 cancer cell after treatment with CS2 and solanine glycoalkaloid (S) with various concentrations (12.5, 25, 40 and 50 $\mu\text{g/mL}$) at 12, 48 and 72 hours. Ea (early apoptosis), La (late apoptosis), Cn (clear nuclear), N (necrosis of cells), Ln (late necrosis). Apoptotic cellular death and necrosis was detected by staining with propidium iodide (PI) and analysed with flow cytometry. Solanine alone increases caspase-3-like activity at highest concentration and low value at lower concentration. Results are mean \pm SD for three consecutive readings. $P < 0.05$ was taken as significance different.

Uncorrected proof