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DRUG DISCOVERY

In vitro anticancer activity of *Melastomastrum capitatum* Fern. loaded chitosan nanoparticles on selected cancer cells

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Background: **Chitosan nanoparticles are used for drug delivery because, they allow drugs to be selectively targeted to organs thereby minimizing injuries.** ***Objectives:*** **This study was carried out in order to determine the anticancer activity of *Melastomastrum capitatum* loaded chitosan nanoparticles on selected cancer cells.** ***Methods:*** **Chitosan nanoparticles were prepared by spray drying method. Glutaraldehyde was used an as cross-linking agent in ratio 1: 4 (drug: polymer). Characterization of *M. capitatum* loaded chitosans was made using FTRIR, Zeta sizer, scanning electron microscope, nano spray dryer and thermal analyzer.** ***Results:*** **Maximum yield of prepared microspheres was 46.40 %; average size ranged from 204 ± 1.15 nm to 248 ± 2.04 nm, Zeta potential 24 ± 0.1 mV to 25 ± 25 ± 0.1 mV, while drug entrapment efficiency ranged from 31.20 ± 0.25 % to 59.60 ± 0.41 %. *M. capitatum* loaded chitosan nanoparticles showed the best anti-ovarian cancer activity with selective index of >100 compared with doxorubicin (p≤ 0.05).** ***Conclusion:*** **Our study showed that chitosan drug delivery system is an excellent way for delivering drug to ovarian cancer with high selective index and best anticancer activity.**

INTRODUCTION

Research into medicinal plants used in treating tumor-related ailments has become imperative due to the emergence of various forms of cancers. In recent time, there has been an increasing amount of cancer research directed towards the investigation of plant derived anti-cancer compounds many of which have been used in traditional herbal treatments for centuries[1]. With increasing recognition of herbal medicine as an alternative form of health care, screening of medicinal plants for biologically active compounds, has become an important source of antibiotic prototypes and cancer-related drugs[2].

Cancer is the name given to a collection of related diseases. In all types of cancer, some of the body's cells begin to divide without stopping and spread into surrounding tissues a process called metastasis. Cancer can start almost anywhere in the human body, which is made up of trillions of cells. In Nigeria, about 10, 000 people died annually from various types of cancers, with breast and ovarian cancers the most prevalence of all cancer cases. Recently, life styles such as self-medication of certain drugs like antibiotics, autoimmune, hereditary, smoking and mutations have been reported to be the major causes of cancers.

Nanotechnology refers to the understanding and control of matter at dimensions approximately from 1-100 nm, or nanoscale. This aspect of biomedical science is rapidly expanding. Nanotechnology in biology and medicine is aimed as serving as an authoritative reference for many people involved in research, teaching, learning, and practice of nanotechnology in life sciences. Nanotechnology has been revolutionizing numerous important scientific fields from biological sciences to medicine as well as medical sciences. This technology, which is at the scale of the precursor to cell, has the potential of developing devices that are smaller and more efficient than anything that is currently available [3,4].

The new progress in biotechnology, genetic engineering, genomics, proteomics, and medicine and medical sciences will depend on how well nanotechnology is understood in the coming years. Chitosan has been investigated extensively as a potential drug carrier, due to its biocompatible properties. Some studies have suggested using chitosan to coat nanoparticles made of other materials, in order to reduce their impact on the body and increase their bioavailability. Many drugs have problems of poor stability, water insolubility, low selectivity, high toxicity, side effects among others. Hence, the main aim of using nanocarriers are minimizing drug degradation upon administration, increasing bioavailability and preventing undesirable side effects of the drug [5,6].

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M. capitatum is a member of one of the largest Families of flowering plants Melastomataceae with an approximate 4,500 species in about 200 genera that are distributed in both tropical and sub-tropical regions of the world. Scanty documentation have resulted in different hypothetical viewpoints on the bio-geography of this Family. It is a shrubby herb that grows up to 1·25 m high, and it is found in dry situations and stream-banks in Nigeria especially in Mambila plateau, Taraba State , Guinea, Mali, Uganda and Angola.

In Nigeria, it is locally called ''*Belkon*'' by the Fulani tribe in Mambila plateau Nigeria who use the leaf to treat ovarian cancer traditionally. The leaf-sap diluted into a little water is used in Ivory Coast as a sedative. Its leaf can cure cancer, reduce cholesterol, acts as an analgesic, and cleans the blood vessels in traditional medicine because, the leaf contains mainly glycosides and alkaloids. In South-south Nigeria, the leaves have been used to heal many wounds[7,8]. Apart from the Mambila plateau, the plant also grows well in Ogurugu Uzo-Uwani Local Government Area of Enugu State; where it is majorly found in mash land and wet areas especially in shallow streams. They also found in Ibaji Local Government Area of Kogi State, Borgu Local Government Area of Niger State, Southern part of Kaduna State, Edo State, South-west Nigerian States of Ondo, Ekiti, Lagos Oyo and Osun. The leaves are the major part use in traditional medicine where it is used for blood purification, as remedy for stomach ache, as antitumor and anticancer agent by the Fulani tribe (these claims have not been scientifically proven or ascertained yet). The leaf has been shown to display analgesic and anti-inflammatory properties, and anti-hypercholesterolemic in mice. The other uses of the plant are not documented or known. The regular use of these plants for cancer management and treatment by the locals in Mambila plateau Nigeria has attracted our attention towards investigating this claim to further provide valid scientific justification for its acclaimed use in cancer treatment.

Yet, despite the prevalence of cancer all over the world, less than half of all the countries have functional plans to prevent the disease and provide treatment and care to patients, hence the need to search for excellent anticancer agents of natural origin by screening medicinal plants for an anticancer compound is imperative. The bottleneck of conventional anticancer drugs includes high toxicity of most of these drugs, due to indiscriminate distribution of drugs toward diseased and healthy cells. Also, anticancer drugs often suffer from poor solubility in water thus, the need to use organic solvents for clinical applications, resulting in undesirable side effects such as venous irritation and respiratory distress. Therefore, designing a distinct carrier system that encapsulates a large quantity of drugs and specifically targeting cancer cells is indispensable for successful cancer therapy, hence the need for this research.

Therefore, this present study was carried out in order to determine the in vitro anticancer activity of *M. capitatum* loaded chitosan nanoparticles on selected cancer cells.

MATERIALS AND METHODS

Materials

Some of the chemicals and apparatus used in this research are: glutaraldehyde, glacial acetic acid, methanol, acetone (analytical grade; Sigma Aldrich), rotary evaporator, deionized water, alpha II Fourier transform infra-red spectrophotometer (Model 9020, Brucker USA), magnetic stirrer (Advantech SR550, UK), GEMINI-20 portal milligram scale(AWS, China), chitosan powder from crustaceans and shrimps [high molecular weight; ChemSaver, USA; Purity: 90 + % deacetylated, Quantity: 100 g; Product no: CHTS100G; CAS no: 9012-76-4; Formula:$(C_6H_{11}O_4N)n$], scanning biological microscope, Malvern Zetasizer, scanning electron microscope (Olympus), , cancer cell lines, among others.

Methods

Collection and identification of plant

Fresh leaves of *Melastomastrum capitatum* were collected from Mambila plateau Sarduana Local Government Area, Taraba State, Nigeria, in the evening hour to correlate with the time the traditional medical practitioners collect the plant for medicinal purposes. It was authenticated with a voucher specimen number of "ABU2761" at the herbarium of Department of Botany, Ahmadu Bello University, Zaria by Mr Namadi Sunusi.

Preparation of plant material

The fresh leaves of *M. capitatum* were air-dried under shade for 2 weeks, and were pulverized into fine powder using an electronic blender (Model 5000 MH, Japan). The powder was then sieved using sieve number 20 mesh to obtain a fine powder and remove any unwanted debris, and was weighed on a scale balance to know the initial weight of the powder. The powdered plant material was then kept in a clean and dried bag for further use.

Extraction of plant material

2.5 kg of powdered leaves of *M. capitatum* were defatted with petroleum ether (60–80 °C) to remove fat, latex and non-polar compounds of high molecular weights. The defatted plant residues were extracted using Soxhlet apparatus in methanol for 12 h at temperature of 40 °C, to obtain the methanol extract (MCME). The solvent was regularly changed until no coloration was observed. The collected extract was filtered through Whatman filter paper No. 1. Finally, the filtrate was concentrated in vacuum using rotary evaporator. The final weight of the extract was then noted and percentage yield was calculated with reference to the initial weight of the powder as given below:

$$\% \text{ Yield} = \frac{\text{Final weight of extract}}{\text{Initial weight of powdered drug}} \times 100 \qquad (1)$$

It was then stored in a clean dried bottle and kept in a desiccator for further use.

Preparation of chitosan nanoparticles loaded M. capitatum leaf extract

Chitosan nanoparticles (CSNPs) were prepared at the Research Laboratory of the Department of Pharmaceutics and Microbiology, University of Ghana School of Pharmacy, Legon, Accra by spraying drying method. Briefly, 1 g of *M. capitatum* leaf methanol extract (MCE) was dissolved in 10 mL deionized water. Similarly, 4 g of chitosan powder made from shrimp shells and crustaceans was accurately weighed on a Gemini-20 portal milligram scale balance into a clean 500 mL beaker, and dissolved in 100 mL deionized water containing 0.5 % v/v glacial acetic acid under magnetic stirring. Prepared solution of MCE was added to the beaker containing CSNPs under constant stirring at 3000 rpm for 2 h. Glutaraldehyde (a cross-linker) was added in drop-wise at concentrations of 500, 1000, and 1500 µg/mL to each formulations (MCS2-MCS4). MCS1 was not cross-linked. The pH of the preparations were adjusted by adding 0.1M NaOH solution and stirred for 30 min. In order to enhanced their targeting, 20 mg of folic acid was dissolved in 20 mL deionized water and 5 mL of

the solution was added to the formulations and stirred for another 15 min at 2000 rpm [9,10]. The CSNPs-MCE complexes formed were sprayed dried for atomization using a nano spray drying apparatus (Model B-90 Shanghai Bilon instrument Co Ltd, China) with the following experimental conditions:

Inlet temperature = 130 ºC
Outlet temperature = 55ºC- 60 ºC
Head temperature = 65 ºC- 70 ºC
Pressure = 37- 38 mbar

The spray dried chitosan nanoparticles loaded with MCE were weighed and stored in clean containers for further use.

Characterization of chitosan nanoparticles loaded with MCE

The formulated MCE loaded CSNPs were characterized in terms of: particle size, surface morphology or shape, percentage drug entrapment, FTIR spectroscopy, percentage yield, swelling index *and in vitro* drug release[11].

Particle size of MCE loaded chitosan nanoparticles

The particle size of MCE loaded CSNPs was determined by light scattering based on laser diffraction using Malvern Zetasizer (Model 3000HS; Malvern instruments Ltd, UK) and fluorescence biological microscope (Olympus) at 500,000x. In this case, MCE loaded CSNPs was firstly diluted with 0.1mM KCl, 10 µL of each formulation was pipetted into separate curvette and then placed in a Malvern zetasizer analyzing chamber having an electrophoretic cell of 12.2 v/cm. The readings were then taken in triplicates after normal dilution. Note that Zeta potential is defined as the electrical potential difference of a particle in dispersion. This parameter which is very useful in the investigation of the physical stability of colloidal dispersion containing CSNPs[12].

Shape or morphology of MCE loaded CSNPs

The shape or morphology of the dried MCE loaded CSNPs was determined using a scanning electron microscope (SEM) (model JSM 6010 LA, JEOL, USA). Briefly, sample of dried MCE loaded CSNPs was placed on a double stick tape over aluminium stubs to get a uniform layer of CSNPs. Sample was coated with platinum for 20 seconds. Then the sample was observed at 10 kV [13,14].

Percentage drug entrapment efficiency (% EE)

The percentage drug entrapment efficiency and drug loading of *Melastomastrum capitatum* loaded CSNPs was determined by centrifugation method. Briefly, re-dispersed CSNPs suspension was centrifuged at 12, 000 rpm for 30 min at 20 ºC in order to separate free drug in supernatant. Supernatant from this procedure was separated by decanting them, and the pellets were washed thrice with deionized water. The concentration of MCE in the supernatant was then determined using Uv-vis spectrophotometer at 570 nm while the entrapped drug was determined by the extraction of pellets using methanol followed by vortexing for 5 min [15]. Percentage (%) entrapment efficiency and drug loading of *M. capitatum* were calculated from the equations below:

$$\% EE = \frac{\text{experimental drug content}}{\text{Total drug content}} \times 100 \quad (2)$$

Fourier transform infra-red spectroscopy analysis of MCE loaded CSNPs

FTIR analysis was carried out using Alpha II (model 9020; Brucker, USA) in order to check the extent of interaction between MCE and chitosans as well as the stability of the extract. In this case, little quantities of homogenously dried crossed and non-crossed formulations were placed in FTIR light pathway and scanned in the wave number range of 4000-400 cm^{-1} [16,17].

Thermal analysis of MCE loaded CSNPs

In order to determine the effect of heat on the prepared MCE loaded CSNPs, differential scanning calorimetry (DSC) analysis on a NETZSCH Photo-DSC204 F1 Phoenix® (Germany) equipped with the Omnicure S2000 lamp was used .Heat flow in the formulation was measured at temperature range 50-200 ºC at 10 ºC/min [17, 18].

Swelling index study of M. capitatum loaded chitosan nanoparticles

The swelling activity of the spray dried MCE loaded chitosan nanoparticles in phosphate buffer solution (PBS) (pH 7.4) was evaluated. Briefly, spray-dried MCSNPs of weight 4 g without containing the leaf extract was dissolved in PBS for 6 hours. The solution was centrifuged at 3000 rpm for 1 hour, and decanted to collect the microspheres. Moisture was removed from the collected microspheres by blotting them with filter paper. After this, the microspheres were then weighed using a GEMINI portal milligram scale balance at predetermined time period 0, 1, 2, 3, 4, 5, and 6 hours. Percentage of swelling index of MCE loaded chitosan nanoparticles in the PBS was then calculated using the formula:

% swelling index = (Final weight - Initial weight/ initial weight) 100. (3)

In vitro drug released study of MCE loaded CSNPs

The amount of drug released by each formulation was determined using dialysis tubation method. Dialysis bags were obtained on purchase from Benrock Medicals and Science Ltd, Nigeria. Prepared MCE loaded CSNPs were each dissolved in 5 mL phosphate buffer solution (PBS) (pH 7.4). The ends of the dialysis bags were tied with one end first. 10 mL of dissolves MCE-CSNPs complex was transferred into the bags and the remaining end of the bags were tied with clean ropes and then placed in a 500 mL beaker containing 150 mL PBS under magnetic stirring at 1500 rpm and 37 ºC. After every one hour, 5 mL of the sample was withdrawn from the beaker and replaced with 5 mL fresh PBS. This was continued for 6 hours. The amount of drug released by the formulations was analyzed from the withdrawn samples using UV-vis spectrophotometer at 570 nm. Data obtained from the *in vitro* drug release for formulation were fitted to various kinetic models [20].

In vitro anticancer studies of M. capitatum loaded chitosan nanoparticles (MCSNPs)

Anticancer activity of chitosan nanoparticles loaded with *M. capitatum* leaf extract was evaluated in order to compare the effect of leaf extract alone on cancer cells and targeting the cancer cells using a carrier in the form of chitosan nanoparticles using MTT bioassay [21]. Briefly, Cancer cell lines (OV7; from human epithelial cell of the ovary, MCF-7; from a woman's breast and HMVII; human vaginal malignant melanoma of a 65 years old woman obtained from Sigma Aldrich, USA) were each cultured in a 96 well cell culture plates at 10^5 cells / per well containing 100 µL of RPMI 1640. The cells then allowed to incubated in a CO_2

Table 1 Optimization conditions of spray dried chitosan nanoparticles loaded with *M. capitatum* leaf extract

Formulation Code	Drug: Chitosan (w/w)	Glutaraldehyde (µg/mL)	Inlet-temp (ºC)	Outlet-temp (ºC)	Head-temp (ºC)	Pressure (mbar)
MCS1	1:4	-	130	60	70	38
MCS2	1:4	500	130	60	70	38
MCS3	1:4	1000	130	55	65	37
MCS4	1:4	1500	130 55	65	37	

Airflow rate = 2 mL/min.

Table 2 The yield, particle size, drug entrapment efficiency, and zeta potential of spray dried chitosan nanoparticles loaded with *M. capitatum* leaf extract

Formulation Code	Yield (%)	Particle size (nm)	Drug entrapment efficiency (%)	Zeta potential (mV)
MCS1	44.40	248.00±2.04	31.20±0.25	24.00±0.1
MCS2	45.80	241.00±2.01	53.20±0.58	25.00±0.1
MCS3	46.40	204.00±1.15	59.60±0.41	24.00±0.1
MCS4	43.40	205.00±1.17	51.20±0.22	24.00±0.1

Results are mean ± SD, n = 3.

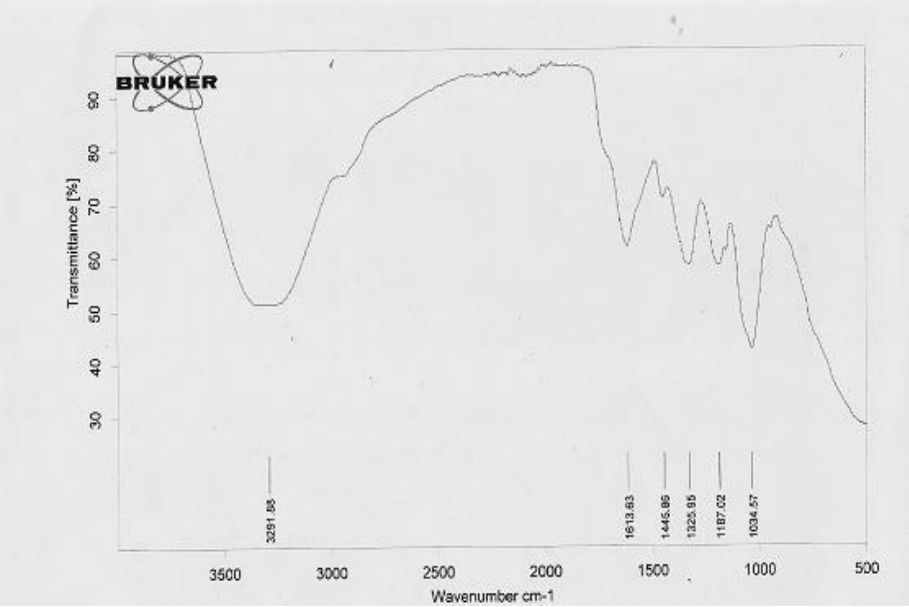


Figure 1 FTIR spectra of chitosan nanoparticles loaded with *M. capitatum* leaf extract

incubator for 24 h at 37 ˚C, and 5 % CO_2. The media were then removed and replaced by fresh media containing different concentrations of *M. capitatum* loaded chitosan nanoparticles and incubated for 48 h. After this, 20 µL MTT dye (3- (4, 5-dimethylthiazol-yl)-2, 5-diphenyltetrazolium bromide) solution containing 5 mg/mL phosphate buffer saline (PBS) were each added to each well using a multi-channel pipette, and further incubated for 2 h. The medium for each was then removed, and 200 µL of Dimethyl sulphoxide solution (DMSO) was added to each well to dissolve the MTT formazan crystals. Finally, the culturing plate was shaken at 200 rpm for 5 min, and the absorbance of each was measured using a UV-vis spectrophotometer at 570 nm. The control well contained doxorubicin anticancer drug. Three readings were taken and recorded as mean of all the values. The percentage cytotoxicity (IC_{50}) and the selective index were then calculated from the formulas below:

IC_{50} = *(absorbance of cell without treatment - absorbance of cells with treatment /absorbance of cell without treatment) 100* 1

Selective index (SI) = CC_{50} *value for Vero E6 cell /* IC_{50} *for cancer cell* 2

Statistical analysis

Raw data obtained were expressed as mean±SD for triplicate readings analyzed by statistical software SPSS version 23. Each experiment was repeated three times.

RESULTS

Optimization condition for formulation

Table 1 shows the optimization conditions for various formulations MCS1, MCS2, MCS3, and MCS4. Inlet temperature was 130ºC while the outlet temperature is from 55-60ºC. Nozzle head temperature was initially 65ºC and increased to 70ºC while the operating pressure of nano spray dryer ranges from 37-38ºC.

Particle size measurements

Nanosized *M. capitatum* loaded chitosan nanoparticles ranged from 204.00±1.15 - 248.00±2.04 while zeta potential ranges from 24.00±0.1 - 25.00±0.1. Percentage drug entrapment was higher in MCS3.

FTIR analysis of M. capitatum loaded chitosan nanoparticles

Formulated microspheres were scanned from 3500 cm^{-1} to 500 cm^{-1} wave number (Fig. 1).

In vitro drug release study

Results from drug release profiles of formulated microspheres showed a controlled drug release in MCS3, thereby making it an excellent tool for drug delivery of the anticancer plant *M. capitatum* to cancer cells (Fig. 2).

Cumulative drug release study

Table 3 showed that MCS3 followed first order kinetics drug release. The release is controlled release within 6 h of study. Hence, this formulation maintained a controlled release of its contents to cancer cells within this time.

Thermal analysis

Higher concentration of glutaraldehyde lowers the temperature of formulated microspheres (Fig. 3).

Morphology study

Microscopic images of formulated cross-linked and uncross-linked showed spherically shaped microspheres containing *M. capitatum* leaf extract (Fig. 4).

Swelling index analysis

There was no significant increase in swelling index as concentration of cross-linking agent increases (Fig. 5).

In vitro anticancer study

Tables 4, 5, 6 and Fig. 6 showed that chitosan nanoparticles loaded with *M. capitatum* leaf extract possessed excellent anti-ovarian cancer activity as compared with the control drug doxorubicin. There were however no significant difference in anticancer activity of MCS3 and doxorubicin ($p \leq 0.05$).

DISCUSSION

Many anticancer drugs have the problem of specific targeting of organs or cells without causing major problems to healthy tissues. This has become a major setback in the treatment and management of cancers globally. Therefore, for effective treatment of cancers, there is the need to load a chemotherapeutic agent into a carrier in the form of chitosan nanoparticles so as to minimize drug side effects and exposure of healthy tissues to negative drug effects. Aside this, chitosan nanoparticles are well tolerated by the body cells because they are biodegradable in nature and are site specific in targeting which makes them an excellent delivery tool for anticancer drugs. This is the major rationale behind this present study.

This study showed the preparation and characterization of chitosan nanoparticles loaded with *Melastomastrum capitatum* leaf methanol extract cross-linked with glutaraldehyde using spray drying method of preparation. Crossing linking microspheres with cross-linking agents help to control the release of drug from the nanoparticles[22]. In this present study, the role of glutaraldehyde was not different from the above. From the results in Table 1, drug: chitosan composition did not affect significantly the optimization conditions of nano sprayer. This means that varying the amount of drug and chitosan in various formulations have not significant effect on the optimization conditions. However, it was observed that a higher concentration of cross linking agent the yield and particle size decrease. It is possible therefore, to say mention that glutaraldehyde reduces the surface areas of microspheres at higher concentration (Table 2). This result was similar to the work of Desai and Park[19] who also observed that particle size of microspheres decreased with increasing amount of cross linking agent because cross linking agent hardens the chitosan nanoparticles leading to decreased water absorption.

In this study zeta potential of the prepared microspheres were found to range from 24 to 25mV. It was independent of the concentrations of the cross linking agent. This was contrary to previous reports that concentration of cross linking agents affect zeta potential of prepared nanoparticles. For effective drug delivery, various factors must be put into consideration such as drug to polymer ratio, drug entrapment efficiency as well as particles sizes, among others[23]. From the study, the drug entrapment efficiency is concentration dependent (Table 2). From the results in the said table 2, MCS3 entrapped more of the plant extract with a value of 59.60 ± 0.41, hence it was used for delivery and targeting into cancer cells because of its optimal efficiency. It has been reported that the lower the viscosity of chitosan loaded drug, the better the entrapment efficiency[24]. In addition, the study further revealed that cross linked preparations had the highest drug entrapment efficiencies.

In this present study, scanning the prepared chitosan loaded leaf extract using Fourier transform infrared (FTIR) revealed that there were some functional groups present at 3500-1000 wave number (cm^{-1}) (Fig. 1) and it further showed that the prepared nanoparticles might posses excellent biological activities due to the presence of the functional groups such as alkynes, aromatic, alcohol and methyl esters. In Table 3; fig.2, the % cumulative drug released by prepared microsphere with code MCS3 was the best in 6h duration and slow, controlled manner. For a decrease in temperature in prepared chitosan nanoparticles, higher concentration cross linking agent favours this, since most cross linking agent decrease the hidden thermal energy of microspheres because of the opposite charges existing between the chitosan and cross linkers[25,26], (Fig.3).

Scanning electron image of formulated microspheres showed spherical shapes with smooth surfaces (Fig. 4). This is similar to the results obtained by Crcarevska *et al.* and Desai and Park[19, 25]. Swelling index of chitosan nanoparticles loaded with Melastomastrum capitatum leaf extract increases with increased concentration of cross-linker. However, at formulation MCS3, the swelling index was the lowest in 6 hours in phosphate buffer solution (PBS) (Fig. 5). From these results, it can be confirmed that MCS3 was an excellent formulation for drug delivery because it has the best characteristics of low swelling index, controlled drug release, better yield, higher drug entrapment efficiency, and low zeta potential.

Many drugs have problems of poor stability, water insolubility, low selectivity, high toxicity, side effects among others. CSNPs are also drug carriers with wide development potentials and have the advantage of slow/controlled drug release, which improves drug solubility and stability, enhances efficacy, and reduces toxicity.

Many researches have studied the properties of chitosan nanoparticles with a view to using them as an anticancer drug delivery agent[27,30]. The biocompatibility, specificity and non toxicity of the material make it attractive as a neutral agent for delivery of active

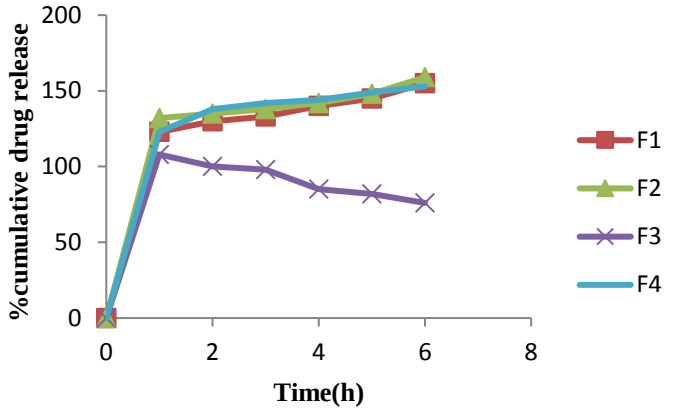


Figure 2 *In vitro* drug release of chitosan nanoparticles loaded with *M. capitatum* leaf extract; F1-F4 (MCS1-MCS4)

Table 3 Cumulative drug release and drug release kinetics of spray dried chitosan nanoparticles loaded with *M. capitatum* leaf extract

Formulation Code (%)	CDR in 6 hrs	Zero order	First order	Higuchi plot	Peppas plot
MCS1	93.40	0.881	1.086	1.072	1.100
MCS2	96.00	0.903	1.152	1.148	1.105
MCS3	98.90	0.930	1.280	1.258	1.165
MCS4	95.20	0.895	1.224	1.214	1.121

CDR; cumulative drug release, hrs; hours.

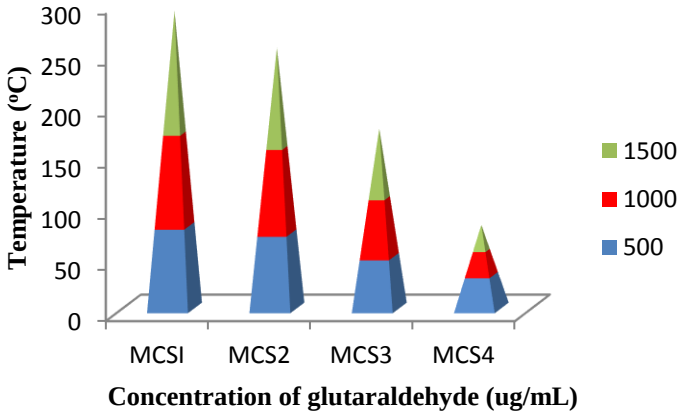
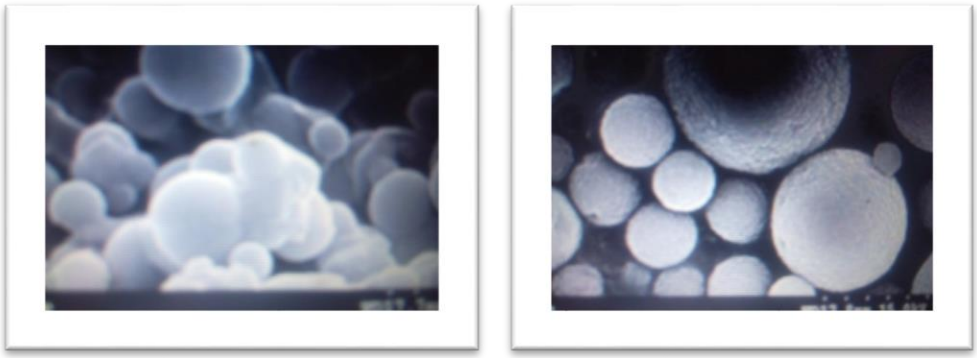


Figure 3 Effects of temperature on various formulations at 50-400ºC



MCS1 (non-cross linked) MCS3 (cross-linked)

Figure 4 SEM images of *M. capitatum* leaf extract loaded chitosan nanoparticles

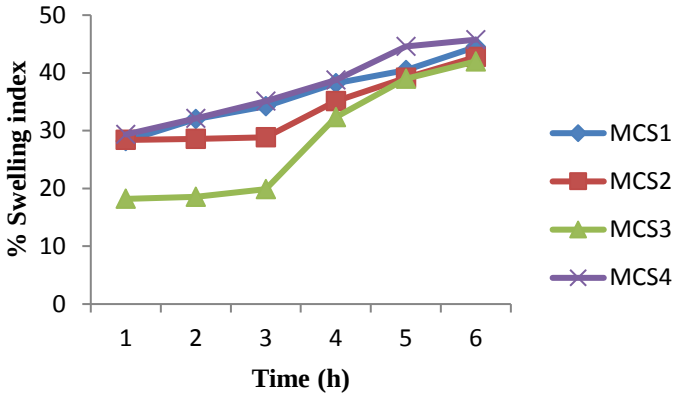


Figure 5 Swelling characteristics of various *M. capitatum* leaf extract loaded chitosan preparations

Table 4 Cytotoxicity effect of *M. capitatum* loaded chitosan nanoparticles on human breast cancer cell line MCF-7

Drug concentration (µg/mL)	Cancer cell lines MCF-7 IC50 (µg/mL)	VeroE6 CC50 (µg/mL)	SI
MCSNPs	66.24±2.12	72.51±3.08	1.09
MCE	278.681±56.60	293.00±29.14	1.05
Doxorubicin	6.69±1.04	8.33±1.11	1.25

Results are mean ± SD, n = 3, SI (selective index), MCE (*M. capitatum* leaf extract without chitosan).

Table 5 Cytotoxicity effect of *M. capitatum* loaded chitosan nanoparticles on human vaginal malignant melanoma HMVII cancer cell line

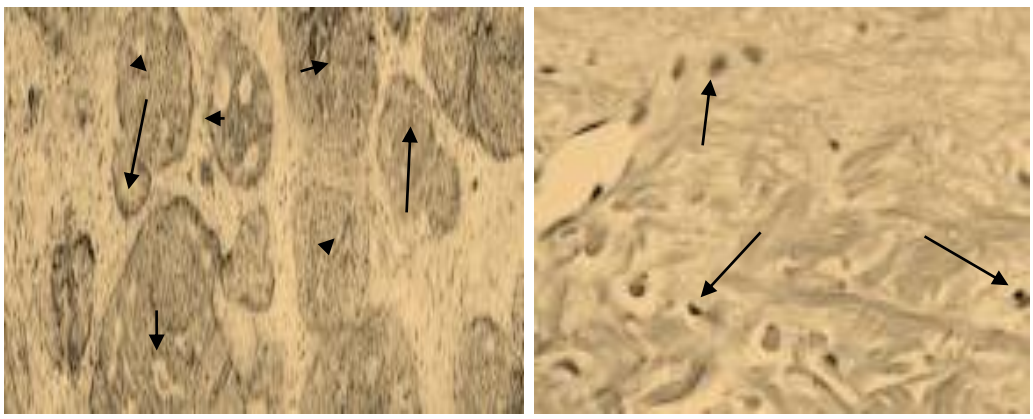
Drug concentration (µg/mL)	Cancer cell lines HMVII IC50 (µg/mL)	VeroE6 CC50 (µg/mL)	SI
MCSNPs	49.56±1.33	72.31±4.61	1.46
MCE	294.86±29.41	315.42±12.02	1.07
Doxorubicin	2.98±0.43	10.18±1.04	3.42

Results are mean ± SD, n = 3, SI (selective index), MCE (*M. capitatum* leaf extract without chitosan).

Table 6 Cytotoxicity effect of *M. capitatum* loaded chitosan nanoparticles on human epithelial ovarian cancer OV7 cancer cell line

Drug concentration (µg/mL)	Cancer cell lines OV7 IC50 (µg/mL)	VeroE6 CC50 (µg/mL)	SI
MCSNPs	0.02±0.01	2.56±0.10	> 100
MCE	10.13±2.20	56.00±3.13	5.53
Doxorubicin	0.06±0.01	1.96±0.10	32.67

Results are mean ± SD, n = 3, SI (selective index), MCE (*M. capitatum* leaf extract without chitosan).



(a) (b)

Figure 6 Effect of MCSNPs on: (a) MCF-7 human breast cancer and (b) OV7 woman epithelial ovarian cancer

agents. Moreover, it is well-known that the pharmacokinetic properties of a drug or excipients change considerably when included in a nanoparticulate system. Thus, there *in vivo* fate is decided by the size, charge and surface modifications of the NPs. The aftermath of this is that the toxicity effects the NPs are changed rapidly, because as all there parameters regulate the manners with which many microspheres carry out their biological functions with various tissues in the body aimed at controlling their metabolic pathways and ending with their exit from the body by excretion[28]. For easy targeting of cancer cells of the granulosa of the ovaries, chitosan nanoparticles are linked with specially receptors designed to identify folic acids, and this make the to induce cellular dead of cancerous cell in the ovaries and also help to prevent spread of cancer cells to healthy tissues of the ovaries or other nearby tissues[29], (Table 6). The function chitosan loaded leaf extract in this research might also be the same due its high selective index against normal cell VeroE6 (Table 6). Also, chitosan has established itself as an excellent drug carrier capable of delivering anticancer drugs to cancer cell with minimal or no side effects. In this study, chitosan loaded leaf extract was potent against ovarian cancer cell with higher selective index than doxorubicin first line anticancer drug (Table 4, 5, 6, figs.6a & 6b). This aspect of drug delivery by loading chitosan nanoparticles with leaf extract from anticancer plant is novel, and it should be exploit further for better cancer therapy option.

CONCLUSION

The study revealed that chitosan loaded drug delivery of anticancer drug is an alternative option for cancer treatment. This aspect of cancer therapy should be improved upon by various researchers towards achieving cancer treatment. The present study will help researchers in exploring nanoparticle delivery approach for cancer treatment, because it is novel. The results from this study maybe extended into various fields of medical sciences toward problem solving based on chitosan drug delivery approach.

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We have no competing interest.

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