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Facile Synthesis of Natural Therapeutics Encapsulated Biopolymeric Okra Mucilage Nanoparticles as Dual ameliorative agent

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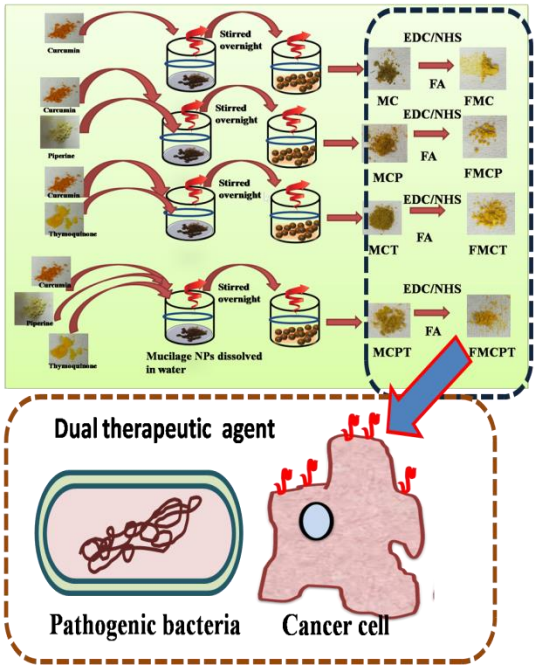
Graphical Abstract



Abstract.

Among the different types of biomaterials, natural excipients Okra mucilage (MNP) is economic and has the potential for controlled drug delivery. We have synthesized MNPs by co-precipitation method and characterized them by XRD, FESEM, FTIR, UV-Vis spectra and DLS. Despite their potential anti-cancer activity, solubility of curcumin, piperine, thymoquinone is very low rendering its limit in application. We have used MNPs where the natural compounds like

curcumin, piperine, thymoquinone can be loaded comfortably and thereby increases its bioavailability. The antibacterial activity of these nanoparticles were evaluated against pathogenic bacterial strains. The cytotoxicity of curcumin ,piperine, thymoquinone encapsulated MNPs was evaluated on triple negative breast cancer cell lines. They were found to induce apoptosis by perturbing the mitochondrial membrane potential. Folic acid was conjugated to curcumin, piperine, thymoquinone encapsulated MNPs, for delivering it specifically to the breast cancer cells. The antimicrobial and anticancer potential of conjugated and non conjugated MNP were assessed by various *in vitro* cellular assays. Our present study confirms that these functionacan be used as a dual therapeutic option for combating pathogenic microbial strains and triple negative breast cancer cell.

Keywords-Okra Mucilage; natural therapeutics; synergistic effect; anticancer;antibacterial activities.

Introduction

Plant derived polymers such as gums and mucilage have various pharmaceutical applications such as diluents, binder, disintegrant in tablets, thickeners in oral liquids, protective colloids in suspensions, gelling agent in gels, bases in suppository [1]. Mucilages which are found in rhizomes, roots and seed endosperms of various higher plant, may act as energy reserves whereas foliar mucilages do not store carbohydrates [2]. Mucilages are polysaccharide hydrocolloids having high water binding capacity due to the high concentration of hydroxyl groups in the polysaccharides. These polysaccharides are preferred to semi synthetic and synthetic excipients as they are biocompatible, non irritant, lack of toxicity, cheap and easily available [3-6]. Such a polysaccharide is okra mucilage which can be extracted from okra that is an erect annual plant, botanically known as *Abelmoschus esculentus* (Family: Malvaceae). Okra mucilage is a polymer of galacturonic acid, rhamnose, and galactose [7]. It can be used as a pharmaceutical excipient i.e. binder, film coating, bio-adhesive and suspending agent [8].

Recent studies show that various kind of natural products have potent medicinal properties and can be used to treat many diseases. One of those natural products is Curcumin (MW 368.38 g/mol) derived from the plant *Curcuma longa* is slightly soluble in water and it has great role in different type of diseases such as cancer, diabetes, arthritis, atherosclerosis and auto immune diseases [9,10] as well as it has antibacterial activity too [11]. One of the very useful medicinal properties of curcumin is its anticancer efficacy. It inhibits tumor progression by activating cell signals and inducing apoptosis in pre-cancerous or cancer cells without affecting normal cells [12-15]. But, its poor water solubility restricts its therapeutic use in cancer treatment [16, 17].

Thymoquinone (MW164.2 g/mol) is a phytochemical present in black cumin *Nigella sativa* with a long history of medicinal use [18]. It has anti microbial, anticancer, anti diabetic, analgesic, anti inflammatory and antioxidant activities [19-21].

Piperine (MW285.34 g/mol) is another natural product having good anticonvulsant, anti microbial and anticancer property [22, 23]. It is a plant alkaloid found in black pepper *Piper nigrum* and long pepper *Piper longum* . It is reported that piperine has bioavailability enhancing activity for some drug [24, 25].

From the therapeutic approach various strategies have been employed to minimize the toxicity to normal cells. Many kinds of cancer targeting agents like small molecules (folic acid, benzamides), peptides (RGD, EGF), peptide domain (FN3 domain, Z domain) are reported [26-28]. Among them folic acid is one of the well accepted cancer targeting agent due to its low cost and it is easy to modify. Folate receptors are over expressed in a variety of cancer cells like epithelial, ovarian, cerviacal, lung [29, 30]. Additionally, these folate receptors are either absent or expressed at a low level in normal cells, which makes them an ideal targeting agent. Thus folate receptors can be employed as effective tool for active targeting of various drug delivery systems.[31]

Here we have evaluated the effect of these natural compound encapsulated biopolymeric mucilage nanoparticles on the prokaryotic and eukaryotic systems. The Curcumin-Piperine-Thymoquinone encapsulated biopolymeric Okra mucilage nanoparticles (MCPT) had more promising effect against Gram-positive (S. aureus) and Gram-negative (E. coli) pathogenic bacterial species than curcumin or curcumin and piperine or curcumin and thymoquinone encapsulated mucilage nanoparticles (MC,MCP,MCT respectively) due to their synergistic effects.

The natural compounds like curcumin, piperine and thymoquinone are reported to possess significant anticancer activities. The encapsulation of these natural compounds within the bioplymeric Okra mucilage matrix enhances their bioavailability. Furthermore the mucilage nanoparticles were conjugated with folic acid in order to target these natural compounds specifically towards the folate receptor over expressed breast cancer cell lines. Our results suggests that the Curcumin-Piperine-Thymoquinone encapsulated folic acid conjugated bioplymeric Okra mucilage nanoparticles (FMCPT) had more promising effect against the breast cancer cell lines than folic acid conjugated curcumin or curcumin and piperine or curcumin and thymoquinone encapsulated mucilage nanoparticles (FMC,FMCP,FMCT) and the non conjugated nanoparticles(MC, MCP, MCT, MCPT) due to their synergistic effects. Our results suggest that the Reactive oxygen species (ROS) generation plays a crucial role in the maintenance of the redox balance in most cancer cells and an elevated ROS level may promote oxidative damage which in turn leads to the mitochondrial membrane potential disruption that culminates in cellular abnormalities or death [32].

In this study, we investigated their antimicrobial as well as anticancer activities against different pathogenic bacterial strains and MDA-MB 468 breast cancer cell lines. Thus, the development of these biopolymeric, non hazardous, eco-friendly functionalized natural compound encapsulated mucilage nanoparticles has the potential to emerge as an effective dual therapeutic agent.

2. Method

2.1. Material

Okra had been collected from market and mucilage was extracted. Ethanol was purchased from Merck. LB broth, Agar powder were supplied by HiMedia. Curcumin, Thymoquinone, Piperine were purchased from Sigma-Aldrich , Cell culture DMEM, RPMI, Fetal Bovine Serum (FBS), JC-1 (mitochondrial staining dye), N-hydroxysuccinimide (NHS), 1-[3-dimethylamino)propyl]-3-ethylcarbodiimide hydro-chloride (EDC), Thiazolyl blue formazan (MTT), and 2′,7′-Dichlorofluorescin diacetate (DCF-DA), were purchased from Sigma-Aldrich. Deionised (Millipore) water was used throughout the experiment with resistivity at least 18MΩ. All glassware used was cleaned with aqua regia solution followed by rinsing with ultrapure Millipore water.

2.2. Bacterial Strains:

Standard cultures for antibacterial assays were procured from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh, India. These include Gram-positive (Staphylococcus aureus 740 and Bacillus subtilis 441) and Gram-negative (Escherichia coli 443 and Pseudomona aeruginosa1688)

2.3. Breast cancer cell line

Triple negative breast cancer cell line, MDA-MB-468 were obtained from the central cell repository of National Center for Cell Science (NCCS), Pune, India and cultured as suggested by the supplier. . All the above cell lines were cultured either in RPMI 1640 or DMEM, containing 10% FBS, 1mM sodium pyruvate, 2mM L-glutamine, non–essential amino acids, 100 units/L penicillin, 100mg/L streptomycin and 50mg/L gentamycin sulfate at 37ºC with 5% CO_2.

2.4. Extraction mucilage from Okra

About 500 g of fresh okra was collected from market and washed with distilled water. After washing these were chopped into small pieces and kept in beakers containing 500 ml of water, then stirred continuously until the mixture became concentrated. Then, the concentrated solution was filtered through strainer. After that, absolute ethanol was added to the filtered solution in continuous stirring condition. Due to ethanol treatment mucilage got precipitated. Precipitated mucilage was collected in a

petridish and kept at 37^0C for overnight to make it dry. On the very next day the dried mucilage was grinded with mortar pestle and now this grinded mucilage is ready to be used .

Figure.1. Schematic representation for the synthesis of Okra Mucilage nanoparticles

2.5. Drug loaded mucilage synthesis

10% of total weight (drug+mucilage) should be the weight of a drug to be loaded e.g. if we want prepare 100 mg of drug loaded mucilage and there are two drugs then 10 mg of each drugs and 80 mg of mucilage would be taken.

To prepare curcumin loaded mucilage (MC), 90 mg of mucilage was dissolved in 20 ml water and vigorously stirred using a magnetic stirrer for 24 hour. After 24 hour, 10mg of curcumin, was added to the mucilage solution. Similarly 10mg each of curcumin, piperine and curcumin, thymoquinone were added to 80mg mucilage solution to prepare curcumin-piperine and curcumin-thymoquinone loaded

mucilage(MCP and MCT) resepectively. To prepare curcumin-piperine-thymoquinone loaded mucilage (MCPT), 10 mg of each drugs were added to 70 mg mucilage solution prepared through above described process.

2.6. Folic acid conjugated drug loaded mucilage synthesis

At first, 8 mg of folic acid and 1 mg each of EDC and NHS was added in 20ml acetone in dark condition to activate the folic acid. The activated folic acid was then drop wise added to 20ml drug loaded mucilage solution containing 90mg drug loaded mucilage and the whole solution was stirred overnight with the help of magnetic stirrer in dark condition. After that, the reaction mixture was centrifuged at 8000 rpm for 8 min and the supernatant was discarded and folic acid conjugated drug loaded mucilage was dissolved in minimum amount of water. Then the solution was taken in a petridish and kept in 37^0C to make dry it. Finally the dried material was collected.

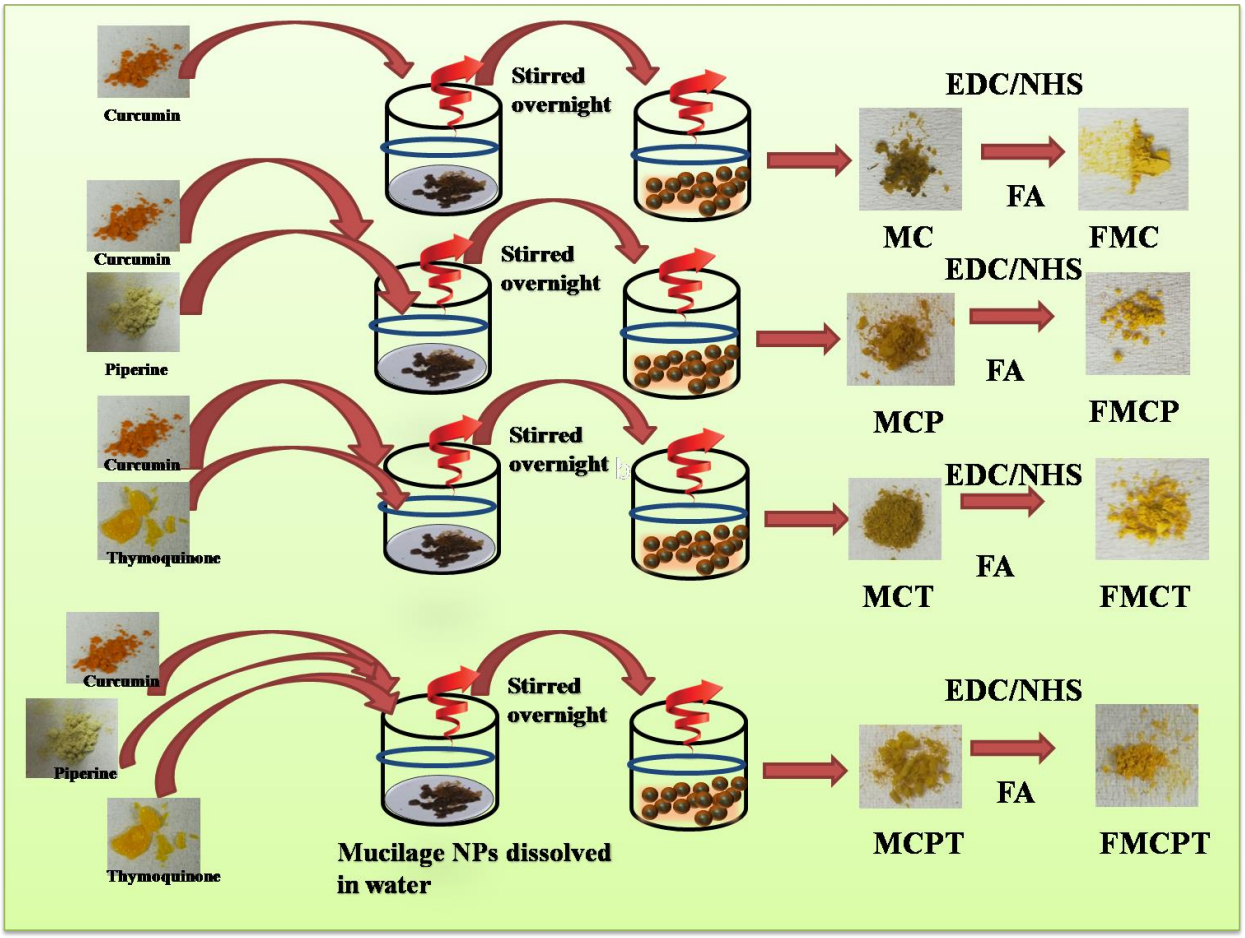


Figure.2. Schematic representation of the encapsulation of natural therapeutics into the Okra mucilage nanoparticles

2.7. Characterization of Okra mucilage nanoparticles

The XRD (X Ray Diffractometer) patterns of the powdered Okra mucilage samples were recorded by X-ray powder diffractometer model-D8, Bruker AXS, Winconsin, USA, using Cu-Kα target employing wavelength of 1.5418 Å and operating at 35kV with scan speed of 1sec/step. Field emission scanning electron microscope (FESEM) was employed for morphological study using INSPECT F50 (FEI, Netherland). The Fourier transform infrared spectroscopy (FTIR) study was done using FTIR-8400S, Shimadzu in the wave number range from 400 cm^{-1} to 4000 $cm^{-1.}$ The Okra mucilage nanoparticles were dispersed in MilliQ water to form a diluted suspension of 2 mg/ml using a bath sonicator for 30 mins. When particles were completely dispersed in water its absorption spectra was taken using UV visible spectrophotometer (Bio-Tek). Average particle diameter and size distribution of Okra mucilage nanoparticles were measured DLS (Dynamic light Scattering) using Zetasizer (NANO ZS90, Malvern Instruments Ltd,UK). The charge of the NP was also measured by the Zetasizer.

2.8. Minimum Inhibitory Concentration (MIC) determination

Test tubes containing 4 ml of LB media was inoculated with 200 µl overnight culture of *E.coli* (DH5α) and *S.aureus* for 4 hour. Then various concentrations (100 µg/ml, 200µg/ml, 300µg/ml, 400µg/ml and 600µg/ml) of drug loaded mucilages was added to each marked tubes. Tubes were then left for shaking at 37^0C for 24 hour. After 24 hour OD of each solution were measured at 600 nm.

2.9. Agar well diffusion method

The susceptibility of pathogenic bacteria to Okra mucilage MC, MCP, MCT, MCPT nanoparticles was examined by the Agar well diffusion method according to a previously reported protocol. The pathogenic strains were grown on LB Broth at 37°C overnight upto a turbidity of 0.5 Mac Farland standards (10^8 CFU per ml). About 100µl of this suspension was used to inoculate 90 mm diameter petridish filled with 30 ml of LB Agar. Wells were punched in the Agar plates and treated with Okra mucilage MC, MCP, MCT, MCPT nanoparticles at their MIC concentrations. The zone of inhibition diameter in the bacterial growth surrounding the disc (excluding the well) was measured.

2.10. Cytotoxicity assay

The viability of MDA-MB 468 cells after exposure to various concentrations of natural compound encapsulated folic acid conjugated and non conjugated Okra mucilage nanoparticles was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, around 1×10^4 cells per well of 48-well plates were exposed to Okra mucilage capped natural compound encapsulated nanoparticles at the concentrations of untreated as control, 20, 40, 60, 80, 100 µg/ml for 24 h of incubation at 37◦C and 5% CO2. Following this, the cells were incubated Zn aiwith 10 µl MTT solution (stock 1 mg/ml) for 4 h at 37◦C and 5% CO_2 following a wash with 1× phosphate-buffered saline (PBS), and the resulting formazan crystals were dissolved in MTT solubilization buffer to measure the absorbance at 570 nm by using a microplate reader (Biorad). The data were formulated comparing with the control ones [33, 34].

2.11. ROS measurement Intracellular ROS generations were checked by DCFDA method

Normally, the DCFDA enters the cell and reacts with the reactive oxygen to give a green fluorescent color compound dichlorofluorescein (DCF) [33]. Briefly, a stock solution of DCFDA (10 mM) was prepared in methanol and was further diluted with PBS to a working concentration of 100 µM. MDA-MB 468 cells were treated at LD_{50} dose of MCPT and FMCPT for 12 h at 37◦C, and washed with ice-cold 1× PBS followed by an incubation with 100 µM of DCFDA for 30 min in the dark at 37◦C. The fluorescence intensity was measured both spectroscopically (Hitachi, Japan) and under a fluorescence microscope in MDA-MB-468 cells (Leica, Japan) at excitation and emission wavelengths of 485 and 520 nm, respectively.

2.12. Mitochondrial membrane potential measurement by JC1 staining

Mitochondria depolarization is specifically indicated by JC-1 dye, which is a cationic dye that exhibits potential-dependent accumulation in mitochondria, indicated by a fluorescence emission shift

from red to green as the mitochondrial membrane gets damaged and loses its membrane potential [33]. Cells were treated after the respective LD_{50} dose of MCPT and FMCPT nanoparticles for 12 h. After the treatment, the cells were washed with phosphate buffered saline (PBS) and incubated with (10 µg/ml) JC-1 for 30 min at 37^0C. Cells were then observed under a fluorescence microscope (Leica, Wetzlar, Germany).

3. Results and discussions-

3.1. Structural morphology analysis of Okra mucilage Okra mucilage nanoparticles

The XRD pattern of GA and MCPT are shown in Fig. 1(XRD). In the XRD spectrum of MNPs, no crystalline peaks are observed, but a small hump (25- 40^0) is seen in MCPT. This confirms the encapsulation of curcumin, piperine, thymoquinone in the cross-linked network of MNPs.

Morphology and particle size of the samples were determined using FESEM. FESEM micrographs [Fig. 1(FESEM)] show spherical distribution of the particles with a size range of 400- 500 nm (for MNP).

3.2. Spectroscopic analysis of Okra mucilage nanoparticles

FTIR spectroscopy is a useful technique to examine the functional groups of any organic molecule. Formation of drug loaded mucilage and conjugation of folic acid (FA) with drug loaded Okra mucilage was confirmed by FTIR study. Fig. 1(FTIR) showed that the pure MNPs exhibit strong band at 3407 cm^{-1} (-OH stretching) and 2937 cm^{-1} (-CH stretching). Vibrational peaks at 1624 and 1427 cm^{-1} (symmetric and asymmetric stretching of –COO-) indicate the formation of Okra mucilage[23]. Conjugation of curcumin, piperine, thymoquinone with Okra is indicated by a peak at 1510 cm^{-1} (C-C stretching) and 1627 cm^{-1} (-C=O vibration) [23]. The FTIR spectra of MCPTF Okra mucilage nanoparticles showed two clear absorbance 1125 cm^{-1} and 1605 cm^{-1} which are similar to the absorbance peak present in FA . These results confirm the conjugation of folic acid modified and curcumin, piperine, thymoquinone loaded MNPs.

Ultraviolet-visible spectroscopy is a characterizing tool to ascertain the optical behaviour of a material. The absorbance spectrum of MNP [fig 1(UV-Vis analysis)] does not show any sharp peak in its spectrum whereas MC, MCP, MCT and MCPT samples exhibit a prominent peak at 423 nm, 343 nm, 430

nm and 344 nm respectively [35,36]. There is a hump at 425 nm in MCP and MCPT UV spectra due to presence Curcumin [37]

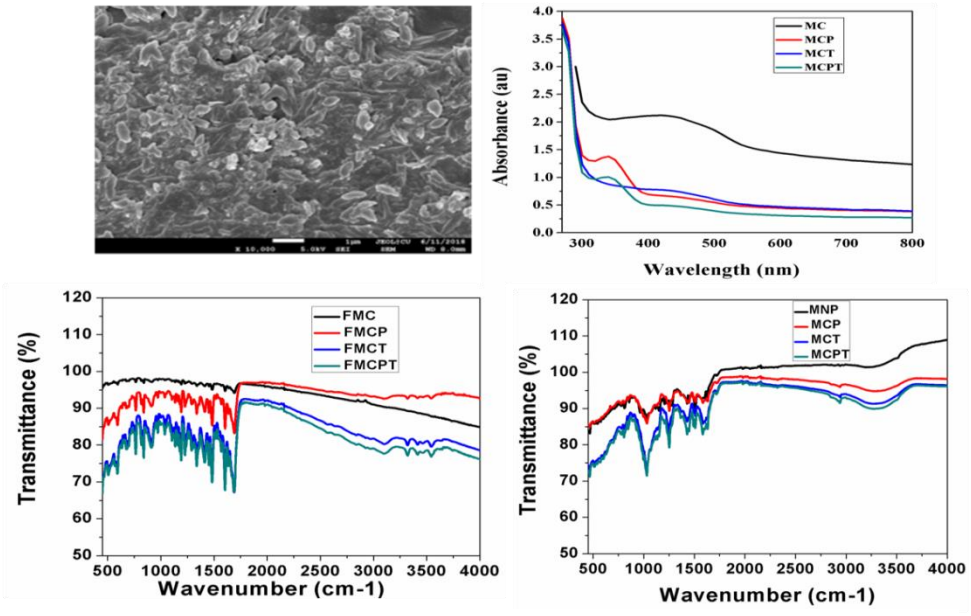
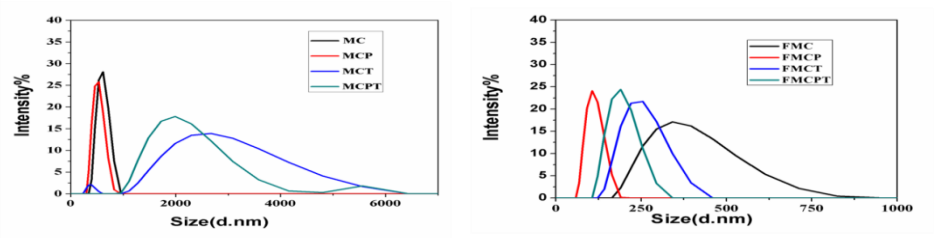


Figure.3. Physical characterizations of the Okra mucilage nanoparticles.

3.3 Colloidal performance and surface charge of synthesized Okra mucilage nanoparticles

Curcumin, piperine, thymoquinone loaded MNPs show a hydrodynamic diameter of 413.3 nm and a zeta potential of -0.348 mV (Table. 1). Curcumin, piperine, thymoquinone loading does not alter the zeta potential, but increases the hydrodynamic diameter. Presence of curcumin, piperine, thymoquinone inside the matrix also increases the size of the Okra mucilage nanoparticles than the bare Okra mucilage nanoparticles. The folic acid conjugation enhances the hydrodynamic diameter of the Okra mucilage nanoparticles to 813.3 nm and the surface charge of the Okra mucilage nanoparticles considerably increases to -7.6 mV. This high negative zeta potential of the Okra mucilageindicates the stability of the particles. The corresponding polydispersity index (PDI) values of the samples are quite low suggesting that they form a homogenous solution which is desirable for any biological application.



Sample Name	PDI	DLS Size (d. nm)	Zeta Potential (mV)
MC	0.299	605.2	1.100
MCP	0.193	617.3	0.219
MCT	0.314	1887	-0.424
MCPT	0.278	2066	-2.640
FMC	0.157	340.6	-2.580
FMCP	0.015	105.9	-3.160
FMCT	0.005	236.3	-1.460
FMCPT	0.061	185.4	0.365

Sample name	*E.coli*	*S.aureus*

Figure.4. Hydrodynamic size and Zeta potential studies of MC,MCP,MCT,MCPT,FMC,FMCP, FMCT,FMCPT.

3.4. Biopolymeric MC, MCP, MCT, MCPT nanoparticles nanoparticles exhibits significant antibacterial activity against pathogenic bacterial strains

To evaluate the bactericidal activity of the synthesized MC, MCP, MCT, MCPT nanoparticless have been treated separately against Gram negative *E.coli* and *Gram positive S.aureus.* In the case of *E.coli,* strain the IC_{50} values of MC, MCP, MCT, MCPT nanoparticles was 240±2.7µg/ml, 224±3.5µg/ml, 218±4.1µg/ml and 176±2.89µg/ml, that is in case of *S.aureus* the MIC values were 236± 2.67µg/ml, 218±3.65µg/ml, 215±2.98µg/ml and 163±2.57µg/ml, respectively.

In this study we observed significant bactericidal activity of all the natural compound encapsulated Okra mucilage nanoparticles. However, the Curcumin-Piperine-Thymoquinone encapsulated bioplymeric Okra mucilage nanoparticles (MCPT) had more promising effect against Gram-positive (S. aureus) and Gram-negative (E. coli) pathogenic bacterial species than single compound encapsulated mucilage nanoparticles (MC,MCP,MCT) due to their synergistic effects [fig 5]

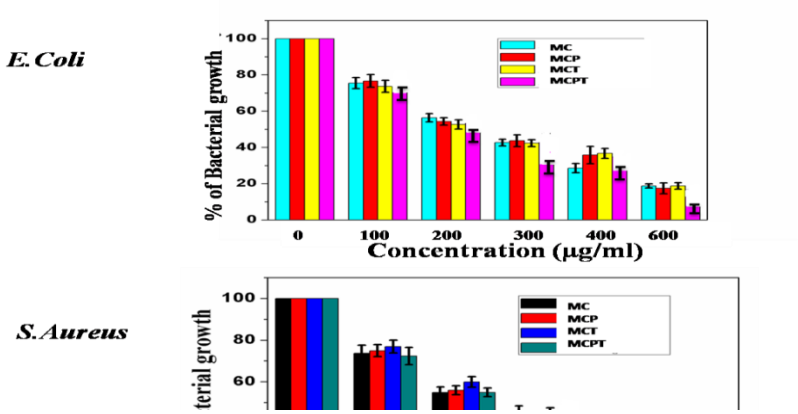


Figure.5. Synergistic effects of natural therapeutics encapsulated Okra mucilage nanoparticles on common pathogenic bacterial strains

Table 2

MC	240 ± 2.7	236 ± 2.67
MCP	224 ± 3.5	218 ± 3.65
MCT	218 ± 4.1	215 ± 2.98
MCPT	176 ± 2.89	163 ± 2.57

3.5.Bactericidal activity of MC, MCP, MCT, MCPT nanoparticles confirmed by Disc diffusion method

This study was performed to visualize the comparison of antibacterial activity of different Okra mucilage capped MC, MCP, MCT, MCPT nanoparticles. The zone of inhibitions against the Gram-positive and Gram negative bacteria are shown in Figure 6. Zone of inhibition having a radii of around 1.16 ± 0.04 cm, 1.86 ± 0.23 cm, 1.94 ± 0.44 cm ,2.14± 0.40 cm (in case of *E.coli*) and 0.91 ± 0.12 cm, 1.23 ± 0.04 cm, 1.19 ± 0.04 cm, 1.45 ± 0.07 cm (in case of *S.aureus*) were observed when strains were treated MC, MCP, MCT, MCPT respectively nanoparticles at their respective IC_{50} values. So, from this study, it is quite evident that the MC, MCP, MCT, MCPT nanoparticles are really proved to be beneficial and can be administered for pathogenic *E.coli and S.aureus*, growth inhibition with MCPT having the more pronounced effect.

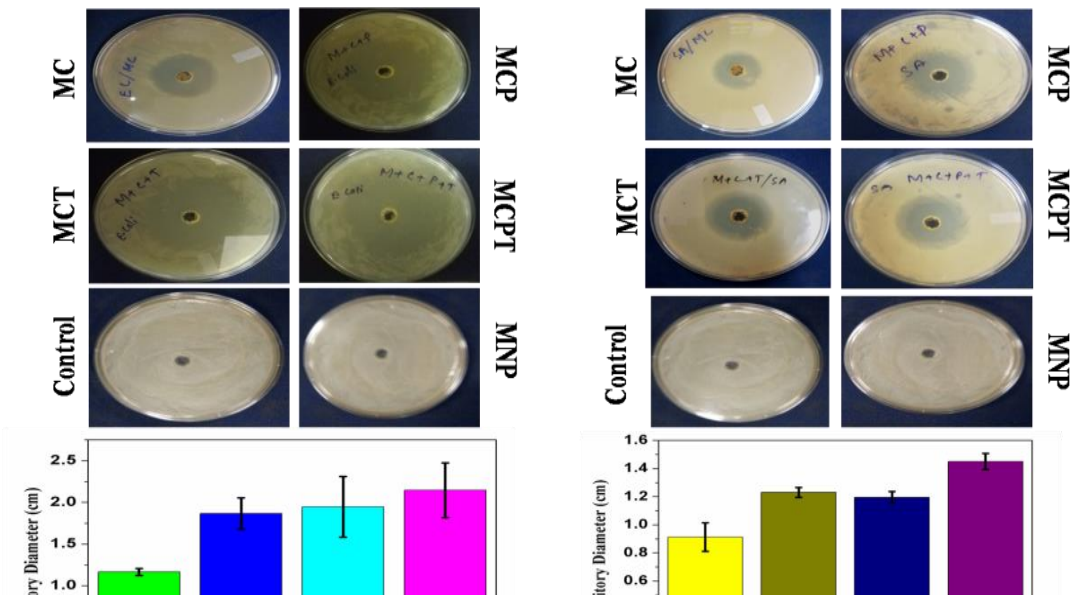


Figure.6. Bactericidal activity of natural compound encapsulated Okra mucilage nanoparticles confirmed by Disc diffusion method

3.6. Selective cytotoxicity of targeted Okra mucilage Okra mucilage nanoparticles towards the folate overexpressed TNBC cells

We have explored the cytotoxic effect of our synthesized MNP, MCPT, MCPT (0-100µg/ml) in human triple negative breast cancer cell lines MDA-MB 468 respectively. It was found that cell survivability was decreased in dose dependent manner in the treated cells. This study concluded that FMCPT have strong cytotoxic effect on both human TNBC as compared to MNP, MCPT [Fig 7]. Bare Okra mucilage nanoparticles have little cytotoxic effect. The LD_{50} dose for each of these Okra mucilage nanoparticles are compared in Table 3. Our results suggests that The Curcumin-Piperine-Thymoquinone encapsulated folic acid conjugated bioplymeric Okra mucilage nanoparticles (FMCPT) had more promising effect against the breast cancer cell lines than single compound encapsulated folic acid conjugated mucilage nanoparticles (FMC,FMCP,FMCT) and the non conjugated nanoparticles(MC, MCP, MCT, MCPT).

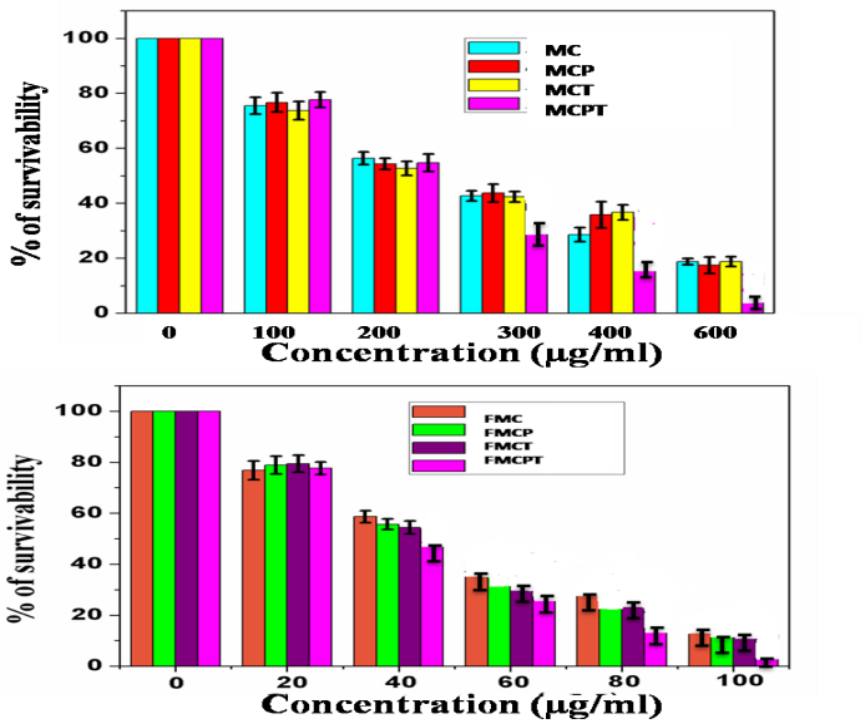


Figure.7.Selective and synergistic effects of the natural compound encapsulated folic acid conjugated MNPs in MDA-MB 468 cells

3.7. Treatment of targeted curcumin, piperine, thymoquinone encapsulated Okra mucilage nanoparticles promotes ROS generation and mitochondrial permeability transition (MPT) in TNBC cells.

Reactive oxygen species (ROS) was measured by both fluorescence microscopy and spectrofluorometry methods by using specific probe, DCF-DA. MDA-MB 468 cells were used for the determination of reactive oxygen production .The cells were treated with MNP, MCPT, FMCPT treatment at their respective LD_{50} dose for 12h. From the fluorescence microscopic image, it was seen that green color fluorescent intensity was increased for FMCPT treated cells in MDA-MB 468 cell line compared to control cells (Fig. 8a). From the spectrofluorometry study, it was seen that ROS intensity increased almost 2.5 folds for the cell line in FMCPT treated cells (Fig. 8b).

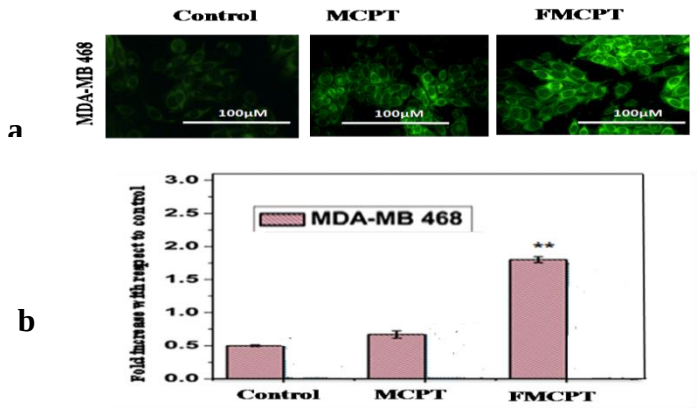


Figure.8.Enhancement of intracellular oxidative stress in MDA-MB 468 cells

Production of ROS directly contributes to mitochondrial damage. Thus, we explored the mitochondrial membrane potential effect of FMCPT, MCPT in MDA-MB 468 cells. Mitochondria depolarization is specifically indicated by JC-1 dye, which is a cationic dye that exhibits potential-dependent accumulation in mitochondria, indicated by a fluorescence emission shift from red to green as the mitochondrial membrane loses its potential due to its damaged membrane. It was observed that FMCPT and MCPT nanoparticle treated cells have an increased green intensity (monomer-form of the dye) compared with the untreated cells which appear red (aggregated-form of the dye) [Fig 9a]. This observation indicated that the FMCPT had more effect on the mitochondrial membrane than MCPT which suggests that the folic

acid conjugated Mucilage nanoparticles could effectively deliver the natural compounds,Curcumin,Piprine and Thymoquinone to the breast cancer cells.Green and Red colour quantification data is also presented for both the cell lines (Fig. 9b).

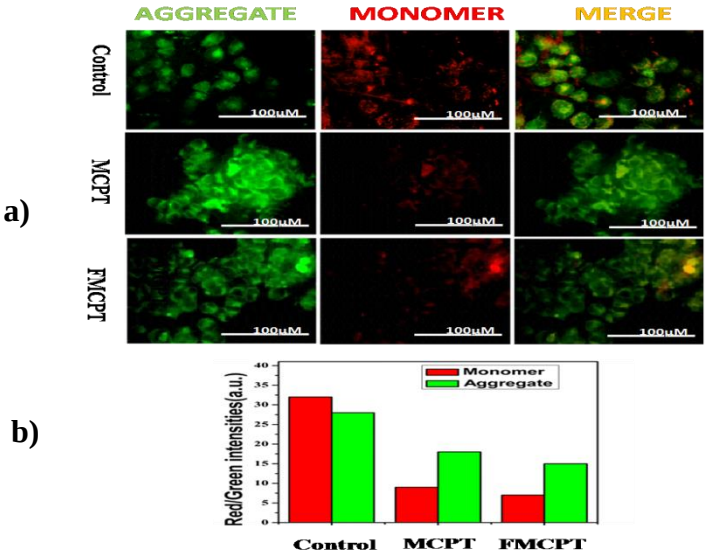


Figure.9.Mitochondrial membrane potential disruption by the delivery of natural therapeutics in MDA-MB 468 cells

Conclusions

The synthesis of biopolymeric natural compound encapsulated Okra mucilage nanoparticle is a very low cost effective process and non toxic. In this study we have observed a significant antibacterial as well as anticancer activity. This may be due to inhibit the growth of gram positive and gram negative bacteria. The Conjugated and non conjugated MNP were characterized by UV-Vis, FTIR, DLS and FESEM. The FTIR, UV–Vis spectral studies confirmed the formation of the natural compound encapsulated nanoparticles. Particle size and stabilization were determined by DLS and zeta potential techniques. FESEM studies revealed rod and uniform shaped MC,FMCP,FMCT,FMCPT nanoparticles with size in the range 25-100 nm.

Despite a notable improvement in the field of cancer diagnosis and treatment, cancer is a growing threat to the world. Furthermore, due to high side effects caused by most promising chemotherapy, metastatic cancer needs more effective chemotherapeutics to minimize these side problems. Again, the

global dissemination of pathogenic bacterial strains is one of the most serious present-day challenges in hospital-acquired infections which needs to be taken care of in a more economical and healthy way. The natural compound encapsulated MNPs were shown to be a potential antibacterial agent when tested against Gram-positive (S. aureus) and Gram-negative (E. coli) bacterial species which was confirmed The Curcumin-Piperine-Thymoquinone encapsulated bioplymeric Okra mucilage nanoparticles (MCPT) had more promising effect against Gram-positive (S. aureus) and Gram-negative (E. coli) pathogenic bacterial species than single compound encapsulated mucilage nanoparticles (MC,MCP,MCT) due to their synergistic effects.

Furthermore, Folic acid conjugated MNP enhances cellular ROS and causes mitochondrial membrane potential disruption in human breast cancer cells which in turn culminates in the death of cancer cells. Our results suggests that The Curcumin-Piperine-Thymoquinone encapsulated folic acid conjugated bioplymeric Okra mucilage nanoparticles (FMCPT) had more promising effect against the breast cancer cell lines than single compound encapsulated folic acid conjugated mucilage nanoparticles (FMC,FMCP,FMCT) and the non conjugated nanoparticles(MC, MCP, MCT, MCPT) due to their synergistic effects. In conclusion, our studies suggest that the natural compound encapsulated mucilage nanoparticles is not only an effective antibacterial agent but also appear as a promising anticancer drug.

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