RESEARCH PAPER

Screening and Evaluation of Drug Loaded Silver Nanoparticle for Treatment of Breast Cancer by Using Triterpenoid Glycosidal Compound

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ABSTRACT

Proposed research work aims with formulation and optimization of anticancer Drug loaded Silver Nanoparticle (0.1N) using triterpenoid derived Bergamot (Citrus bergemia). Both drug 18β-Glycyrrhetinic acid and ursolic acid from triterpenoid glycoside group were selected by considering anticancer activity. Triterpenoid glycoside are significant in the treatment of breast cancer treatment, they aid in preventing cancer cells from proliferating especially in models such as MCF-7 breast cancer cell line. These substances cause tumour cells to undergo apoptosis or programmed cell death and interfere with continuation of cell cycle, also shows anti-inflammatory and antioxidant properties that show growth and spread of tumour. Triterpenoid glycoside reportedly can improve effectiveness of formulation delivery with higher chemotherapeutic effect. Another key ingredient is bergamot oil with its anticancer properties, it prevent cancer cells from proliferating and aids in their apoptosis. By lowering oxidative stress its potent antioxidant activity stops cancer from spreading. It can also improve efficacy of formulation. To achieve homogenous particle size and distribution high speed homogenization and ultra-probe sonicator is implemented for formulation of Drug loaded Silver Nanoparticle (0.1N) followed by ultra-centrifugation and lyophilized to attend free flowing and dry silver nanoparticles. The Prepared AgNPs were evaluated with surface morphology Field Emission Scanning Electron Microscopy (FE-SEM), Particle size, Zeta potential, PDI, FTIR, Differential Scanning Calorimeter (DSC), X-Ray Diffraction study (XRD), In-vitro study (MCF-07), with research finding Drug loaded Silver Nanoparticle (0.1N) shows significant cytotoxic activity against MCF-07 cells. Obtain values with IC50, TGI and LC50 value indicate strong growth inhibition, effective suppression of cell proliferation, induce cell death.

Keywords: Lyophilizer, Triterpenoid glycoside, MCF-07 cell line, Bergamot oil (Citrus bergemia), Silver-Nanoparticle, Ultra-probe sonicator, X-Ray Diffraction.

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Introduction

Breast cancer is very diverse tumor that starts in the milk duct epithelium. It varies greatly between individuals (inter-tumor heterogeneity) and within each individual tumor (intra-tumor heterogeneity). Inter-tumor heterogeneity is basis for the histopathological classification of breast cancer. Invasive ductal carcinoma is the most common histologic form of invasive breast cancer(40-75%)(1) . The WHO classification also includes 21 additional specialized subtypes with unique morphologic features, the most prevalent of which is invasive lobular carcinoma (5-15%).

Natural products have great potential for contemporary medication development because of their structural diversity, variety of sources and biological activity. These products and their derivatives include a wide range of bioactive substances that come from plant sources (such as phenolic acid, alkaloids, polysaccharides, saponins, quinones and terpenes) microorganisms, fermentation products (such as polysaccharides , antibiotics and pigments and marine organism(such as sterols, terpenes and saponins). Cells

Flavonoids, polyphenols, alkaloids , terpenes and saponins derived from plant have been shown to have anti-tumor effects(2).

Cancer is cauterized the uncontrolled growth and spread of uncontrolled growth of the abnormal cell. In a normal cell are grow, divided and die but in the cancerous cell are only multiply and potentially forming the masses called as tumor(3). Healthy cells within the body result in unchecked growth that produces a lump known as tumor. Tumor can develop and spread into nearly healthy tissue or to other areas of the body through the lymphatic and blood system if treatment is not received. They can also have an impact on digestive, neurological and circulatory system. The world health organization estimates that malignant neoplasms cause 107.8 million disability adjusted life years for women globally with breast cancer accounting for 19.6 million of these DALYs. With 2.26 million. It is anticipated that 29% of all new malignancies in women in the US will be breast cancer alone.

Age standardized incidence rate of breast cancer have high and positive correlation with the human development index according to the 2018 GLOBOCAN data. The ASIR was highest in extremely high HDI countries (75.6 per 100,000) according to statistics from 2020(4). Cancer is a genetic disorder which due to change or mutation of DNA which control the function of cell. Over the caner there are more than 100 distinct diseases to uncontrolled growth of cell, which contentiously dividing and spread to body. Cancer cell are differing as that normal cell with their Size, Shape and nucleus. This cell is growth rapidly before they mature, cancer cell is always remaining immature state. Cancer cell having ability to reduce the immunity and grow into a tumor(5). Globally in the year of 2022 on report it has been noted 20 million of cancer cases and the 9.7 million deaths due to cancer, over this 2.3 million of Female Breast cancer is detect which is nearly 11.6 percent over this the 6.9 percent of death rate(6).

There are notable differences in the incidence of breast cancer between rural and urban populations with five cases per 100,000 in rural areas and thirty cases per 100,000 in urban areas, according to data from ICMR (Indian Council of Medical Research) National Cancer Registry Programmed. In India's northeastern regions and large metropolitan areas, this disparity is especially noticeable. The ICMR-national center for Disease informatics and Research hospital based Cancer registries study from 2021 states that half of all female cancers are gynecologic cancers, primarily breast cancer(7). Breast cancer is very diverse tumor that starts in the milk duct epithelium. It varies greatly between individuals (Antitumor hetergrogenicity) and within each individual tumor (intratumor heterogeneity).

Intertumor hetergrogenicity is basis for the histopathological classification of breast cancer. Invasive ductal carcinoma is the most common histologic form of invasive breast cancer (40-75%). Depending upon the class their having differ type of breast cancer, Origin it having Ductal and Lobular, Invasiveness have In situ and Invasive, according to Receptor status it having ER/PR+, HER2+, Triple-negative. Molecular type it classifies as Luminal A, Luminal B, and HER2-enriched, Basal-like. Special type it having Inflammatory, Paget's, Mucinous, etc. Breast cancer having some risk factor including Genetic, Hormonal, Lifestyle, Reproductive, Envirocm-1ental, Demographic and Medical history(8). High expression of genes linked to estrogen receptor activity and low expression of genes linked to proliferation and HER2 receptor expression are characterized of the luminal type A. Luminal type B is distinguished by a positive ER status, low expression of genes linked to this receptor and higher than type A expression of genes linked to the proliferation measured by Ki-67 marking. Meltdown and Ki-67 expression were identified by a panel of panelists in St. Galen as variables that might be utilized to distinguished between luminal type A and subtype B cancers.

This is crucial for the prognosis evaluation, which is superior in type A(9). In the breast cancer various Genes are involved like BRCA1 and BRCA2 it having tumor suppressor gene involved in DNA repair, mutation significantly increases hereditary risk factor of breast cancer, and HER2 these genes have proto-oncogene which promotes cell growth. This lead to aggressive breast cancer it can cure by specific therapies(10). TP53 and other oncogenes, TP53 are regulate to cell cycle and apoptosis(11). In the mutation happen which result uncontrolled cell division. Breast cancer are commonly described as the three stages, Initiation, promotion and progression. In the Initiation stage involved irreversible genetic mutation in normal epithelial cell, also mutation is occurring the gene key in BRCA1 and BRCA2 and TP53 which this are caused by Radiation, Carcinogens, Inherited mutations(12). In Promotion Stage mutated cell are rapidly get

proliferation mainly driven hormonal stimulation which lead to increase cell number and abnormal cell structure which is reversible if stimuli remove. In Progression stage acquire additional mutations and become malignant which create invasion of surrounding tissue, Angiogenesis and metastasis to lymph nodes and distant organs which become clinically detectable and aggressive(13).

Natural products have great potential for contemporary medication development because of their structural diversity, variety of sources and biological activity. These products and their derivatives include a wide range of bioactive substances that come from plant sources (such as phenolic acid, alkaloids, polysaccharides, saponins, quinones and terpenes) microorganisms, fermentation products (such as polysaccharides , antibiotics and pigments and marine organism(such as sterols, terpenes and saponins). Cells Flavonoids, polyphenols, alkaloids, terpenes and saponins derived from plant have been shown to have anti-tumour effects(2). In this research article the Citrus bergemia natural components are use with micro-emulsion bases silver nanoparticle. Citrus Bergemia is a citrus fruit which belong to citreae tribe in the Aurantioidae subfamily of Rutaceae plant family(14). It having bioactive compound which contain Flavonoids, Polyphenol, Essential oils, it having a potential as anti-cancer effect which inhibit growth of breast cancer, promotes cell death in tumor cell which involved pathway of TP53 and caspase enzyme. It neutralizes free radical which reduce oxidative stress linked to cancer development.it also shows anti-inflammatory effect which decreases inflammatory mediators, also help to reduce tumor-promoting envirocm-1ent. It also modulates signal modulating pathway which affect cancer cell cycle regulation and Angiogenesis inhabitation(14, 15).

Material and method

18β-Glycyrrhetinic acid (GA-18) and was purchase from Yucca enterprises, Mumbai. India, and Ursolic acid was purchase from Yarrow chemicals, Tween 20, PEG-400, Glycerol, Bergamot oil was purchased from Loba Chem. Pvt. Ltd. (Mumbai, India), whereas Ethanol, Methanol, Silver Nitrate, Phosphatidylcholine, And Sodium Taurocholate Pluronic F68, and all other solvents and reagents used were of analytical grade and was purchased from Loba Chem. Pvt. Ltd. (Mumbai, India).

Selection of oil, surfactant, and Co-Surfactants

A number of volatile oils, including lemongrass, cinnamon, clove, and cinchona, bergamot and eucalyptus oil were tested in order to determine best liquid for the formation of containing silver nanoparticles. Each oil sample solubility was assessed visually by maintaining the test tube at room temperature for 24 hr. 18β-Glycyrrhetinic acid and ursolic acid hours which was 37°C. Tween 20, Tween-20, was chosen as surfactant and Glycerol, PEG-400, PEG-4000 was chosen as a Co-surfactant. The stability of each oil phase for drug solubility were examined. Based on how efficiently they emulsified with a certain surfactant, oil with requisite drug solubility were further evaluated(16, 17).

Formulation of Drug loaded Silver Nanoparticle (0.1N)

Oil phase: On the basis of screening study with different oil Bergamot oil is selected along with Tween 20, PEG- 400 as surfactant and Co-surfactant respectively. Both drug sample in equal quantity (mg each) were added into Bergamot oil (10 ml) and entire mixture is allow to mix properly with magnetic stirrer at 1000 RPM. To drug oil mixture there is addition of Tween-80(10 ml) with dropwise manner with syringe is carried out, to this uniform phase addition of 10 ml of 0.1 N AgNO3 is proceeded.

Aqueous phase: 40 ml PEG-400 and distilled water 20 ml is allow to mix uniformly with continuous stirring 1000 RPM with magnetic stirrer. After mixing of both Oil phase and aqueous phase, solution mixture is proceeding further in high speed homogenization at 30,000 RPM and Ultra-probe sonication for period 45 minute to allow uniform mixing and to attend Nano-size range of proposed formulation. Next, allow sepration of brown colour silver nanoparticles by ultra-centrifugation with 75,000 RPM for period of 20 min. Brown colour silver nano-particles were collected and next subjected for drying along with lyophilization(18-20).

Physicochemical Characterization of Drug loaded Silver Nano-particles (0.1N)

According to the protocols described in official compendia, drug loaded silver Nano-particles (0.1N) were examined using a variety of physicochemical parameters, including general appearance, colour, size, shape as per all determination were made in triplicate.

DSC Study of both Phytochemical

For both coated and uncoated samples, differential scanning calorimetery reading were taken while the samples were heated to 100 C. the process of preparing the sample involves weighing the coated and raw grains ,using forcep to place each one into an alumninum crucible and then palcing each into DSC test device in turn(21).

FTIR Spectroscopy analysis of both Phytochemical

Both phytochemical were analyzed by KBR pellet method in the wave number range of 4000-400 cm to indentify the funtional grous. For the measurements prepared pellet was loaded on holder of fourier transform infrared (FTIR-4800,Shimadzu ,Japan)(22).

XRD study of both Phytochemical

The pure drug sample was coarsly powdered and gently placed on a sample holder to ensure a smooth uniform surface , to reduce preferred orientation effects. the sample were scanned at a rate of 2 °/min with a step size of 0.02 ° over a 2 ɵ range of 5 °C to 80 °C. to find distinctive peaks that corresponded to each drugs crystalline structure ,diffraction patteren were captured and examined. To prevent moisture interference during sample prepration analysis, all drug samples were evaluated at a room tempratre(18).

Particle size, Polydispersity Index and Zeta potential

Mean particle size, Polydispersity index and Zeta potential were calculated using Malvern Zetasizer. To prevent multiple scattering effects, the formulated Drug loaded Silver Nanoparticle (0.1N)formulations were diluted suitably usually 1:10 dilution using deionized water , The mean particle size was measure at a fixed 90 C angle, to accurately determine their size the produced silver nanoparicles were scanned., while the zeta potenical was ascertained by transferring the sample to elctrophoretic cells and applying an electric potential zeta potential was calculated using the smoluchowski equation and the nanpoparticles mean electrophoretic mobility, PDI All measurements were recorded at room temperature was used for all measurement. Particle size, PDI and zeta potenical measurment(23)

Morphological Evaluation Field Emission Scanning Elctron Microscope (FE-SEM)

One kind of electron microscope that uses a high energy electron beam to scan a smaple surface in a raster scan patteren is called a field Emission Scanning Elctron Microscope . Field emission guns were used to emit elctron. The emission from these kinds of electron emitters can be up to 1000 times that of tungsten filament. They needed far higher vaccume conditions ,trough. Metal aperatus and magnetic lenses are used to restrict and concentrate the elctron beam after it leaves electron gun into thin ,monochromoatic beam lastly each sort of electron beam after it leaves the elctron detector is inerted into a microscope which gathers signals to create an image of material. (24).

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR is a comparatively quick method for the first stage of sample charectrization. KBR pellet method is implimented for sample prepration for drug loaded Drug loaded Silver Nanoparticle (0.1N) by finely grounded with sectroscopic grade potassium bromide in ratio approximately 1:100. The KBR grinding. The spectra were recorded over a wavenumber range of 4000-400 cm-1, the spectra were analyzed to identify functional group and molecular interaction.

Differential Scanning Calorimetery (DSC) of Drug loaded Silver Nanoparticle (0.1N)

Differential scanning calorimetery measurements were recoreded during heating to 100 for non coated and coated samples. Sample preprations consisited of weighing both coated and new granules , inserting them in turn into DSC test apparatus. Differential scanning Colorimetery were used to invstigate Drug loaded Silver Nanoparticle (0.1N)(Mettler Toledo Ltd. USA). A DSC pan was filled with a known amount of Drug loaded Silver Nanoparticle (0.1N)(2-4 mg) covered with lid and crimped. Nitrogen ultra pure inert gas was used to analyze the reference and sample pan at a heating rate of 10 °C per minute . the melting point and kind of reaction exothermic or ndothermic were then determined from DSC peak. There were twon broad ,flat peaks with a baseline that sloped gardually (25).

X-Ray Differection study (XRD) of Drug loaded Silver Nanoparticle (0.1N)

XRD a powerful analytical method for evaluating the purity nature and crystalline phases of sample particles, was emplyed to investigate the crystallinity and purity nature of ursolic acid&18β-Glycyrrhetinic acid. Thus it demonstarte that whereas the generated in clear crystalline for 18β-Glycyrrhetinic acid and ursolic acid shows low crystalline near to amorphous form. by its strong intense diffraction peaks art 2 ɵ value.(26)

In-vitro study(MCF-07) of Drug loaded Silver Nanoparticle (0.1N)

Cell lines were kept in an appropriate culture medium that was enhanced with 2mM L-glutamine and 10% fetal bovine serum. 5000 cells per well were seeded onto 96 well microtiter plates with 100 µL of medium for screening investgation. Plates were then incubated for 24 hours at 37 °C with 5 % CO2 high humidity. The experimental medications were first dissolved at 100 mg/mL, then diluted to 1 mg/mL and frozen. The stock solution was thawed and subsequently diluted to concentrations of 100-800 µg/mL prior to use. To reach final concentrations of 10-80 µg/mL, 10 µL of each dilution was applied to wells holding 90 µL of media. Plates were incubated for 48 hours following medication treatment. After fixing the cells with cold trichloroaccetic acid, the test was stopped by

incubation at 4 °C. After being cleaned and allowed to air dry, the plates were stained with sulforhodamine B dye. Acetic acid washes were used to remove excess dye, while trizma base was used to dissolve bonded dye using a reference of 690 nm, absorbance was measured at 540 nm. The percentage ratio of treated to control absorbance reading was used to calculate cell growth(27).

Result & Discussion

Ursolic acid and 18β-Glycyrrhetinic acid based Drug loaded Silver Nanoparticle (0.1N)was formulated by using Tween 20 /20 as surfactant and glycerol as co-surfactant. Pre-formulation study was done on the Ursolic acid and 18β-Glycyrrhetinic acid. The formulated Drug loaded Silver Nanoparticle (0.1N) was evaluated for organoleptic, morphological, and physico-chemical characteristics like particle size, infrared spectroscopy, In-vivo and In-vitro study etc.

Physicochemical Characterization of Drug loaded Silver Nano-particles (0.1N)

As per protocols described in official compendia, drug loaded silver Nano-particles (0.1N) were examined using a variety of physicochemical parameters, including general appearance with free flowing water soluble, typical brown colour rod shape, approximate 50-300 nm size range silver nanoparticle are successfully formulated.

Differential Scanning Calorimetery (DSC) of Drug & drug loaded Silver Nanoparticle (0.1N)

Melting point of Ursolic acid and 18β-Glycyrrhetinic acid was reported at 170-174 °C and 33-38 °C which was determined by using digital melting point apparatus. The thermal behavior and physical condition of ursolic acid, 18β-Glycyrrhetinic acid and the associated Drug loaded Silver Nanoparticle (0.1N) formulation assessed using differential scanning calorimeter. The crystalline nature and thermal purity of pure ursolic acid were demonstrated by DSC thermogram which showed a strong endothermic peak at its distinctive melting point 267.37 ° C. 18β-Glycyrrhetinic acid also displayed a clear endothermic peak that matched its melting point indicating that its crystalline structure was clearly defined. 300.66 ° C. There was a notable change in thermal behaviour of drug loaded silver nanoparticles. Indicating peak of Drug loaded silver Nanoparicle at 165.70° C, both drug distinctive melting endotherms either expanded decreased in strength or shifted to lower temperature. These peaks occasionally vanished indicating a loss of crystallinity and potential transformation of the drug into an amorphous or molecularly dispersed state inside nanoparticle matrix.

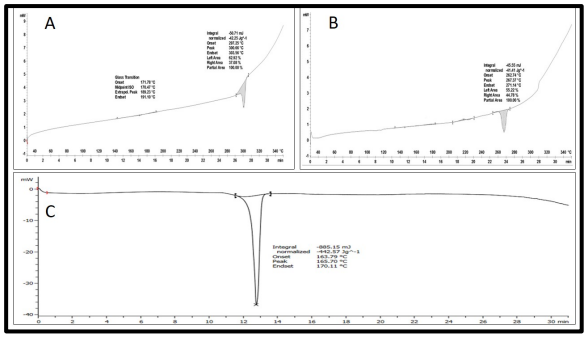

Fig. 1: A Showing *DSC study* of 18β-Glycyrrhetinic acid B. showing the *FTIR* spectrum of Ursolic acid and C. showing FTIR spectrum of Drug loaded Silver Nanoparticle (0.1N)

Fourier Transform Infrared Spectroscopy (FTIR) of Drug & Drug loaded Silver Nanoparticle (0.1N)

The FTIR spectrum of 18β-Glycyrrhetinic acid shows a large peak at 3452.70 cm^{-1} which is indicating hydroxyl group (O-H) stretching. Aliphatic (C-H) stretching vibrations are responsible for the absorption at 2929.97 cm^{-1}. A carbonyl(C=O) group is confirmed by a prominent peak at 1729.24 cm^{-1}, while C=C bending vibrations are represented by the peak at 1462.09 cm^{-1}. Band at 1837.26 cm^{-1} related to carbonyl possibly result of molecular interactions. Ursolic acid FTIR spectra shows a wide absorption band at 3454.62 cm^{-1}, which is hydroxyl group (O-H), the aliphatic C-H stretching vibrations of methyl and methylene group are responsible for peak at 2871.14 cm^{-1}. The presence of a carbonyl(C=O) functional group is how by prominent band at 1775.53 cm^{-1}. The triterpenoid structure C=C stretching vibrations are responsible for absorption at 1558.54 cm^{-1}. Alcohol or ester group C-O stretching vibrations are represented by the peak at 1091.75 cm^{-1}. The existence of important functional group in ursolic acid is confirmed by these distinctive peaks. The FTIR spectrum of drug loaded Drug loaded Silver Nanoparticle (0.1N) showed a broad peak at 3438.53 $cm^{-1,}$ attributed to O-H stretching, indicating involvement of hydroxyl groups in nanoparticle stabilization. Aliphatic C-H stretching vibrations are represented by peak at 2867.15 cm^{-1}. , the absorption 1645.44 cm-^{1} indicating C=C stretching. 1352.06 $cm^{-1,}$ 1290.50 cm-1 represent C-H and C-O-c, 881.46 cm^{-1} peaks indicating C-H aromatic.

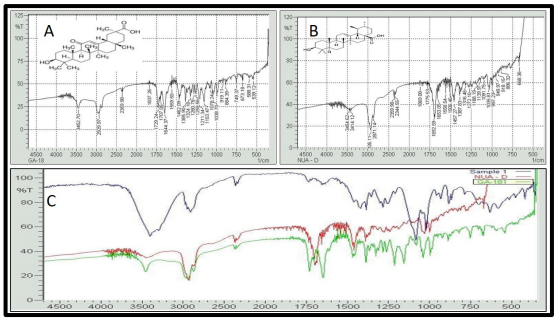

Fig. 2: A Showing *FTIR Spectra* A. showing 18β-Glycyrrhetinic acid B. showing the *FTIR* spectra of Ursolic acid and C. showing FTIR spectrum of Drug loaded Silver Nanoparticle (0.1N) with overlay of both drug.

X-Ray Differection study (XRD) of drug & Drug loaded Silver Nanoparticle (0.1N)

X-ray diffraction examination was used to detect crystalline quality of the pure drug ursolic acid and 18β-Glycyrrhetinic acid, drug loaded silver nanoparticle. The XRD pattern of pure ursolic acid revelled distinct and major diffraction peaks at specific 2 ɵ , 18β-Glycyrrhetinic acid also represent moderate crystalline nature. Drug loaded Drug loaded Silver Nanoparticle (0.1N) shows a significant reduction in peak strength and partial broadening of unique peaks of both drug.

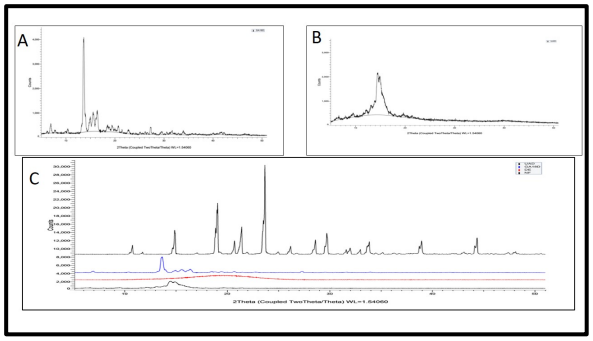

Fig. 3: A Showing *XRD study* A. Showing 18β-Glycyrrhetinic acid B. showing the *XRD study* of and C. showing *XRD study* of Drug loaded Silver Nanoparticle (0.1N) with overlay of both drug 18β-Glycyrrhetinic, Ursolic acid, Drug excipient and Drug loaded Silver Nanoparticle

Particle size, Polydispersity index and Zeta potential of drug loaded Silver nanoparticle

Stability and homogeneity of the produced silver nanoparticles their size, size distribution and surface charge were measured. The successful creation of nanoparticles within the nano scale range was confirmed by formulation mean particle size 241 nm. A comparatively narrow size range and strong homogeneity of the nanoparticle population were indicated by the polydispersity index (PDI) which was determined to be less than 0.300. The optimized silver nanoparticles had a zeta potential of -24mV, indicating modest colloidal stability. Particles are repelled electrostatically by the negative surface charge.

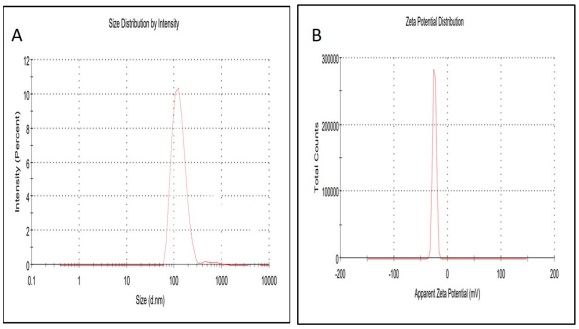

Fig. 4: A. Showing Mean particle size of Drug loaded Silver Nanoparticle (0.1N) and B. Showing zeta potential value

Morphological Evaluation Field Emission Scanning Electron Microscopy (FE-SEM)

The morphology and size distribution of the produced silver nanoparticles were examined using field Emission scanning electron microscopy analysis. The micrographs clearly showed that nanoparticles between 50-300 nm in size range were formed. Demonstrating effective synthesis in the intended although a little amount of polydispersity was noted the particle morphology was generally consistent and mostly rod shape.

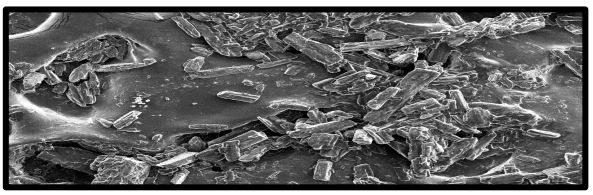

Fig. 5: Field Emission Scanning Electron Microscopy (FE-SEM) Surface morphology study indicating rod shape Drug loaded Silver Nanoparticle (0.1N)

In-vitro cell line study

The breast cancer cell line MCF- 07 was used to analyse different concentrations (10-80 µg/ml) of silver nanoparticles based on the combination of 18β-Glycyrrhetinic acid and ursolic acid. The results showed that in comparison to individual 18β-Glycyrrhetinic acid and ursolic acid based silver nanoparticles produces a synergistic result of cell growth inhibition.

Table no: 01 Human Breast Cancer Cell line (MCF-07) Tabular % Control Growth report

	Human Breast Cancer Cell Line MCF-7															
	% Control Growth															
	Drug Concentrations (µg/ml)															
	Experiment 1				Experiment 2				Experiment 3				Average Values			
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
UA	77.8	46.2	4.1	-26.7	82.1	44.6	3.3	-0.7	75.2	53.7	13.7	5.4	78.3	48.1	7.1	-7.4
GA	13.2	12.8	-0.1	-25.0	15.4	13.2	3.8	-3.9	19.2	19.4	14.7	3.9	15.9	15.1	6.1	-8.3
UGB	-70.6	-24.5	-0.5	2.5	-24.2	-17.1	5.2	9.7	9.0	8.8	15.5	22.3	-28.6	-10.9	6.8	11.5
NF1	10.7	9.4	6.6	-30.7	13.3	12.2	9.2	1.1	16.1	21.4	16.6	2.3	13.3	14.3	10.8	-9.1
ADR	-70.4	-80.2	-82.4	-60.6	-61.6	-64.0	-67.9	-55.5	-58.0	-57.4	-59.0	-44.4	-63.3	-67.2	-69.8	-53.5

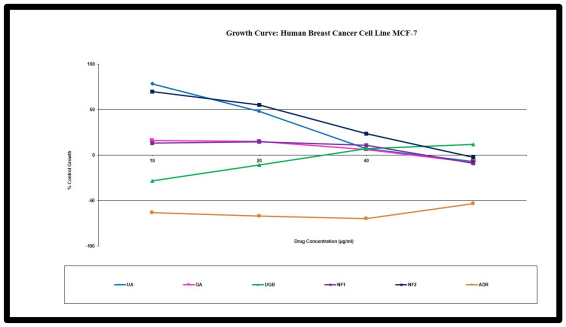

Fig. 6: In vitro cytotoxicity (Sulforhodamine B, SRB) colorimetric assay MCF-7

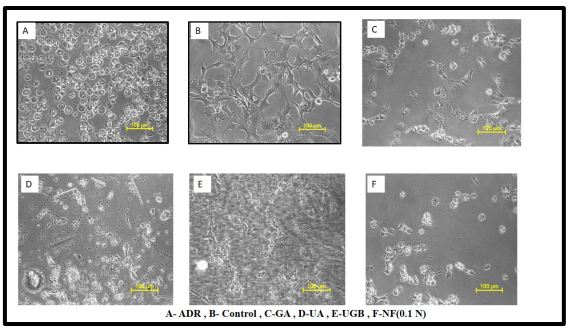

Fig.07 Image of Breast cancer cell line(MCF-07) indicating % growth inhibition by Figure A - ADR, Figure B-Control, Figure C-18β Glycyrrhetinic acid, Figure D – Ursolic acid, Figure E-Ursolic acid , 18β-Glycyrrhetinic acid & bergamot oil mixture , Figure F- Drug loaded Silver Nanoparticle (0.1N)

Conclusion:

The present study successfully demonstrate the formulation and evaluation of drug loaded silver nanoparticle (0.1N) incorporating triterpenoidal glycosidal compounds for the treatment of breast cancer. The optimized nanoparticle shows particle size 241 nm with a zeta potential of -24.7 mV indicating good stability along with a polydispersity index (PDI) of less than 0.300, confirming uniform size distribution. Comprehensive characterization using Field Emission Scanning Electron Microscopy shows well define surface morphology. While FTIR analysis confirms successful drug incorporation without chemical incompatibility. Differential Scanning Calorimetric (DSC) and X-ray diffraction study indicate change in crystallinity suggesting effective encapsulation of drug within nanoparticle form. In vitro cell line study using MCF-07 breast cancer demonstrate prepared drug loaded silver nanoparticle shows significant cytotoxic activity IC50 , TGI and LC50 values which indicate strong growth inhibition ,effective suppression of cell proliferation and induction of cancer cell death. Overall proposed research work developed Silver nanoparticle from triterpenoide glycosidal derived phytochemical in combination with bergamot oil hold promising potential as an efficient therapeutic system for breast cancer treatment.

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