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Biogenic Silver Nanoparticle from Citrus Bergemia Essential Oil: Synthesis, Characterization and Antibacterial Assessment

Ms. Pooja S. Murkute*[1], Dr. Mohammed Ismail Mouzam[2]
[1]Ph.D. Research Scholar Y.B. Chavan College of Pharmacy (Chh.Sambhajinagar)
[2]Associate Professor, Y.B. Chavan College of Pharmacy (Chh.Sambhajinagar

Abstract:

Objective: *The proposed Research work mainly emphasis on Synthesis, characterization of Biogenic Silver Nano-particle from citrus bergemia for various anti-microbial infectious condition. By considering alleged advantages for the environment and human health, natural preservatives as opposed to synthetic ones are becoming more and more popular for preserving the quality and safety of food. Bergamot oil a plant essential oil and extract which contain a high concentration of flavonoids such as neoeriocitrin, neohespiridin, and naringenin are primary preparation employed Realizing the full potential of essential oil derived bioactive compounds in the fight against bacterial infections require an understanding of their chemical variety and mode of action.*

Method: *Glycyrrhetenic acid, (GA-18), Ursolic acid, Tween 80, PEG-400 and (0.01N) $AgNO_3$ were selected to prepared antibacterial silver nanoparticle. The silver nanoparticle was prepared by using micro-emulsification technique by using high speed homogenization and then it is transferred for drying purpose to allow free flowing Nano-particle.*

Results and Conclusion: *Particle size, PDI and zeta potenical measurment, Morphological Evaluation (Field Emission Scanning Electron Microscope (FE-SEM)), FTIR Spectroscopy analysis, Antimicrobial study were used to evaluate prepared silver Nano-particle. Particle size, PDI and zeta potenical measurment gives significant particle size value of 151 nm , 0.300 PDI and 34mV , Significant in-vitro growth suppression of bacillus substillus was demonstrated by the silver nano-particle. Different zone of inhibition(mm) were recored for different sample concentration, as per reported data generated Bergamot oil containing microemulsion based Silver Nano-particle shows considerable antibacteial effect.*

Keywords: *Nano-emulsion, Antimicrobial study , Triterpenoid glycoside, , Bergamot oil, bacillus substillus, Silver-Naoparticle*

INTRODUCTION:

Given that fruits with similar morphologies may belong to very distant species and that very similar or genetically nearly indistinguishable varies may give rise to very difficult morphologies the history of citrus species distribution in Asia and cultivar's. this makes morphology based classification extremely difficult(1). Citrus bergamia is often known as bergamot. it's an orange sized fruit with a yellow peel. Up to 93-96 % of volatile chemicals, including monoterpenes (25-53% of limonene) as well as trace amounts of linalool and linalyl acetate are present in bergamot essential oil and bergamot juice. Non-volatile substances such pigment waxes, comarines and psoralens make up a variable fraction of BEO. Bergamot extract which contain a high concentration of flavonoids such as neoeriocitrin, neohespiridin, and naringenin are primary preparation employed(2). Citrus bergamia a member of the rutaceae family is commonly referred to as bergamot. The trees have round yellow fruit ,star shaped white blooms and large , dark green , ovate leaves that resemble lemons(3) . Traditionally bergamot oil was extracted by hand using a technique known as slow folding, or sfumatura. More contemporary methods use machines called peelers to mechanically remove the oil by scraping the outside of fruit as water runs over it. This creates an emulsion which is then place into centrifuges to separate the essence from the water. The rinds of 100 bergamot oranges can yield about 3 ounces (85g) of bergamot oil.The bergamot orange is probably a hybrid of lemon. According to genetic study on the lineage of contemporary citrus cultivars, the bergamot orange is most likely hybrid of citron and bitter orange as well as bitter orange. Extracts have been utilized as a fragrant ingredient in tea, cuisine, snus, cosmetics and fragrances.it may increase photosensitivity when use topically raising the dangers of sun exposure(1).

Bergamot essential oil contains significant levels of the phototoxic chemical bergapten, which gets its name from bergamot orange, bergapten a linear furanocoumarin derived from psoralen, is commonly found in plants associated with phyto-photodermatitis. It should be mentioned that bergamot essential oil has a higher concentration of bergapten than any other citrus based essential oil(4).

The fruit known as bergamot has a greenish yellow skin is very acidic and smells exquisite and fresh. Its most significant output is bergamot essential oil, which is obtain by steam distillation or cold processing

from the fresh fruit pericarp and a portion of its mesocarp. Because it can be employed in combination with other essences in addition to fixing the aromatic bouquets of perfume, bergamot essential oil is used as flavouring agent in a variety of goods, including soft drink, tea, and confection. In early gery tea recipe bergamot essential oil and its extracts are used to provide a pleasing and revitalizing aroma(5).Up to 93-96 % of volatile chemicals, including monoterpenes (25-53% of limonene) as well as trace amounts of linalool (2-20%) and linalyl acetate (15-40%) are present in bergamot essential oil and bergamot juice. Additionally BEO contains non-volatile substances such as pigment ,waxes,coumarines and psoralens in varying percentage (4-7%) (2).Significant antiseptic, antibacterial, antiviral, antioxidant, anti-parasitic, antifungal and insecticidal properties have been found for essential oil. As a result, essential oil can be an effective means of lowering bacterial resistance. Essential moil also known as volatile oil are aromatic, oily liquid that are extracted from plant material, including leaves, buds, fruit, flower, herbs, wings, bark, wood, root and seeds. The most popular technique for commercially extracting essential oils was initially created by Arabs in the middle ages and is known as steam or hydro-distillation(6, 7).

Realizing the full potential of essential oil derived bioactive compounds in the fight against bacterial infections require an understanding of their chemical variety and mode of action. The essential oil shows variety modes of action including as the breakdown of bacterial cell membranes and causes the loss of vital cellular components, ultimately resulting in cell lysis. Additional significant ways that essential oil work include interfering with biological function like preventing protein synthesis, cellular respiration or inhibiting function(8).

The essential oil are highly volatile chemicals with intricate aromatic structures that come from plants. The overall fraction of pungent molecules generated in specific plant cells is known as volatilomes (9, 10). These are secondary metabolites that by attracting polarizers and providing protection from diseases are crucial to the dynamics of plants in their environment(11). Several techniques can be used to extract them from every part of the plant, including the roots and leaves. Numerous plant nutritional and environmental parameters. As well as stressors affect the presence yield and chemo-type of essential oil(12).

Breeding and selection strategies are used to promote particular composition for commercial essential production(13). Gas chromatography and mass spectrometry investigations have already produced the structural profiles of commercial essential oil(14). Their chemical structure typically identifies two or three main compounds that makeup over 20% of the entire molecule and are typically in charge of their biological characteristics(15).

Essential oil is a fascinating class of natural bioactive molecule that are attracting a lot of industries, including aromatherapy, cosmetics, food preservation and pharmaceuticals(16, 17). These aromatic liquids and bioactive compounds come from variety of different plant sources and contains a broad spectrum of chemical elements that give them unique smells and medicinal qualities(18). Because of their antibacterial, antifungal, and antioxidant properties, essential oil have established themselves as important substituent for artificial food additives, which is in line with the global trend toward healthier more sustainable and environmentally friendly consumption habits(19). Emulsions of essential oils are mixture of essential oil and water or other aqueous solutions. Furthermore, to guarantee that essential oil are uniformly dispersed throughought the aqueous phase and do not separate over time, stable emulsions must from(20, 21). essential oil emulsions can be made using a variety of techniques including surfactant assisted emulsification. Phase inversion temperature method, micro-fluidization, high energy mixing, low energy mixing & high pressure homogenization(22). To create stable and long lasting emulsions it is also essential to choose the right emulsifying agent, modify the formulation ratios and employ the right mixing process. Furthermore, the stability evaluations of capsulated essential oil concentrate on how long they last in storage and after being added to food matrices(23). Essential oil are complex, volatile, natural compounds with potent scents that are created by aromatic plants as secondary metabolites. Leaves, stems, flowers, roots, seeds, fruit rinds, bark, resins and more all provide essential oils(24). In the middle times Arabs discover how to make essential oils using steam or hydro-distillation. In nature, essential oils serve as insecticides, pesticides antiviral agents and antibacterial to protect plants from herbivory. Additionally, they might repel certain insects while drawing others to help with pollination and distribution. The most labour intensive and time consuming procedure is essential oil extraction(25). maceration, cold pressing, effleurage, hydrodistillation, solvent extraction and supercritical CO2 extraction are the most often used extraction technique(26).Because of their bactericidal, fungicidal and therapeutic properties aromatic oil are widely employed in food preservation, embalming and as antibacterial analgesic, sedative anti-inflammatory and anesthetic therapies(27). Notwithstanding the effectiveness of essential oil as

preservative in food systems their strong scent, high reactivity, hydrophobicity, low solubility and potential negative interactions with different food ingredient that alter organoleptic properties have led to certain restrictions in their practical use(28). To overcome these limitations a number of recent technological advancements utilizing different distribution methods have been implemented(29). One of the cutting edge and novel delivery methods for essential oil is Nano encapsulation which enhances antibacterial efficacy in food system by improving stability, dissolution and controlled release of essential oil scent(30). Drug delivery, biological science, gene delivery, chemical industries, optics, mechanics, catalysis and other fields all use nanoparticles(31). Silver nanoparticle are most well-known metal nanoparticles because of their potent antibacterial and anti-inflammatory properties. In order to prevent bacterial infection, AgNP containing creams, ointment is given to burns and wounds(32). AgNP are used in variety of physical, biological, and pharmaceutical domain. AgNP is traditionally synthesized using a physical and chemical methodology to create nanoparticle with precise, regulated sizes and shapes(33). However, there is a need for more biologically compatible nanoparticles due to usage of hazardous chemicals, high pressure and energy in processes including laser ablation, hydrothermal synthesis, solvo-thermal synthesis, pyrolysis and inert gas condensation(34, 35). The concentration and content of AgNP will differ depending on the type of plant since different plants have different metabolites that can help reduce silver ions, stabilize and cap AgNP, this is particularly true for the medicinal plant(36), which is abundant in antioxidant found in therapeutic plants(37). Numerous anti-inflammatory, antibacterial, antiviral, antiaging and anticancer properties are demonstrated by the medicinal plants(38). Other biomolecules such as polysaccharides, aldehyde, ketone, protein, enzyme, amino acid and caffeine in addition to the polyphenols present in plants are charge of decreasing and capping AgNP(39). The complex biomolecules included in medicinal plants help stabilize nanoparticles and reduce metal ions(40). One possible technique for eliminating microorganism is the continuous release of silver ions by silver nanoparticles(41). Silver ions can stick to the cytoplasmic membrane and cell wall because of their affinity for sulphur protein and electrostatic attraction. The bacterial envelope nay be disrupted by the adhering ions which may increase the cytoplasmic membranes permeability(42). Respiratory enzymes may become inactive following the uptake of free silver ions into cells, producing reactive oxygen species but halting the synthesis of adenosine triphosphate. Deoxyribonucleic acid (DNA) alteration and rupture of cell membrane can be triggered primarily by reactive oxygen species. Given that sulphur and phosphorus are crucial elements of DNA, issues may arise when silver ions interact with these elements(43). Silver nanoparticles not only have the ability to emit silver ions but they can also kill germs. When silver nanoparticles attach to the cell surface they can gather in the pits that develop on cell wall(44). Eleven denaturation of cell membranes may result from the accumulation of silver nanoparticles. Since silver nanoparticles are nanoscale in size they can also pass through bacterial cell wall and alter the structure of cell membrane(45). Eleven cell lysis may occur as a result of organelle rupture cause by cytoplasmic membrane. Denaturation. Silver nanoparticle may also have a role in signal transduction of bacteria(46). Tyrosine residues on peptide substrate can be dephosphorylated by nanoparticles and phosphorylation of protein substrate influences bacterial signal transduction. When signal transduction is disrupted cell death may result(47).

Silver nanoparticles are effective against a broad range of microorganism including Bacteria Example E. coli, Pseudomonas aeruginosa, staphylococcus aureus, fungi candida albicans, Aspergillus species, some antiviral effects have been shown (e.g. against HIV, influenza, SARS-CoV-2. AgNP can disrupt and prevent the formation of bacterial biofilms which are typically resistant to antibiotics(48). Mechanism of antimicrobial action Disruption of cell membranes Host cell has a thick cell wall and the product protein is not produced extracellularly cell disruption is necessary(49). The cells can be disrupted and their content released using a variety of unit actions. Direct physical disruption techniques, such as high pressure homogenization, ball mill grinding and cell wall breakage from ice crystal formation through freeze/thaw of a cell paste are among the most popular large scale cell disruption activities(50, 51). When AgNP are exposed to bacteria, the nanoparticles adhere to the membrane and cell wall, the AgNP positive surface charge is essential for attachment. AgNP can adhere to cell membranes more easily because of the positive charge that creates an electrostatic attraction between them and the micro-organism negatively charged cell membrane(52). Such interaction results in morphological alterations that are characterized by cytoplasmic shrinkage and membrane separation, which ultimately cause the cell wall to rupture(53). Additionally, potassium(K^+) ion transport and release from microbial cells can be changed by silver ions(54). In addition to influencing transport activates, increased permeability may have more noticeable consequences such as the loss of cellular components like protein, ions, reducing sugar and occasionally

ATP the cellular energy store. Indeed, the proteomic data on microbial cells treated with AgNP revealed an accumulation of immature precursor proteins that destabilize the outer membrane E. coli. this accumulation of immature membrane precursor proteins that destabilize the outer membrane of E. coli ATP(55). This accumulation of immature precursor proteins indicate that proton motive forces are dissipated and that cellular ATP is depleted, possibly as a result of ATP synthesis inhibition or leakage(56). The thickness and makeup of the bacteria cell wall also affect the antimicrobial activity of AgNP. Compared to gram positive bacteria like S. aureus, gram negative bacteria E. coli are more vulnerable to AgNP(57). This results from variations in the structure of peptidoglycan a crucial part of the cell membrane(58).Essential oil and their bioactive constituents are highly favoured among various plant based products as antimicrobial food preservatives. Essential oil and their bioactive components have limited practical applicability due to their high volatility and instability despite their strong antimicrobial efficacy and preservation potential against fungal and mycotoxin contamination. This suggest the need for the development of strategies to overcome the difficulties associated with essential oil application(59). The negatively charge peptidoglycan layer that makes up the cell wall of gram positive bacteria is relatively more abundant than that of gram negative bacteria. In summary the fact the gram positive bacteria have a cell wall that is far thicker than that of gram negative bacteria explains why they are less susceptible to antibiotics therapy(37).

MATERIAL AND METHOD:

Glycyrrhetenic acid (GA-18) and Ursolic acid was purchase from Yucca enterprises, Mumbai. India, Tween 80, PEG-400, Glycerol, Clove oil, 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.

Selection of oil, surfactant, and Co-Surfactants

For the formulation of Glycyrrhetenic acid and Ursolic acid containing silver nanoparticle suitable liquid was selected by testing of a series of natural fixed and volatile oils such as lemongrass oil, cinnamon oil, clove oil, cinchona oil, Bergamot oil, eucalyptus oil for the Glycyrrhetenic acid. Solubility in each oil sample was tested by visual observation by keeping the test tubes at ambient temperature 37°C ± 0.5°C after 24 h. Pluronic F68, tween 80 and glycerol, PEG-400, phosphatidylcholine, were tested and glycerol was selected as surfactant and co-Surfactants, respectively(60-63).Various oil phases were evaluated for adequate drug solubility. Oil with desired medication solubility were further vetted based on how well they emulsified with a particular surfactant. The gretest nanoemulsifying area found in the phase diagram created for a particular oil phase and were used to screen co-surfactant.

Formulation of Nano-emulsion containing Silver Nano-particle

Oil phase: On the basis of screening study with different oil and Bergamot oil is selected along with Tween 80, PEG- 400. 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 addition of Tween-80(10 ml) with dropwise manner with syringe is carried out. To this uniform phase addition of 10 ml of 0.01 N $AgNO_3$ is proceeded.Aqueous phase: 40 ml PEG-400 and distilled water 20 ml is allowing to mix uniformly continuous stirring 1000 RPM with magnetic stirrerAfter mixing of both Oil phase and aqueous phase, solution mixture is proceeding further in high speed homogenization to allow uniform mixing and to attend Nano-size range of proposed formulation. Mixing into High speed homogenization. Light Brown color silver Nano-particle were seprated with centrifugation technique and allow it for simple drying method (64)

Particle size, PDI and zeta potenical measurment

The particle size ,PDI and zeta potencial were determined by malvern zetasizer.the prepared silver nanoparticle were scan for accurate size determination. The samples were mixed with distilled water before being characterized at 25◦C . While the zeta potenical was determined by moving sample to electrophoretic cells and applying an electrial potencial of ±150,the mean particle size was assesed at a fixed 90◦C angle.using the smoluchowski equation and the mean electrophoretic mobility of the nanoparticle ,PDI, zeta potenical was determined.(64-66)

Morphological Evaluation

Motic microscopes which are frequantly used for brightfield microscopy can be utilized to study silver nanoparticles. When the particles are appropriately pigemnted this is especially true. Particle size analysis can benefit from the use of a motic microscopy as it meansures particle size and shape of silver nano-particle.

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FTIR Spectroscopy analysis

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)(67, 68).

Antimicrobial study

The disc diffusion method was first use to asses the silver nanoaprticle antibacterial effectiveness against various microorganism. These kind of bacteria are gram positive and infectious disease often invovle gram negative organism. Following mention table displays the findings of the inhibition zone diameter. In perticular silver nanoparticle shows various level of antibacterial activity agaisnt every bacterial strain tested, with the variable diameter of inhibition of zone(mm). microbiological sample bacillus substillus were selected ,the concentration for silver nanoapaticle range from 10 to 40 mg/mL.

RESULT & DISCUSSION:

Nano-emulsion containing Silver Nano-particle was formuated by using tween80 surfactant , PRG-400 Co-surfactant. Pre-formulation study was done on the both drug. The formuated silver nanoparticle was evaluted for organoleptic,morphological,physico-chemical charecteristic parameter like PDI, Particle size,zeta potenical, infrarred spectroscopy,antibacterial study.formulation batches were mention in follwong table.

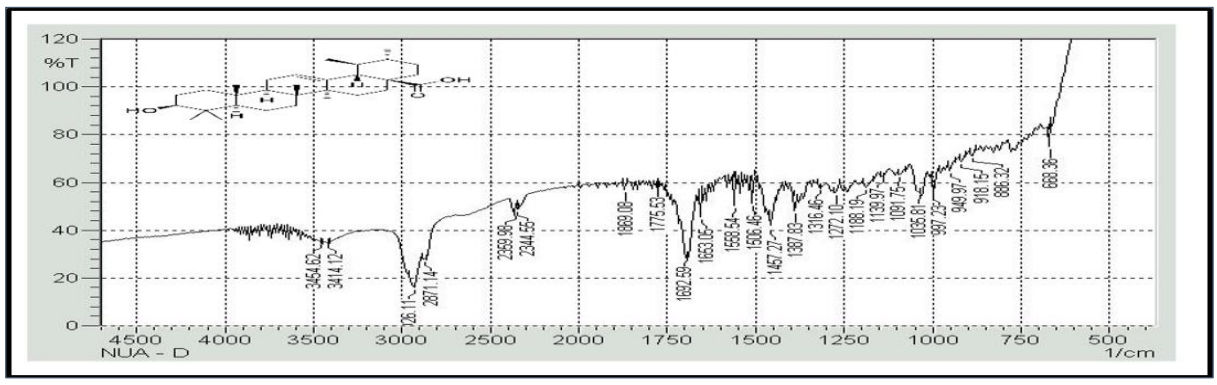
Physicochemical charectrization of drugs.

Melting point of both drug was reported at 284-288 ◦C and 293-294 ◦C which was determined by using digital melting point appartatus.

FTIR

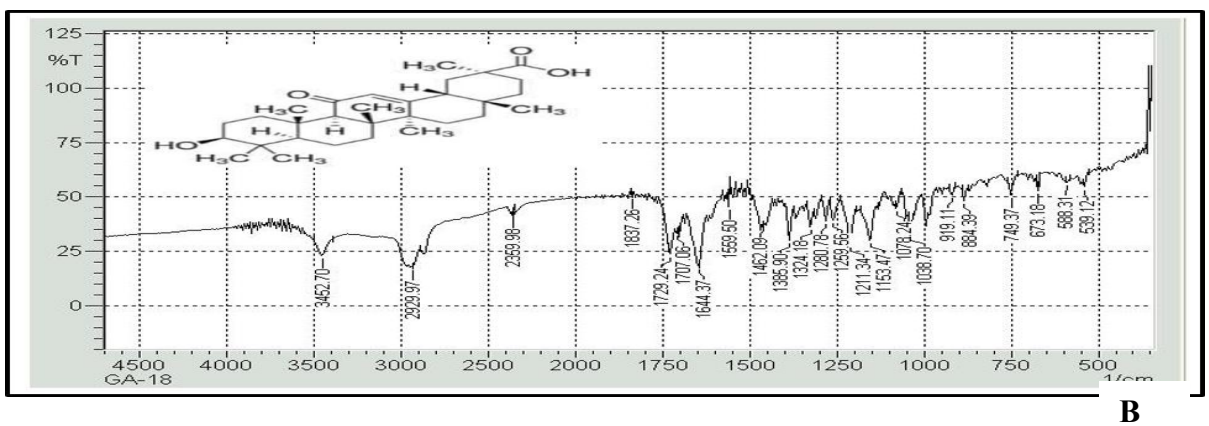
To confirm and identify the functional groups present in the Ursolic acid & 18 β-Glycyrrrhetenic acid; FTIR spectroscopy was done. Fig. 1 A shows the FTIR spectrum of Ursolic acid exhibited band at 3414.12 cm^{-1} mainly attributed to Hydroxyl groups. The peak appeared at 1653.06 is of the asymmetric valence vibrations of the alkene group and the peak at 1692.59 cm^{-1} is due to carboxylic acid stretching of ursolic acid. While Fig. 1 B shows the FTIR spectrum of 18 β-Glycyrrrhetenic acid exhibited band at for hydroxyl group 3454.62 cm^{-1}, and 2929.97 cm^{-1} mainly attributed to $-CO_2H$, C=O (stretch) and O-H respectively. A Characteristic band at 1729.24 cm^{-1} corresponding to the stretching of Ketone of 18 β-Glycyrrrhetenic acid additionally it shows peak at 1259.56 cm^{-1} value C-O stretching bond.

Figure 1: FTIR spectroscopy Image of both drug sample A Figure Indicating Ursolic acid and B Figure Indicating 18 β-Glycyrrrhetenic acid.



A

Physicochemical Characterization of Nano-emulsion Containing silver Nano-particle



B

From all formulated Nano-particle batches, batch no was found to be very stable as free flowing brown colour silver NP were obtain which gives strong long term stability study. Batch no __ was subjected for further analysis.

Genral apperance,pH

The formulated silver Nano-particle was represented as brown colour free flowing particles with aromatic odor and sweet taste. The pH of silver Nano-particle were found to be 9 with basic in nature.the comparable different values of silver nanoparticle indicate dark brown colour silver nanoparticle.

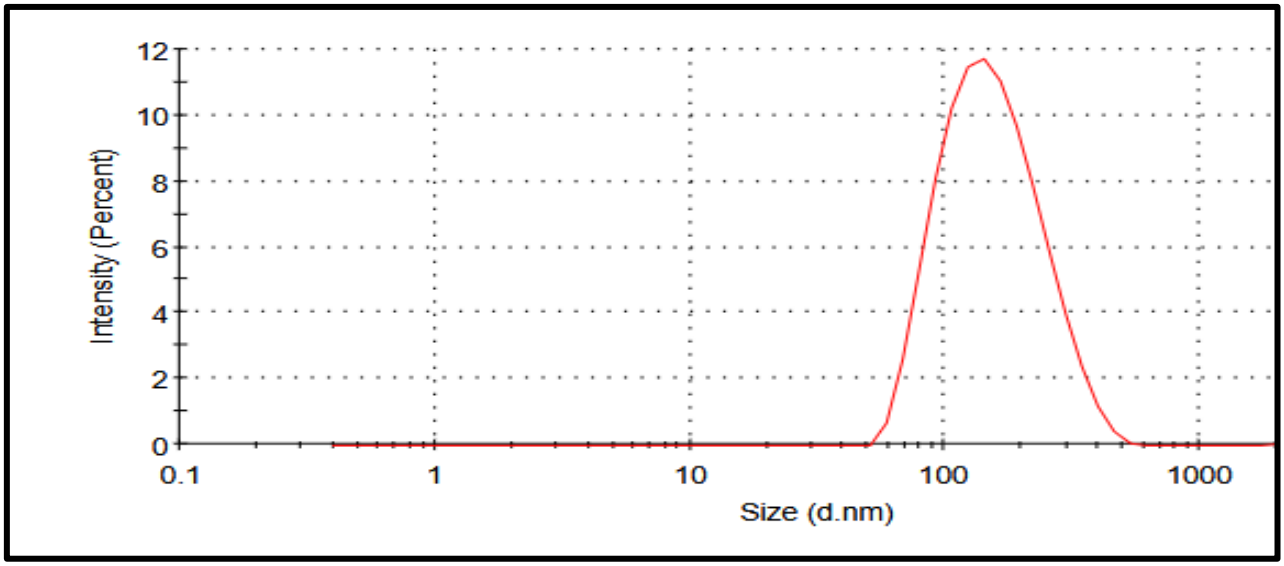
Particle size,Polydispersity Index and Zeta potenical

To determine the mean Particle size, Polydispersity Index is important to forecast physical stability. The mean particle size and polydispersity index of optimized nano-particle was found to be 151 nm,0.300 respectively. Zeta potencial value value is the indicator of stability of formulated silver nanoparticle. Large negative value of zeta potencial indicating good physical stability of silver nanoparticle due to the electrostatic repulsion of each single particle.zeta potential of optimized nanoparticle was found to be -34mV indicating that dispersion will have greater long term stability.

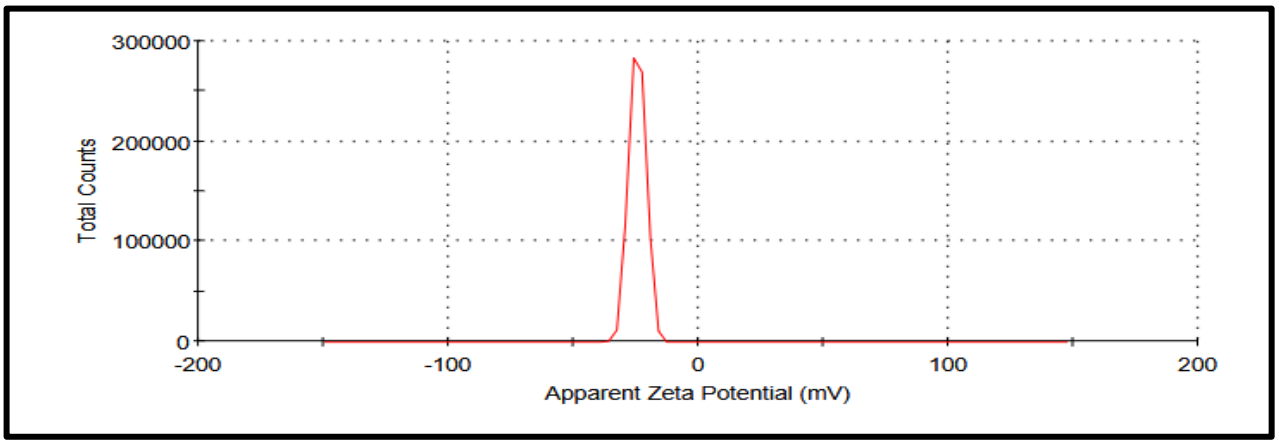
Figure 2: A Figure Indicating Mean particle size and B Figure Indicating Zeta potential of Ag.NP Nano-formulation

Morphological Evaluation

The avergae shape of silver nanoparticle were shows rod shape particles.



A



B

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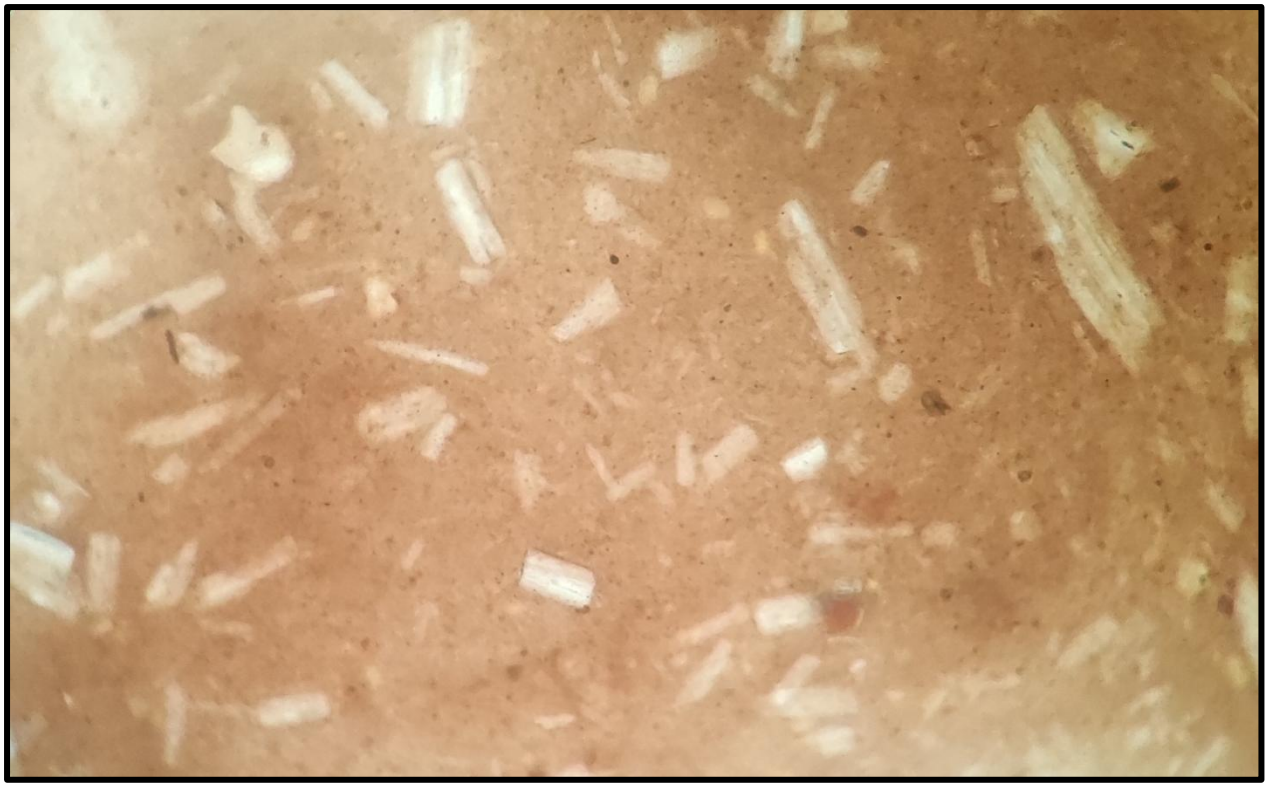

Figure 3: Motic-Microscopy Image of Silver Nano-particle indicating Rod shape arrangement

FTIR of Nano-particle

The measurement of particle formation can be accomplished effectively using fourier transform infrared (FTIR) spectroscopy. It has been discovered that the particle size significantly affects the width and intensity of peaks in an infrared spectrum. The peak width shrinks and intensity rises as particle size increases.The free flowing silver nanoparticle were examined using FTIR to determine which functional groups were present. Free flowing AgNP were crushed along with anhydrous potassium bromide to form a pellet and absorption frequancy was measured in FTIR and ranged from 4000 cm^{-1} to 400 cm^{-1}. The process of interpreting infrared spectra entails comparing the spectrum absorption bands to those of target sample. Figure __ shows the FTIR spectra of the both drug ursolic acid & 18 β-Glycyrrrhetenic acid. Based silver nanoparticle in comparison to the individual drug ursolic acid & 18 β-Glycyrrrhetenic acid spectra. There were some comparable peaks and some shifting of the peak pattern in the produced silver Nano-particle spectrum, confirming the presence of both ursolic acid & 18 β-Glycyrrrhetenic acid. The infrared absorption range for a nitro group is 1350-1550 cm^{-1} wereas present peak shown confirmation of nitro group with value 1384.94 cm^{-1}. IR spectrum of silver nanoparticle shows strong peak at value 2337.80 cm^{-1} and two other shoulder peak value at 2910.68 $cm^{-1}$and 1641.48 cm^{-1}. Based silver nanoparticle exhibited band at 3290.67cm^{-1} & 3400.62 cm^{-1} mainly attributed to Hydroxyl Groups, A Characteristic band at 1726.25 cm^{-1} corresponding to the stretching of Ketone silver nanoparticle additionally it shows peak at 1641.48 cm^{-1} value C-O stretching bond.

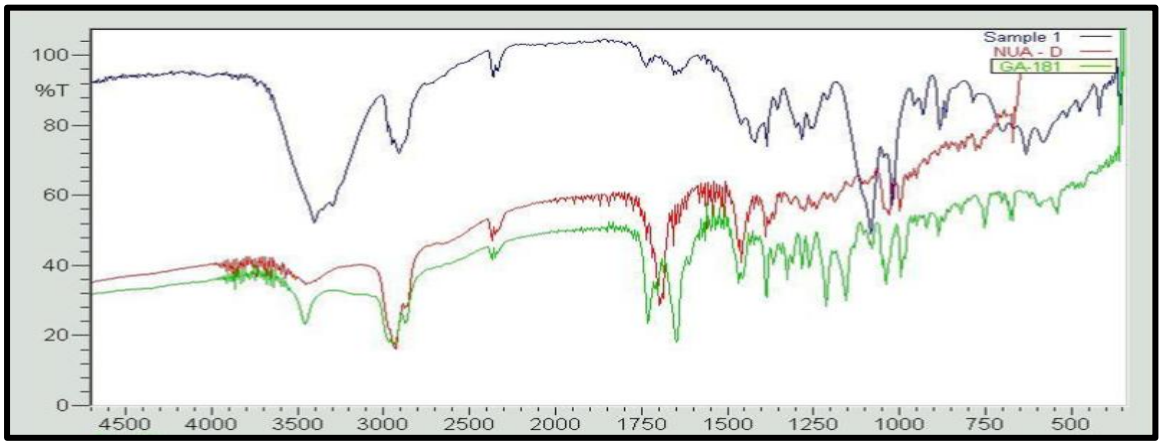

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Figure 4: FTIR spectrum indicating overlay of Ursolic acid indicating with name-NUA-D red colour line, 18 β-Glycyrrrhetenic acid indicating with name-GA-18 green colour line and Ag.NP Nano-formulation indicating with name-sample 1 Purple colour

Antimicrobial study

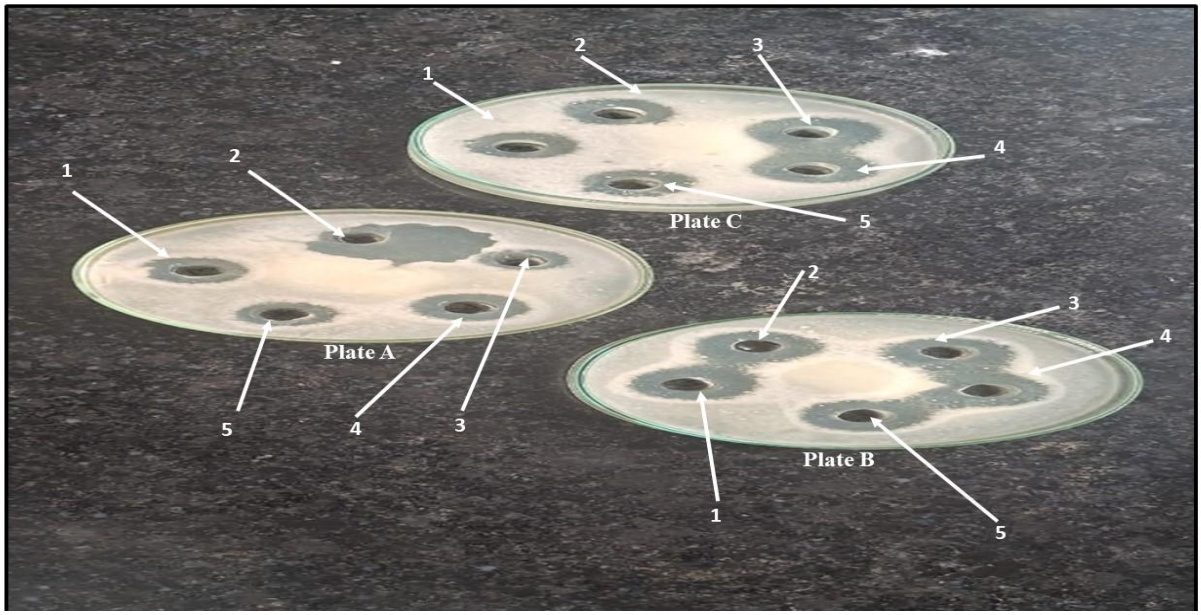

Figure 5: Antibacterial activity of AgNP sample Plate A, Plate B and Plate C indicating different level of Zone of inhibition

Table 1: Zone of Inhibition study of Silver nano-particle against bacterial growth with disc diffusion method.

Sr No.	Unit of standard mg/mL	Diameter of zone inhibition (mm)		
		Plate A	Plate B	Plate C
1	10	6	10	10
2	20	8	12	14
3	30	9	19	21
4	40	12	22	32
5	Standard (50 mg/ml)	18	18	16

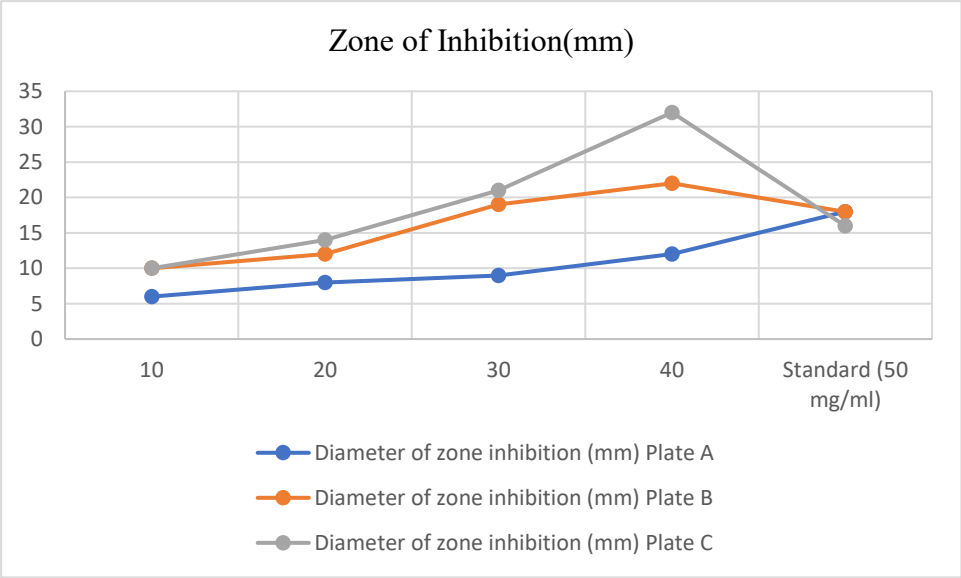

Figure 6: Different level of Zone of inhibition(mm) for Plate A, B and C

CONCLUSION:

Proposed research work design with aim to formulate effective Silver nanoparticle with micro-emulsification technique targeting against microbial infection. In the present study ursolic acid and 18 β-Glycyrrrhetenic acid based silver nanoparticle has been successfully develop and characterized. The FTIR spectroscopy of both drug exhibits typical spectra of both ursolic acid and 18 β-Glycyrrrhetenic acid. The pH of Silver nanoparticle was found to be basic and the Zeta potential of optimized Silver nanoparticle was found to be, –31 mV indicating good long-term physical stability of Silver nanoparticle due to the electrostatic repulsion of individual particles with particle size 151 nm. Morphological Evaluation of Silver nanoparticle shows rod shape arrangement of formulated sample. Anti-microbial study shows good active zone of inhibition of Silver nanoparticle comparative to standard sample. the good synergistic action shown by the ursolic acid and 18 β-Glycyrrrhetenic acid against microbial infection.

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Conflict of interest statement:

Author declares no conflict of interest

Author Contributions

Ms. Pooja S. Murkute involve in original conceptualization, Methodology, Writing Original draft, Dr. Mohammed Ismail Mouzam contribute for formal analysis and supervision.

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