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## Research Article

# Formulation and Evaluation of Quercetin Loaded Silver Nanoparticles in Hydrogel Effective Treatment of Acne

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### ABSTRACT

Acne vulgaris is one of the most prevalent skin diseases which affect almost 80% of adolescents in the world during their lifetime. Development of specific association between antibiotic and bacteria during repeated treatment develops antibiotic resistance. Quercetin is flavonoid widely used as medicine in ancient time. Treatment of acne has been considered as a major research area in pharmaceutical and personal cosmetic care industries. The aim of the present work was to evaluate the, green synthesis of Quercetin silver nanoparticle and to develop herbal topical gel formulation to treat acne. Quercetin is selected based on its antibacterial activity. Silver Nano particle was synthesized using 1 mM aqueous silver nitrate solution from Quercetin and formation of silver nanoparticle was confirmed by UV spectroscopy. The synthesized silver nanoparticles were stable, spherical shape with average particle size of 94.34 nm and the polydispersity index was found to be 0.307. Synthesized silver nanoparticles was incorporated into gel base and evaluated for its physical properties such as pH, viscosity, spreadability and antibacterial activity against Propionibacterium acne. The results obtained in the developed formulation showed no lumps, had uniform color dispersion and were free from any fiber and particle. It was also observed to have good spread ability, pH was found to be  $6.71 \pm 0.04$  to  $7.10 \pm 0.02$  similar to pH of the skin. The antibacterial study of the optimized formulation showed inhibitory activity against Propionibacterium acne. Synthesized silver nanoparticle of Quercetin showed higher activity than the pure Quercetin and plain silver nanoparticles. Hence, silver nanoparticle of Quercetin in aqueous gel-base can be used as an appropriate formulation for treatment of acne vulgaris.

### INTRODUCTION

Skin in the human being is the most susceptible part for entering various microorganisms into the

body. Acne vulgaris is one of the most prevalent skin diseases which affect the young adults in the age group between 11 and 30 years.

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Among these age group 50.9% of women and 42.5% of men are susceptible to this acne vulgaris. Hormonal influences, altered keratinization, inflammation and immune changes are the multiple factors involved in the formation of acne. Acne vulgaris is a common dermatological condition associated with depression, anxiety and other psychological sequences. Microorganisms such as *Propioni bacterium*, *Staphylococcus* and *Escherichia* species are responsible for the development of acne. Clinical manifestations of acne include the presence of comedones, papules, pustules, nodules, and scarring, which can significantly affect an individual's appearance.<sup>1</sup>

Individuals may seek various methods to maintain healthy skin, including medical treatment, drug administration, or skin care products. Skin care involves cleaning, protecting, maintaining, and improving the skin's condition to maintain its homeostasis. Various acne facial skin care products, including serums, creams, acne patches, and face masks, are now available in the market. Among various types of face masks, sheet, clay, mud, peel-off, exfoliating, and sleeping masks are popular choices.

Conventional acne treatments include topical and oral medications. Topical medications, such as benzoyl peroxide, retinoids, and antibiotics, reduce inflammation, unclog pores, and kill bacteria. However, topical treatments can cause skin irritation, dryness, flakiness and antibiotic resistances. Moreover, conventional treatments may not be effective for all types of acne or, in severe cases, require a combination of treatments and long-term use. Therefore, alternative treatments that are safe, effective, and well-tolerated are needed to address the limitations of conventional acne treatments.<sup>2</sup>

Nanotechnology is an important field of modern research dealing with synthesis, strategy and

manipulation of particle's structure ranging from approximately 1 to 100 nm in size. Within this size range all the properties (chemical, physical and biological) changes in fundamental ways of both individual atoms/molecules and their corresponding bulk. Novel applications of nanoparticles and nanomaterials are growing rapidly on various fronts due to their completely new or enhanced properties based on size, their distribution and morphology. It is swiftly gaining renovation in a large number of fields such as health care, cosmetics, biomedical, food and feed, drug-gene delivery, environment, health, mechanics, optics, chemical industries, electronics, space industries, energy science, catalysis, light emitters, single electron transistors, nonlinear optical devices and photo-electrochemical applications. Tremendous growth in these expanding technologies had opened applied frontiers and novel fundamentals. This includes the production of nanoscale materials afterwards in investigation or utilization of their mysterious physicochemical and optoelectronic properties.

The nanoparticles used for all the aforesaid purposes, the metallic nanoparticles considered as the most promising as they contain remarkable antibacterial properties due to their large surface area to volume ratio, which is of interest for researchers due to the growing microbial resistance against metal ions, antibiotics and the development of resistant strains. Among the all-noble metal nanoparticles, silver nanoparticle are an arch product from the field of nanotechnology which has gained boundless interests because of their unique properties such as chemical stability, good conductivity, catalytic and most important antibacterial, anti-viral, antifungal in addition to anti-inflammatory activities which can be incorporated into composite fibers, cryogenic superconducting materials, cosmetic products,



food industry and electronic components. For biomedical applications; being added to wound dressings, topical creams, antiseptic sprays and fabrics, silver functions as an antiseptic and displays a broad biocidal effect against microorganisms through the disruption of their unicellular membrane thus disturbing their enzymatic activities.

Generally, nanoparticles are prepared by a variety of chemical and physical methods which are quite expensive and potentially hazardous to the environment which involve use of toxic and perilous chemicals that are responsible for various biological risks.

Hence, it is becoming a responsibility of every researcher to emphasize on an alternate as the synthetic route which is not only cost effective but should be environment friendly in parallel. Keeping in view of the aesthetic sense, the green synthesis is rendering itself as a key procedure and proving its potential at the top.<sup>3</sup>

Generally, “green” bio-synthesis of AgNPs can be aided using bacteria, fungi, algae or plants (currently being used extensively) as intracellular or extracellular sources for the reduction of Ag<sup>+</sup> to Ag<sup>0</sup>. Generally, the mechanism of bio-reduction can be divided into the three main steps: (1) silver ion reduction and nucleation, (2) the growing step and aggregation, and (3) capping and stabilization in the terminal step. The crucial role is always played by the phytochemicals of the plant, namely, secondary metabolites such as sugars, polyphenols, proteins, phenolic acids, ketones, terpenoids, amides, etc. Moreover, in the majority of cases, a reducing agent from the plant extract plays a role as both the capping and stabilising agents.<sup>4</sup>

## MATERIAL AND METHODS

Quercetin (AR grade) was procured from Sigma Aldrich Ltd., Mumbai. Carbopol (AR grade), polyethylene glycol 600 (LR grade), ethanol (LR grade), ethyl acetate (LR grade), triethanolamine (LR grade), *n*-butanol (AR grade), methyl paraben (AR grade), silver nitrate (AgNO<sub>3</sub>, AR grade), potassium bromide (KBr, AR grade), nutrient broth (LR grade), nutrient agar (AR grade), and sodium hydroxide (LR grade) were purchased from S.D. Fine Chem. Ltd., Mumbai.

### Identification of Quercetin

#### Organoleptic Characteristics: -

For the sake of identifying the medication, visual observations were used to conduct organoleptic research on factors like general appearance, such as colour, nature, and odour. For colour analysis, a small amount of the drug was taken on butter paper and observed in an area with adequate illumination, whereas for observing the odour, a small quantity of the drug was smelled in order to obtain the fragrance.<sup>5</sup>

#### Determination of melting point:

Melting point of Quercetin was determined by capillary method.

**Capillary Method:** In this method the capillary tube was sealed with gentle heating from one end. Then the small quantity of drug Quercetin was filled into the sealed capillary tube. Capillary tube was tied to the tube containing the oil phase in such a way that the sealed part of the capillary tube containing Quercetin was dipped into the oil. The apparatus's temperature was steadily raised, and it was noticed at what point the medication began to melt and at what point the full amount of the drug melted.<sup>6</sup>



## **Spectroscopy Used for UV–Visible Analysis**

### **Identifying the Wavelengths of Quercetin**

For identifying Quercetin wavelengths, a UV–Vis spectrophotometer with a 1 cm quartz cuvette is employed. The experimental conditions entail dissolving 10 mg of Quercetin in an appropriate solvent, like water and ethanol, with a standard concentration. The wavelength range spans 200–800 nm, baseline correction is applied, and measurement was made at room temperature. A common standard scan speed for UV–Vis spectrophotometers is 200 nm/min.

### **Preparation of Silver Nanoparticles (AgNPs) of Quercetin**

The stock solution of quercetin (2 mM) was prepared using 1 mM sodium hydroxide solution at pH 8 and stored at room temperature. Next, 1 mM of 10 ml silver nitrate was prepared, to which 1 ml of quercetin solution was added in a drop-wise manner under constant stirring at room temperature. This facilitated the bio-reduction of silver ions ( $\text{Ag}^+$ ) to neutral ions ( $\text{Ag}^0$ ) in the solution, which was evidenced by the appearance of brown colour. The resultant colloidal AgNPs were filtered through a Whatman filter paper and stored in refrigerator (2–8°C) until further analysis. The experiment was repeated three times, and the reliability of this method was confirmed.<sup>7</sup>

### **Characterization Techniques:**

#### **UV-Vis spectral analysis<sup>8</sup>:**

The silver nanoparticles were confirmed by measuring the wave length of reaction mixture in the UV- Vis spectrum of the Shimadzu spectrophotometer after suitable dilution at a resolution of 1 nm (from 300 to 600 nm) in 2 ml quartz cuvette with 1 cm path length.

### **SEM analysis:**

Surface morphology of the synthesized AgNPs were determined using a scanning electron microscope (Carl Zeiss EVO 18) at an accelerating voltage of 20 kV. The nanoparticle solution was diluted 5-fold with deionized water. Subsequently, a few drops of dispersed AgNPs were placed on an aluminium stub, which was air dried at 60°C for 5 min. The sample was then scanned, and photomicrographs were captured.

### **Particle size and zeta potential analysis<sup>9</sup>:**

The hydrodynamic diameters of the silver nanoparticles (suspended in solution) as well as their zeta potentials were measured 24 hrs after the nanoparticle's synthesis using the DLS Malvern-zetasizer. Thus, 200 L of the silver nanoparticles solution was mixed with 800 L of di-ionised water in a beaker and with the help of a syringe the diluted AgNPs was introduced into the disposable folded capillary cuvette (DTS 1070) carefully while ensuring that no air bubbles were trapped in the cuvette. The cuvette was wiped dry, and introduced into the machine for the required measurements. For a measurement, the workspace was changed to reflect which particular measurement was desired, be it the nanoparticles sizes or zeta potential.

### **Formulation of Quercetin silver nanoparticles loaded gel**

Topical gel formulations were prepared by cold mechanical method with defined quantity of Carbopol 940 and polyethylene glycol 600. The specified quantity (1 g) of polymers was weighed separately and sprinkled slowly on surface of purified water. To this defined quality of double distilled water was added with vigorous stirring and left overnight for dissolving the polymer. To the polymer solution, Quercetin silver



nanoparticles were added with continuous stirring. Required quantity of methyl paraben was added and mixed well by using magnetic stirrer. After complete dispersion, the pH of the gel was adjusted to neutral pH 7 by using triethanolamine. Distilled water was added and made up to 100 g. The formulation composition is shown in table

**Table 01: Composition of Quercetin silver nanoparticles loaded gel**

Ingredients (mg)	QSNG1	QSNG2	QSNG3
Quercetin silver nanoparticles	500	500	500
Carbopol 940	250	500	750
Polyethylene Glycol 600	0.2	0.2	0.2
Methyl Paraben	0.08	0.08	0.08
Triethanolamine	1.0	1.0	1.0
Distilled Water	100 ml	100ml	100ml

### Evaluation of gel formulations<sup>10</sup>:

#### Physical evaluation:

Physical parameters such as color, appearance and consistency were checked visually.

#### pH:

Aqueous solution (1%) of the formulation was measured by using a calibrated digital pH meter at constant temperature.

#### Viscosity:

Brookfield Viscometer (Brookfield Engineering Laboratories, USA) with spindle #C 50-1 was used to measure the viscosity of the formulated topical gel at a speed of 50 rpm in room temperature. Measurement of viscosity was done in triplicate.

#### Spreadability:

Glass slides with standard dimension (length of 6.0 cm) were taken. Topical gel formulation was placed on the one side of the glass slide and sandwiched with the help of another slide. Remove

the adhering gel on the outer surface of the glass slides by wiping. Slides are fixed in a stand that only upper slide to slip off freely without any disturbance by force of weight (20 g) tied to it. Time taken for the movement of upper slide to the distance of 6.0 cm was measured. Measurement of spreadability was done in triplicate and calculated by using the following formula:

$$\text{Spreadability} = (\text{Weight} \times \text{Length}) / \text{Time}$$

Where,

- S=Spreadability
- m=Weight tied to the upper slide (20 g)
- l=Length of the glass (6.0 cm)
- t=Time taken in seconds

#### Anti-microbial activity<sup>11</sup>:

Modified agar well diffusion method was used to detect the anti-bacterial activities of optimized formulation. In this method, plates of brain heart infusion media were seeded with 48 h broth culture of *Propionibacterium acnes* (*P. acnes*).

The plates were dried for 1 h. In each of the plates, four equidistant wells were excavated with a sterile 8 mm borer. Into each plate, 0.5 ml of solutions of saline, pure Quercetin, plain silver nanoparticles, Quercetin –AgNPs loaded gel and Clarithromycin marketed gel were introduced.

The plates of *P. acnes* were incubated for 48h. The diameter of the zones of inhibition (in mm) was measured for evaluating the antibacterial activity. The experiment was repeated three times and the mean was recorded.

## RESULTS AND DISCUSSION

### Organoleptic Characteristics



To verify the authenticity of the medication, we compared the observed characteristics with the established standards outlined in the pharmacopeia. It was determined that the observed attributes met the specified requirements. The color of Quercetin was observed to be yellow crystalline, whereas the odor was found to be pungent. The observed results were found to be comparable with that of the pharmacopeia. The drug's sensory characteristic is a crucial procedure for confirming both the drug's identity and its overall quality

### Melting Temperature

The melting point of Quercetin was determined by capillary method using digital melting point apparatus in triplicate and found to be 315<sup>0</sup> C. The reported melting point for Quercetin was 315-316<sup>0</sup> C. Hence, experimental values were in good agreement with official value. Thus, obtained melting point is in agreement with literature melting point, confirming the purity of drug

### Determination of $\lambda$ MAX

Suitable analytical method was developed for Quercetin using UV spectrophotometer. Analytical wavelength for Quercetin as 371 nm ( $\lambda$ max) was identified as shown in figure no 01.

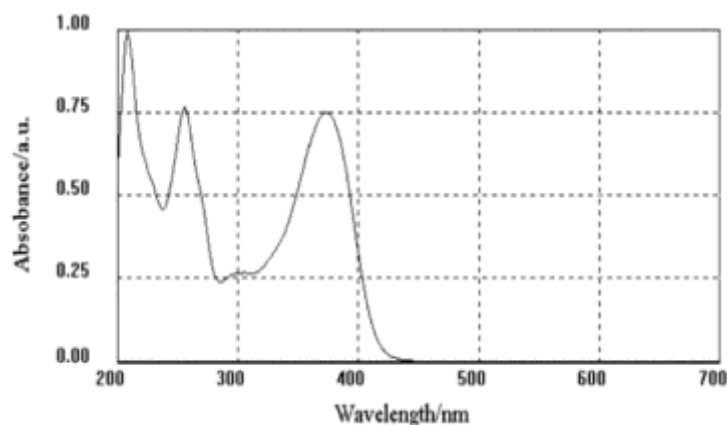


Figure no. 01: Analytical wavelength of Quercetin

### Preparation of silver nanoparticles:

#### Visible Observation:

Green synthesis of AgNPs was prepared from Quercetin. On mixing plant extract with the silver

nitrate solution, a change in the solution color from pale yellow to dark brown was observed which indicates the reduction of silver ions and formation of silver nanoparticle. Formation of silver nanoparticle is shown in (Figure 2).



Fig no 02: Colour change of Quercetin before and after addition of AgNO3

## Characterization of Quercetin silver nanoparticles:

### UV-Visible spectroscopy:

The successful synthesis of Quercetin AgNPs was confirmed by colour change and spectroscopic analysis of the reaction medium and isolated nanoparticles. Indeed, after stirring the mixture of AgNO<sub>3</sub> and Quercetin for 10 min, an obvious colour change was observed. The colour of the reaction mixture changed from yellowish to brown, suggesting the conversion of ionic silver (Ag<sup>+</sup>) to metallic silver (Ag<sup>0</sup>) that self-ensemble into colloidal particles (AgNPs). This observation is consistent with the established literature, which stipulates that silver ions are reduced in the presence of Quercetin due to the reducing

properties of some secondary metabolites i.e. flavonoid.

In fact, the brown colour of AgNPs arises from the concomitant vibration of free electrons of the metallic silver that are in resonance with the light wave. This explains the origin of the surface plasmon resonance (SPR) absorption often observed with metallic nanoparticles, which is commonly verified using UV-Vis spectroscopy to complement the visual observation (colour change) in establishing AgNPs formation. As shown in Figure 02, the synthesized AgNPs exhibited distinctive UV-Vis absorption bands with maximum absorbance at 443 nm. The observed UV-Vis bands are due to the SPR absorption and confirm the presence of AgNPs, alike the colour change.

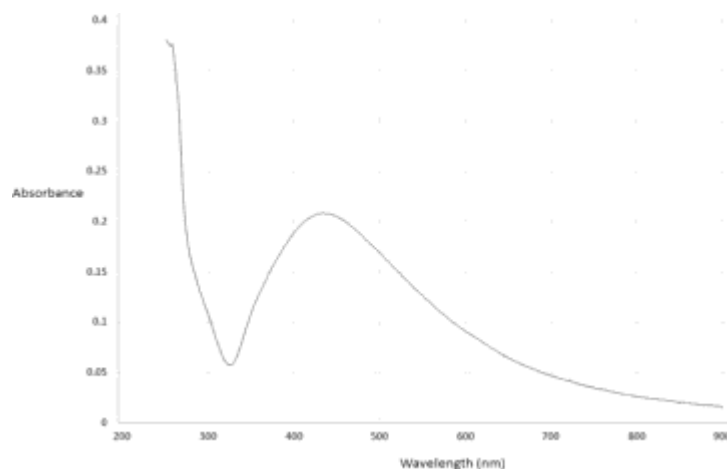
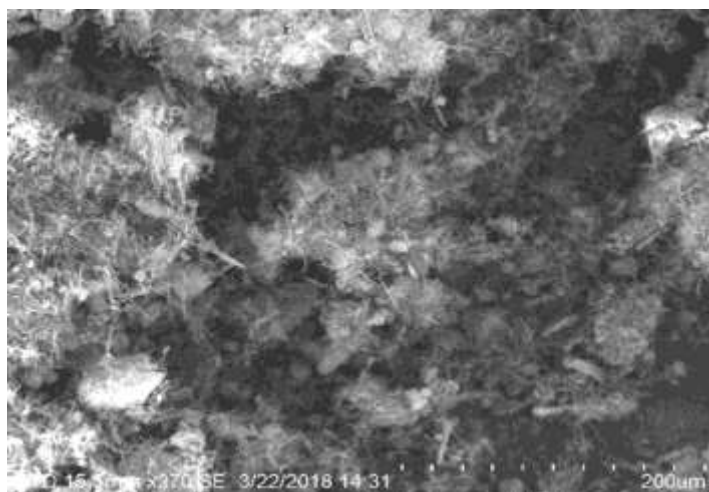


Figure 03: UV-Visible spectrum of synthesized Quercetin AgNPs.

### SEM ANALYSIS:

A scanning electron microscope has been used to identify the size, shape and morphology of nanoparticles. It reveals that the Quercetin silver

nanoparticles are well dispersed and predominantly spherical in shape, while some of the NPs were found to be having structures of irregular shape as shown in Fig. 4.

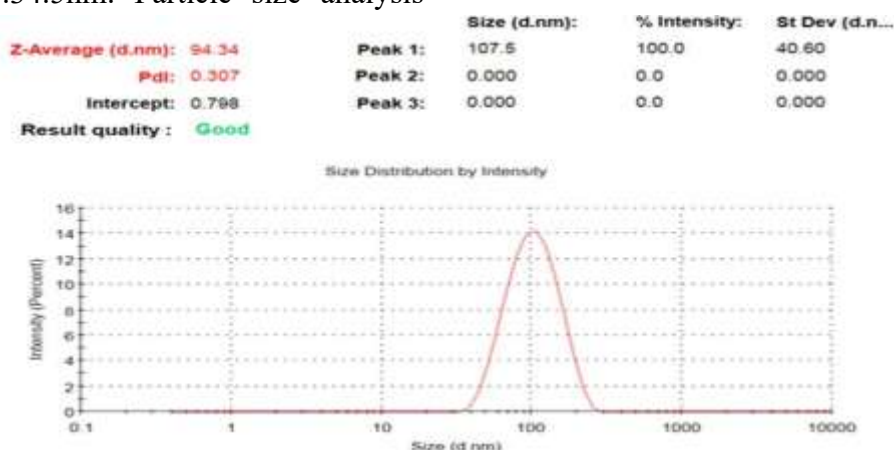


**Fig 04: -SEM photograph of synthesized Quercetin AgNPs**

### Particle size distribution

The particle size is one of the most important parameter for characterization nanoparticles. The average particle size of Quercetin AgNPs was found to be 94.34.5nm. Particle size analysis

showed the presence of nanoparticles with polydispersity indices PDI value 0.307 with intercept 0.798. Percentage intensity of particle size distribution of synthesized Quercetin AgNPs were depicted below.



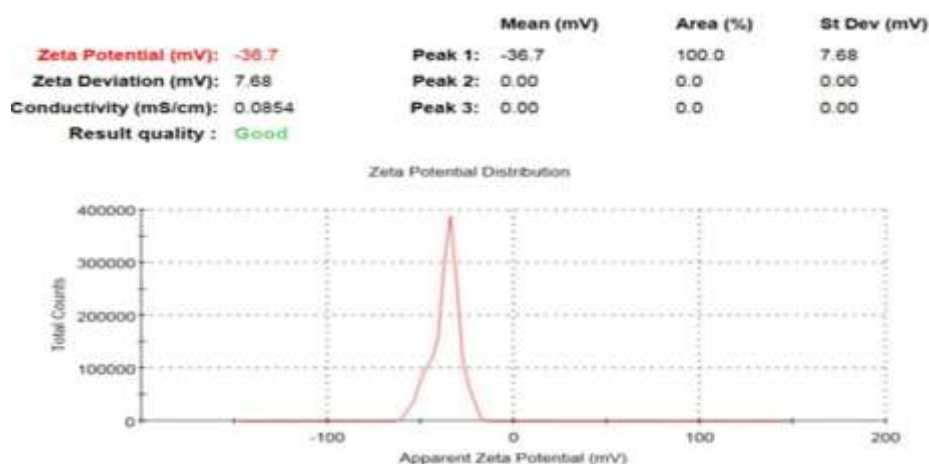
**Fig no 05: Particle size distribution of biologically prepared Silver nanoparticles**

### ZETA POTENTIAL ANALYSIS:

Zeta potential is a key indicator for determining the stability of aqueous silver nanoparticles. For Quercetin AgNPs zeta potential measured was found to be -36.7 mV with peak area of 100% intensity. These values indicate the moderate

stabilization of nanoparticles. Zeta potential distribution of Quercetin AgNPs were depicted below.

Zeta potential (Surface potential) has direct relation with the stability of a form/structure as mentioned below fig no 06



**Table:02: A table showing the stability of the NPs according to the potential charge**

Zeta potential [mV]	Stability behavior of the colloid
from 0 to $\pm 5$	Rapid coagulation or flocculation
from $\pm 10$ to $\pm 30$	Incipient instability
from $\pm 30$ to $\pm 40$	Moderate stability
from $\pm 40$ to $\pm 60$	Good stability
more than $\pm 61$	Excellent stability

**Evaluations of Quercetin silver nanoparticles loaded gel:**

Among the various topical formulations, gel is preferred both in cosmetic and in pharmaceutical preparations due to its faster release rate of drug substances. Gel has various advantages because of its thixotropic property, greaseless, easily spreadable, easily removable, emollient, nonstaining and compatible with several excipients.

Physicochemical parameters such as homogeneity of color, presence of any foreign particle and fibers, pH, spreadability and viscosity are evaluated.

**Physical evaluation:**

Visual inspection results indicate that prepared topical gel formulations has uniform color

distribution and free from any lumps, fibers and foreign particles.

**pH:**

pH was found in range of  $6.71 \pm 0.04$  to  $7.10 \pm 0.02$  for gel prepared by Carbopol and polyethylene glycol as gel base, which is near to the pH of the skin and hence is found to be compatible with skin.

**Viscosity:**

The viscosity was performed to assess the effect of the type and concentration of the gelling agent on the physical properties of the final silver nanoparticle loaded gel products and their viscosity. Table 03 shows the viscosities of gel formulations at low and high shear rates. Generally, the formulation with increase in Carbopol concentration have higher viscosity, this may be because Carbopol is a cross-linked polymer of acrylic acid with high molecular weight that has the ability to absorb and retain water upon neutralization, resulting in a viscous gel.

**Table 03: Results of viscosity of gel**

Formulation	Viscosity* (cp)
QSNG1	2122
QSNG2	2364
QSNG3	2528



### Spreadability:

The spreadability is expressed of time in seconds based on the slip off from the gel by upper slide under certain load. Time taken for the separation of the two slides is less which indicates the topical formulation has better spreadability. The spreadability value was found to be

$7.24 \pm 0.33$  (g.cm/sec) and  $9.10 \pm 0.38$  (g.cm/ csec) for gel prepared by polyethylene glycol and Carbopol. The observed results were comparable with the earlier literature.

**Table 04: Results of spreadability of gel**

Formulation	Spreadability* (gcm/sec)
QSN1	$7.24 \pm 0.33$
QSN2	$7.65 \pm 0.16$
QSN3	$9.10 \pm 0.38$

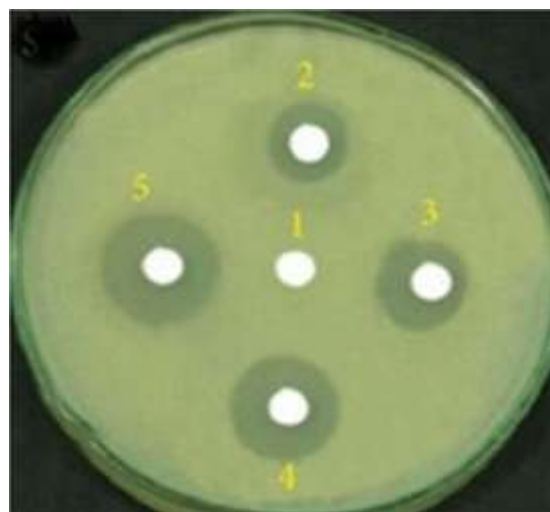
### Anti-microbial activity:

Based on the physicochemical properties of the formulations QSN2 was selected as optimized formulation for antibacterial activity.

The antibacterial activity of the pure Quercetin, plain silver nanoparticles and synthesized Quercetin –AgNPs loaded gel was checked by the disc diffusion method against *P. acnes*, as shown in Figure 02. Quercetin –AgNPs loaded gel showed high antibacterial activity in terms of zone of inhibition against *P. acnes* when compared to the pure Quercetin and plain silver nanoparticles. The maximum zone of inhibition at the highest concentration of Quercetin – AgNPs loaded gel was 17 mm as shown in Table 05. The pure Quercetin and plain silver nanoparticles showed very less activity against the *P. acnes* than Quercetin –AgNPs loaded gel.

**Table: 04 Anti-microbial activity:**

Sr. No	Name of the sample	ZOI (mm)
01	Saline	0.0
02	Pure Quercetin	09
03	Plain silver nanoparticles	08
04	Quercetin –AgNPs loaded gel	17
05	Clarithromycin marketed gel	19



**Fig 07: Anti-microbial activity:**

### CONCLUSION

The present study reveals a simple, rapid and economical method to synthesize Quercetin silver nanoparticle loaded gel. From the results obtained in this research, one can affirm that Quercetin can play an important role in the stabilization of silver ions to silver nanoparticle. As a promising source of bioactive compound. The antibacterial activity is well demonstrated by agar well diffusion method. The synthesized silver nanoparticle using Quercetin showed higher activity than the pure Quercetin and plain silver nanoparticle.

Therefore, it was concluded that our formula could be very promising topical alternative for the treatment of bacterial infection. However, further preclinical, clinical and long-term stability studies should be performed.

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