# Development of Quercetin-Loaded Silver Nanoparticles Gel from Leaf Extract of *Azadirachta indica* for Antifungal Activity

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## Abstract

Background: Neem is a medicinal plant that consists of all aerial parts of the plant known as *Azadirachta indica,* family *Meliaceae.* It contains a lot of chemical constituents like the leaf part consisting of a chemical constituent like quercetin and the oil of *Azadirachta indica* contains Nimbin and nimbidine. The bark contains tannins, gallic acid, and diterpenoids active against klebsiella and staphylococcus species. In this article research is done on the neem plant and gel is prepared from the leaf extract of Neem (*Azadirachta indica*).

Objective: The aim of this review is to highlight the potential of Quercetin a chemical constituent obtained from*Azadirachta indica*in the treatment of fungal infections and prospects associated with the delivery of Quercetin.

Methods: We reviewed the literature from journal publication websites and electronic databases, such as Bentham, Springer, Science Direct, PubMed, Scopus, etc. The development of Quercetin-Loaded Silver Nanoparticles Gel were done by following various techniques like TLC, HPLC, FTIR etc. Evaluation of gel was also done.

Results: Results were concluded on the basis of the data obtained. Quercetin was found to have the potential for anti-bacterial, anti-fungal, anti-viral, anti-inflammatory, anti-cancer, and antioxidant activity. The use of prepared formulation was found to be straightforward and repeatable.

Conclusion: In conclusion, the procedure for making quercetin-loaded silver nanoparticle gel was discovered to be straightforward and repeatable. There are a number of health benefits of Quercetin that makes this group of bioactive compounds the most promising and attractive group for researchers.

**Keywords:** *HPLC, UV- Spectroscopy, Azadirachta indica, Quercetin, Silver Nanoparticles, Zeta- potential.*

## 1.0 Introduction

Neem is an ancient medicinal plant. It consists of all aerial parts of the plant known as *Azadirachta indica*,family Meliaceae [1]. It is commonly called neem or 'Dogon Yaro' in Nigeria, is a plant that has found varied use in ecological, medicinal, and agricultural sectors. It is found in India, Pakistan, Bangladesh, Shri Lanka, Thailand, Malaysia, South Africa, and East Africa [2]. It contains a lot of chemical constituents like the leaf part consisting of a chemical constituent [3] like quercetin and the oil of *Azadirachta indica* contains Nimbin and nimbidine [4]. The bark contains tannins, gallic acid, and diterpenoids active against klebsiella and staphylococcus species [5].Quercetin is an active constituent of leaf extract of neem [6] used to prevent fungal infections like Tinea cruris (Jock itch) throughout the body [7]. Quercetin was the first polyphenolic flavonoid purified from fresh leaves of neem [8] and was known to have antifungal and antibacterial activities and anti-inflammatory[9].Now comes nanoparticles, nanoparticles are not simple molecules [10] but are composed of three layers i.e. The surface layer may be functionalized with a variety of small molecules, metal ions, surfactants, and polymers [11]. The shell layer is a chemically different material from the core in all aspects [12], and the Core is essentially the central portion of the nanoparticle and usually refers to the nanoparticle itself [13].Silver nanoparticles are of various types such as carbon-based nanoparticles, metal nanoparticles, silver nanoparticles, gold nanoparticles, polymeric nanoparticles, lipid-based nanoparticles, etc [14]. Among all of them, one of our interestsis silver nanoparticles [15]**.** Silver is a strong antibacterial and also toxic to cells [16]. Silver has the ability to damage bacterial cell walls [17], inhibit bacterial cell growth [18], and disrupt cell metabolism because of the interaction between silver ions

with macromolecules in cells [19], such as proteins and deoxyribonucleic acid (DNA) [20]. Therefore, silver nanoparticles are indicated to have stronger antibacterial capabilities [21]. Nanogels are newly emerging nano-sized hydrogel-like polymeric materials and have overlapping properties of both nanoparticles and hydrogels [22]. Nanogels provide a crossover for combinations of hydrogels and nanomaterials [23]. Nano-gels consist of physically or chemically cross-linked three-dimensional polymer networks that can also be functionalized and integrated easily with pharmaceutical agents[24].

## 2.0Material and Methods

**Table 1: List of materials used**

| S. No | Material | Source |
|---|---|---|
| 1 | Silver nitrate ($AgNO_3$) | Finar limited, India |
| 2 | Ethanol | Fischer Scientific India Pvt. Ltd., Mumbai |
| 3 | Methanol (HPLC Grade) | Fischer Scientific India Pvt. Ltd., Mumbai |
| 4 | n-octanol LR Grade | SD Fine-chem Ltd., Mumbai |
| 6 | UV-Visible spectrophotometer | Shimadzu |
| 7 | Orthophosphoric Acid | (HPLC Grade) |
| 8 | Nylon 0.22µm membrane filter | Phenomenex Luna |

### 2.1 Pre-formulation study

#### 2.1.1Authentication of Plant

The leaves of about 800gm were collected from the plant *Azadirachta indica.* After that, the herbarium sheet was prepared. The sample was packed properly so that it could not get damaged and the herbarium sheet was sentto theCollege of Horticulture and Forestry, Neri, Hamirpur (H.P. 177001) for authentication of the Plant. The sample was examined by the Assistant Professor and after finding out the correct results the sample was authenticated by him and provided an authentication certificate.

#### 2.1.2 Preparation of Extract of Leaves

The *Azadirachta indica* extract solution was prepared using 30g of *Azadirachta indica* leaves that had been rinsed with deionized water and finely cut into small pieces [25]. The chopped *Azadirachta indica* were boiled in 200 mL of deionized water for 25 minutes and allowed to cool [26]. The cooled leafbroth was filtered and stored in a refrigerator at 4 ◦C. The resulting extract was used as an *Azadirachta indica* extract solution [27]. The extract prepared was then extracted with 3x50ml of ethyl acetate [28]. The resulting ethyl acetate extract was dried

over anhydrous $Na_2SO_4$ and evaporated until itdried [29]. This was done using a rotary evaporator at 400˚C [30].

**2.1.2.1 Characterisation of extraction**

The sample was characterized for physical characterization like appearance, color, and odor.

**2.2 Identification and Isolation of Quercetin**

After extracting neem with methanol, the following process was done for the identification of different compounds present in neem [31]. The neem contains various compounds such as:

**A.** Primary Compounds

**B.** Secondary Compounds

**A. Primary Compounds**: The primary compounds present in neem are fat derivatives, carbohydrates, and proteins.

**B. Secondary Compounds:** The secondary compounds present in neem are flavonoids, steroids, saponins, terpenoids, alkaloids, and tannins [32].

**2.2.1 Identification Tests for Primary and secondary compounds present in neem extract**

**2.2.1.1Identification tests for primary compounds:** Identification tests for primary compounds are given below.

**Table 2: Identification tests for fat derivatives**:

| Sr. No. | Test | Procedure | Observation | Inference |
|---|---|---|---|---|
| **a)** | **Solubility Test** | 1. Taken 3 test tube with 5ml sample solution, to the first one, add 5ml water and observe<br>2. To the second one, add 5ml alcohol and observe<br>3. To the third one, add 5ml chloroform and observe | Sample is sparingly soluble in alcohol and chloroform. | Fats are present. |
| **b)** | **Translucent Spot Test** | Take sample on filter paper and press. | Greasy spot observed. | Fats are present |

| | | | | |
|---|---|---|---|---|
| **c)** | **Acrolein Test** | Take sample in test tube+ add potassium bisulfate to it+ heat the mixture | Pungent and irritating odour. | Fats are present. |

**Table 3: Identification tests for carbohydrates**

| Sr. No. | Test | Procedure | Observation | Inference |
|---|---|---|---|---|
| **a)** | **Molisch's Test** | Take a test tube+ 5ml sample+ 2-3 drops of Molisch reagent+ add conc. $H_2SO_4$ | Presence of violet ring observed at the junction. | Carbohydrates are present. |
| **b)** | **Fehling's Test** | 2ml sample in a test tube+2ml of Fehling's solution A and B+ place on water bath for 10 minutes. | Formation of red ppts. | Carbohydrates are present |
| **c)** | **Benedict's Test** | Take sample in test tube+add 5ml Benedict's reagent+ boil this solution for 10 min. Cool and observe. | Red ppts. observed. | Reducing sugars are present. |
| **d)** | **Tollen's Test** | Sample in test tube+ 2-3ml Tollen's reagent+ place on water bath for 10 min. | Shiny silver mirror observed. | Reducing sugars are present. |
| **e)** | **Iodine Test** | Sample in test tube+ 2-3 drops of iodine solution. Observe colour change. | No colour change. | Starch is absent |

**Table 4: Identification tests for Proteins**

| Sr. No. | Test | Procedure | Observation | Inference |
|---|---|---|---|---|
| **a)** | **Biuret Test** | Take sample in test tube+ add NaOH and 5-6 drops of copper sulphate+ shake gently and keep aside the mixture for 4-5 mins. | Bluish violet colour observed. | Proteins are present. |
| **b)** | **Xanthopr-oteic Test** | Take sample in test tube+ add few drops of conc.$H_2SO_4$+ shake the test tube+ heat it on burner. | Yellow ppts. | Proteins are present. |
| **c)** | **Millon's Test** | Take sample in test tube+2-3 drops of Millon's reagent+ shake gently and observe the changes. | Brick red colour observed. | Proteins are present. |
| **d)** | **Ninhydrin Test** | Take sample in test tube+ add 1-2 ml of ninhydrin solution+ shake well+ boil and observe the change. | Deep blue colour appears. | Proteins are present |

**2.2.1.2 Identification tests for secondary compounds**

**Table 5: Identification test for flavonoids**

| Sr. No. | Test | Procedure | Observation | Inference |
|---|---|---|---|---|
| **a)** | **Shinoda test** | Sample + add magnesium turnings and conc.HCl dropwise and mix properly | Crimson red colour observed. | Flavonoids are present. |
| **b)** | **Alkaline reagent** | Sample + add few drops of NaOH | Intense yellow colour was | Flavonoids are present. |

| | | | | |
|---|---|---|---|---|
| | **test** | | observed. | |
| **c)** | **Zinc HCl Test** | Sample + mixture of zinc dust and conc. HCl | Red colour was observed. | Flavonoids are present. |

**Table 6: Identification test for Steroids and Terpenoids**

| **Sr. No.** | **Test** | **Procedure** | **Observation** | **Inference** |
|---|---|---|---|---|
| **a)** | **Libermann -Burchard test** | Sample+ sufficiet drops of acetic anhydride. Boil and cool. Now add conc. $H_2SO_4$ | Deep red colour was observed. | Terpenoids are present. |
| **b)** | **Salkowski test** | Sample+ few drops of conc. $H_2SO_4$ | Yellow coloured lower layer observed. | Terpenoids are present |

**Table 7: Identification test for Saponins**

| **Sr. No.** | **Test** | **Procedure** | **Observation** | **Inference** |
|---|---|---|---|---|
| **a)** | **Froth Formation Test** | Take 2ml sample in water in a test tube, shake well and observe. | Stable froth(foam) is formed. | Saponins are present. |

**Table 8: Identification test for Alkaloids**

| **Sr. No.** | **Test** | **Procedure** | **Observation** | **Inference** |
|---|---|---|---|---|
| **a)** | **Dragendorff's Test** | Sample + Dragendorff's Reagent (Pot. Bismuth Iodide solution) | Reddish brown ppts. | Alkaloids are presents. |

| | | | | |
|---|---|---|---|---|
| **b)** | **Mayer's Test** | Sample + Mayer's Reagent (Pot. Mercuric iodide solution) | Cream colour ppts. | Alkaloids are present |
| **c)** | **Wagner's Test** | Sample + Wagner's reagent (Iodine pot. Iodide solution) | Reddish brown ppts. | Alkaloids are present. |
| **d)** | **Hager's reagent** | Sample + Hager's reagent (Sat. solution of picric acid) | Yellow ppts. | Alkaloids are present. |
| **e)** | **Picrolonic acid test** | Sample + picrolonic acid | Yellow ppts. | Alkaloids are present. |

**Table 9: Identification test for Tannins**

| Sr. No. | Test | Procedure | Observation | Inference |
|---|---|---|---|---|
| **a)** | **Ferric Chloride Test** | Sample + ferric chloride solution. Observe the solution. | Blue colour observed. | Tannins are present. |
| **b)** | **Phenazone Test** | Sample + sodium acid phosphate. Warm and filter this mixture. To the filtrate, add 2% phenazone solution. | Bulky ppts. formed. | Tannins are present. |
| **c)** | **Gelatin Test** | Sample + 1% gelatin | Precipitates | Tannins are |

| | | | | |
|---|---|---|---|---|
| | | solution containing 10% NaCl | formed. | present. |

### 2.2.2 Isolation of Quercetin from *Azadirachta indica* extract by Column chromatography

Previously prepared neem (Azadirachta indica) extract was used to isolate quercetin from it. Since ethyl acetate (1g/ml) is known to be a solvent for quercetin, ethyl acetate was utilised as the mobile phase, while silica gel was utilised as the stationary phase [33].

First of all, the silica gel taken was carefully moistened with the mobile phase i.e. ethyl acetate. The column was packed sufficiently with a cotton pad at the bottom [34]. The sodium sulphate was added from the top of the column for trapping the molecules of water and the coarse gel was also added to protect the column [35]. Near the end of the bottom of the column, a collecting tube was placed to collect the elute. The blank column was eluted with hexane for 10 min. Subsequent elution was carried out in a manner. Then after that the neem extract (test) was placed on the top of the packed stationary phase [36]. Gradient elution was as follows hexane, hexane: ethyl acetate (5:1), hexane: ethyl acetate (3:1), hexane: ethyl acetate (1:1), ethyl acetate, ethyl acetate: alcohol (5:1), ethyl acetate: alcohol (3:1), ethyl acetate: alcohol (1:1) and alcohol. The amount of collected fraction was 4.0 ml and 29 fractions were collected [37].

### 2.2.3 Identification of quercetin by thin-layer Chromatography (TLC)

The fractions collected before were subjected to TLC. Toluene: ethyl acetate: methanol (4:0.5:0.5) solvent system was used. Because of the similarity of the fractions, 14, 15 and 16 were mixed together and then put through column chromatography once again [38]. The identical procedure for column chromatography was used as in the earlier experiment. But this time the mobile phase and elution manner was different. This time the stationary phase was prepared with silica gel and ethyl acetate [39]. Ethyl acetate was used to elute the blank column for 10 minutes. Ethyl acetate, ethyl acetate: alcohol (6:4), ethyl acetate: alcohol (4:6), ethyl acetate: alcohol (2:8), alcohol, alcohol: methanol (6:4), alcohol: methanol (4:6), and methanol (2:8) were the order of the gradient elution [40]. Total fractions collected were 21 and the volume of the collected fraction was 2.0 ml. Toluene: ethyl acetate: methanol (4:0.5:0.5) was the solvent system utilised for TLC on the collected fractions. Following a second round of column chromatography using fractions 14, 15, and 16, 21 fractions were

also obtained. The fractions 7, 8, 9, 11, 12, and 13 were combined, and TLC was applied twice [41]. Quercetin was separated from an alcohol extract of *Azadirachta indica* using TLC silica gel 60 F254 plates (10x06 cm). After that, the plates were put in a room filled with iodine vapours to see what hue the spot was (light blue). To compare the colour of the spots in the sample and standard quercetin, a 5% ethanolic ferric chloride solution was sprayed onto the formed plate. Mixed portions were concentrated and kept for further examination [42].

### 2.2.4 Identification of quercetin by HPLC

The concentrated plant quercetin was subjected to High-Performance Liquid Chromatography. Based on the solubility, we have chosen the mobile phase for quercetin, which is soluble in ethanol, methanol, and water. and carried out the analysis using gradient elution and reverse phase [43].

**Preparation of Mobile phase A**: 1.0 ml of orthophosphoric acid was pipetted into a 2000 ml volumetric flask. The pipette was then three times washed with water before being filled to volume with water [43].

**Mobile phase B**: Methanol

**Chromatographic conditions:** Flow Rate: 2.0ml/min Injection volume: 10µL Column temperature : 25°C Detection : 370nm Runtime : 25min Column : 250x 4.6,5 µm,C18 Diluent : Methanol

**Table 10: Gradient Programme**

| Sr No. | Time (in min) | Mobile phase A | Mobile phase B |
|---|---|---|---|
| **1** | 0 | 60 | 36 |
| **2** | 6 | 60 | 36 |
| **3** | 12 | 50 | 46 |
| **4** | 15 | 60 | 36 |
| **5** | 20 | 60 | 36 |
| **6** | 25 | 60 | 36 |

**Preparation of Standard Quercetin**:Added around 50 ml of diluent after weighing and transferring 10 mg of standard quercetin into a 100 ml volumetric flask. The mixture was sonicated for five minutes, cooled to room temperature (21 ºC), and then diluted to the

desired amount with diluent. After that, pour 5.0 ml of the aforementioned solution into a volumetric flask with a capacity of 50.0 ml, and dilute it with diluent to volume [44].

**Preparation of sample**: Added around 50 ml of diluent after precisely weighing 5 mg of the test sample into a 100 ml volumetric flask. The mixture was sonicated for 5 minutes, cooled to room temperature, and then diluted to the desired volume. Individual injections of the test sample solution, standard solution, and blank diluent were made into the column. Reported the findings after recording the chromatogram at 273 nm [45].

### 2.2.5 Preparation of silver nanoparticle

In the preparation of AgNPs samples, AgNO3 (1Mm, 3mM, 4mM, 6Mm,9Mm) was first dissolved in 20 ml of deionized water [46] and mixed with 20 ml of *Azadirachta indica*extract solution under vigorous stirring at room temperature for 30 min [47]. The mixtures were added to the sealed volumetric flask of 100ml, which was heated and maintained at various time and temperature conditions, and then gradually cooled to room temperature [48]. A black precipitate was collected by centrifuging at 15000 rpm for 30 min and finally dried in air at $60^0$C for 4hr [49].

### 2.2.6Characterisation of silver nanoparticle

The silver nanoparticles were characterized for the physical characterization like appearance, color and odor. UV-Vis spectroscopy, FTIR, was done for silver nanoparticles. Particle size was also determined.

**Table 11: Composition of Different Formulations of Nanoparticle**

| Formulation Code | Silver Nitrate (mM) | Amount of silver nitrate (mg) | Volume of extract (ml) | Volume of water (ml) |
|---|---|---|---|---|
| F1 | 1 | 6 | 20 | 20 |
| F2 | 3 | 20 | 20 | 20 |
| F3 | 4 | 27 | 20 | 20 |
| F4 | 6 | 40 | 20 | 20 |
| F5 | 9 | 60 | 20 | 20 |

### 2.2.7 Preparation of Drug-Loaded Silver Nanoparticle

Silver nanoparticle solution of 1mM was prepared in 10ml by changing the variation in concentration of Quercetin dihydrate drug (10mg, 20mg,30mg,40mg,50mg) in each different culture tubes and centrifuge it at 5000rpm for 15 min [50].

**Table 12: Composition of Different Quercetin Dihydrate loaded silver Nanoparticles**

| Sr. No | Formulation Code | Amount of Drug (mg) | Volume of nanoparticle solution (ml) |
|---|---|---|---|
| 1 | F2A1 | 10 | 2 |
| 2 | F2A2 | 20 | 2 |
| 3 | F2A3 | 30 | 2 |
| 4 | F2A4 | 40 | 2 |
| 5 | F2A5 | 50 | 2 |

### 2.2.8 Preparation of Quercetin dihydrate-loaded silver nanoparticle gel

Formulation of Quercetin dihydrate entrapped silver nanoparticle (formulation prepared by Quasi emulsion solvent method) [51]. Formulations of silver nanoparticles containing the drug were formed into the gel base which is composed of Quercetin dihydrate loaded silver nanoparticles with 2ml silver nitrate solution 1% & 2% Carbapol 934, Triethanolamine (quantity sufficient) [52].

**Table 13: Composition of Different Quercetin dihydrate loaded silver nanoparticle gel**

| Sr. No | Ingredients | Formulation Code | |
|---|---|---|---|
| | | F2A5B6 | F2A5B7 |
| **1** | Quercetin dihydrate loaded silver nanoparticle (ml) | 2 | 2 |
| **2** | Quercetin dihydrate (mg) | 20 | 20 |
| **3** | Carbopol 934 (%w/v) | 1 | 2 |
| **4** | Triethanolamine (mL) | q.s. | q.s. |

### 2.2.9Characterisation of Quercetin dihydrate loaded silver nanoparticle

Percentage yield, drug entrapment efficiency, particle size, TEM, FTIR and zeta potential values of Quercetin loaded silver nanoparticle were find out.

### 2.2.10Evaluation of Quercetin dihydrate loaded silver nanoparticle gel

Evaluation of Quercetin loaded silver nanoparticle gel was done by performing various tests on the formulation such as visual appearance, pH determination, viscosity, spreadability study, in-vitro release studies, and drug release kinetics.

## 3.0Resultsand Discussions

### 3.1 Physicochemical Parameters of *Azadirachta indica*Leaves

#### 3.1.1 Organoleptic Property

Organoleptic properties of *Azadirachta indica* leaves were carried out in order to check the physical properties as shown in Table 14:

**Table 14. Organoleptic Property of *Azadirachta Indica* leaves**

| | |
|---|---|
| Taste | Extremely Bitter |
| Odour | Unpleasant |
| Colour | Green Powder |

**Result:** *Azadirachta indica* leaves is extremely bitter, unpleasant in odour and green powder in colour.

#### 3.1.2 Total Ash Value

Total Ash Value were carried out to analyze the purity of *Azadirachta indica* leaves as shown in Table 15.

**Table 15: Total Ash Value of *Azadirachta indica***

| **Plant Part** | **Observed Ash value** | **Reference Ash value** |
|---|---|---|
| *Azadirachta indica* | 9.00%±1.26 | 10.00% |

**Result:** Total ash of *Azadirachta indica* leaf powder is found to have 9.02% ±1.26.

#### 3.1.3 Acid-Insoluble Ash

Acid Insoluble ash value of *Azadirachta indica*is shown in Table 16:

**Table 16: Acid Insoluble Acid value of *Azadirachta indica***

| **Plant Part** | **Observed Acid Insoluble value** | **Reference Acid Insoluble value** |
|---|---|---|
| ***Azadirachta indica*** | 1.50%±0.29 | 2.00% |

**Result**: Acid Insoluble ash value of *Azadirachta indica* leaf powder is found to be 1.50%±0.29.

**3.2Preparation of Extract of Leaves**

**3.2.1 Characterization of extract of leaves**

**3.2.1.1 Organoleptic Property**

*Azadirachta indica* leaves extract was discovered to have organoleptic properties that matched the literature.

*Azadirachta indica*leaves extract organoleptic characteristics was discovered to be listed in Table 17.

**Table 17: Organoleptic Properties of Extract of *Azadirachta indica***

| **Odor** | **Odourless** |
|---|---|
| Color | Colourless to Brown colour |

**Result:** The extract of *Azadirachta indica* show odorless in nature and show brown colour from colorless.

**3.3Isolation and Preformulation Study of Drug**

**3.3.1 Isolation of the drug (Quercetin):** The drug was isolated from the *Azadirachta indica* methanol extract. The results are concluded below.

**Results:** Isolation of quercetin was done by column chromatography using silica gel and ethyl acetate as stationary and mobile phases respectively. The amount of collected fraction was 4.0 ml and 29 fractions were collected.

**3.3.2 Identification by TLC**

The identification of the drug Quercetin was done by thin layer chromatography technique.

**Results:** Thin layer chromatography When the developed TLC plates were saturated with iodine vapours, they showed spots which coincided with that of the reference quercetin and ethanol extract of *Azadirachta indica* (Fig.1). The plates developed under UV light showed fluorescent spots in ethanol extraction of *Azadirachta indica* and results are presented in (Fig. 2). And the thin layer chromatogram of the concentrated test sample is presented in (Fig .3).

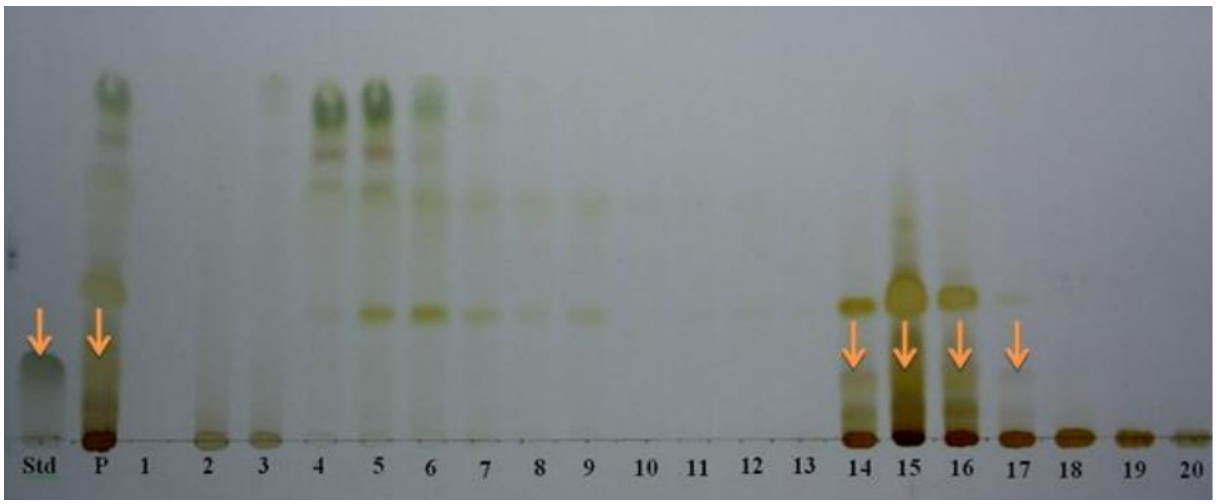



**Fig 1. Thin layer chromatogram of ethanol extract of *Azadirachta indica***

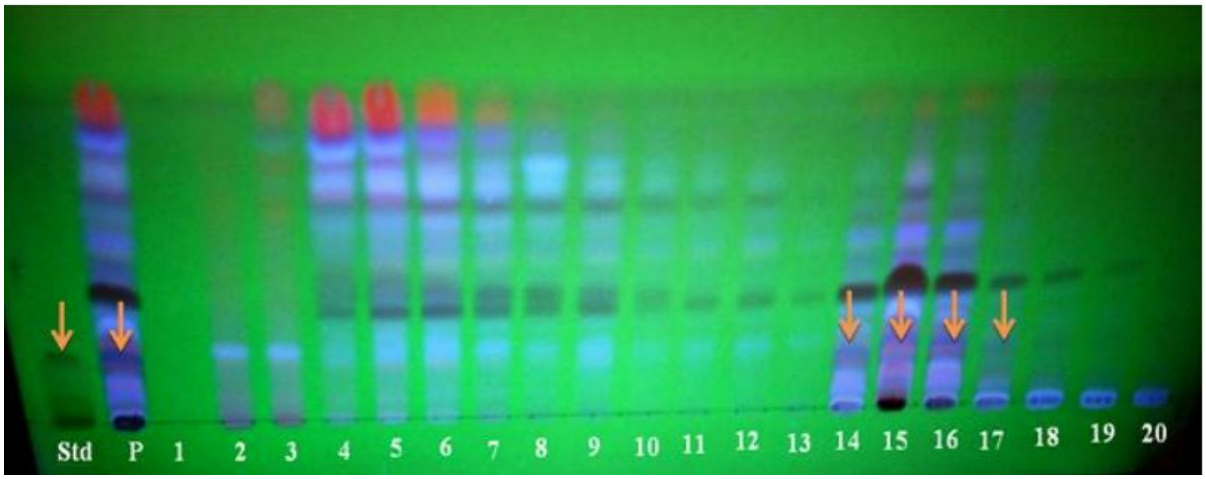



**Fig 2. Thin layer chromatogram of ethanol extract of *Azadirachta indica* under UV trans illuminator**

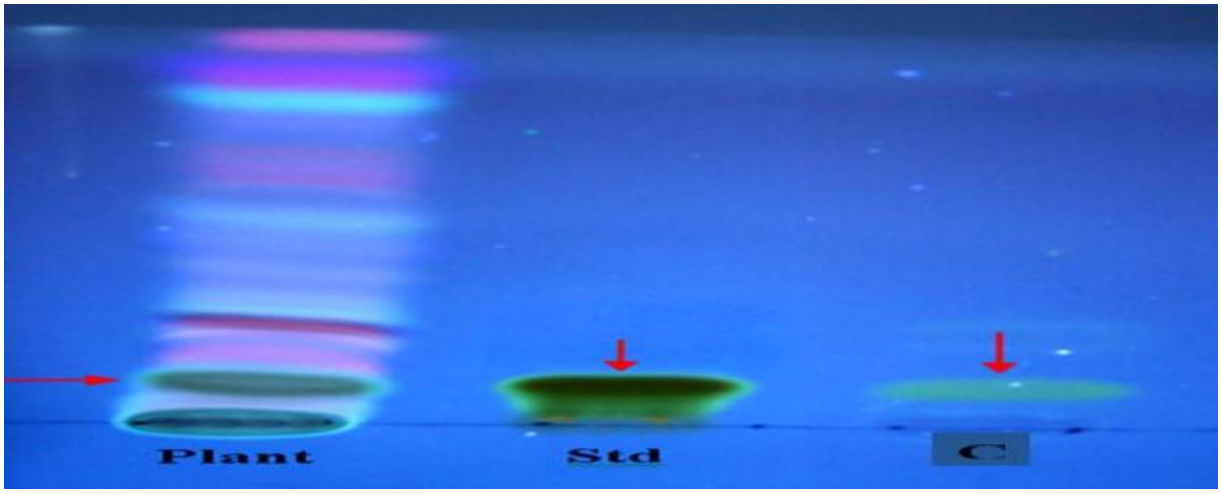



**Fig 3. TLC of concentrated quercetin of *Azadirachta indica* under UV trans illuminator**

### 3.3.3 Identification of quercetin by HPLC-UV

After a retention duration of around 15 minutes, quercetin was eluted. By using HPLC-UV, quercetin was eluted with a retention duration of 15 minutes. Peak purity was also checked, and the standard and sample spectra were matched and validated using the DAD detector. Additionally, it is discovered to contain 995 ± 1.17 µg/g of quercetin. By using the area normalisation approach, purity was determined to be 99.7% pure. The HPLC chromatograms of the test sample and the reference quercetin are shown in (Figs. 4 & 5), respectively. Figures 6, 7, and 8 show the spectra of standard quercetin, the test sample, and both the standard quercetin and the test sample.

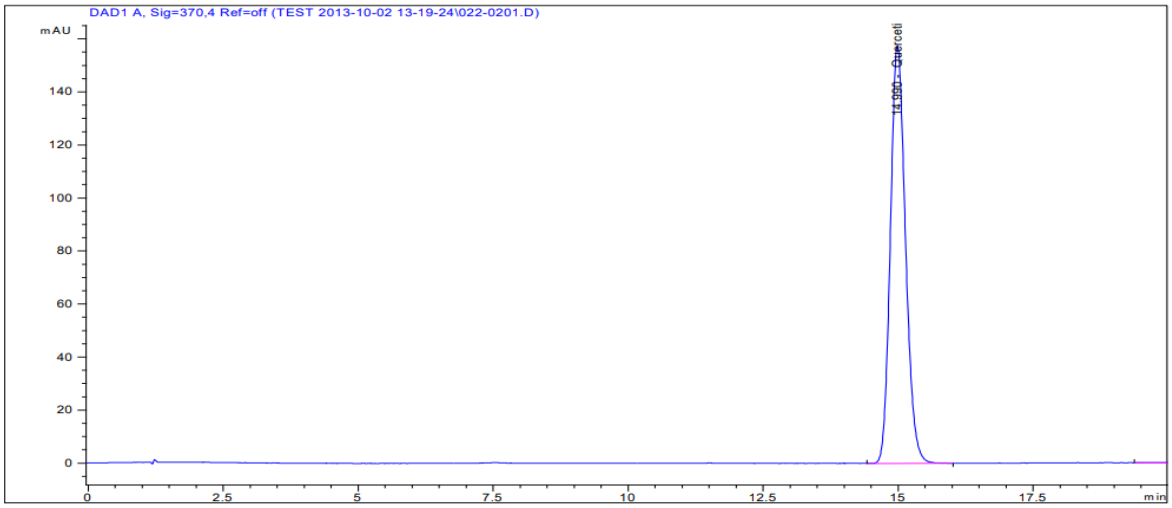



**Fig 4. HPLC chromatogram of Standard quercetin at RT of 15 min**

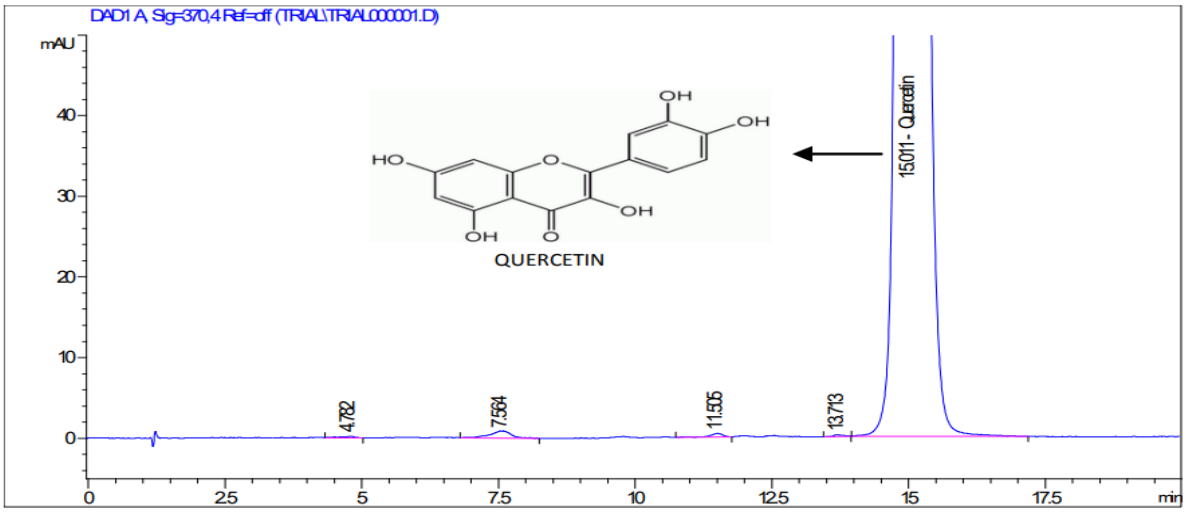



**Fig 5. HPLC chromatogram of test Sample**

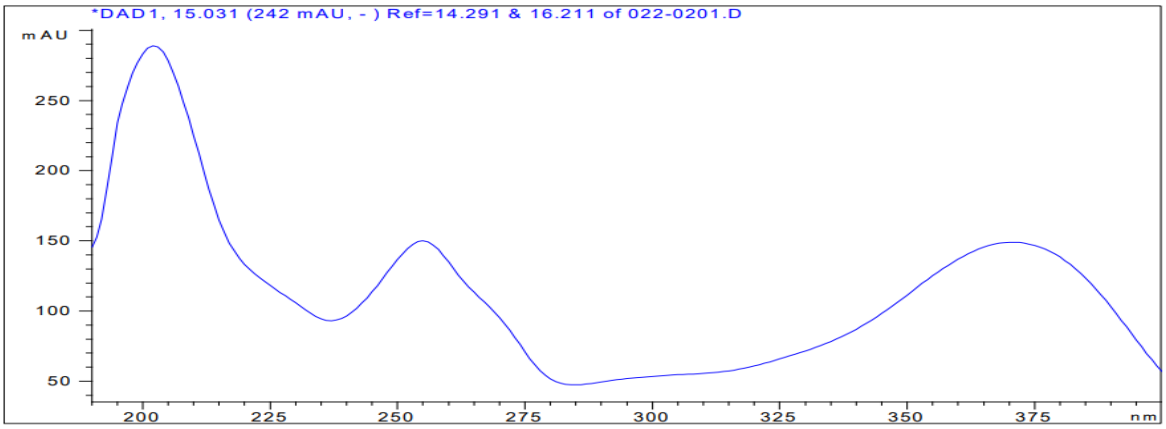



**Fig 6. Spectra of standard Quercetin.**

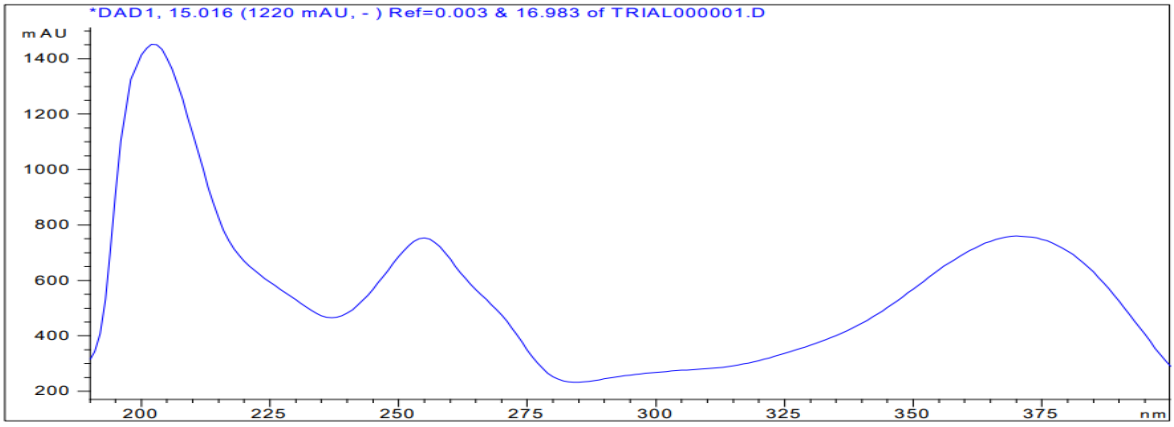



**Fig 7. Spectra of test sample**

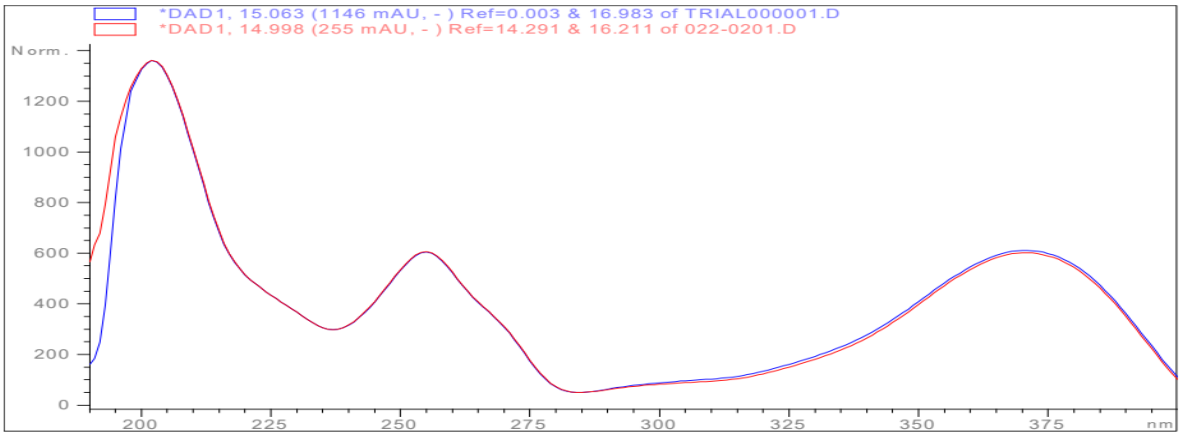



**Fig 8. Spectra of standard quercetin and test sample.**

**Results:** Our quantitative HPLC analysis revealed that the *Azadirachta indica* sample contained about 99.7% of pure quercetin.

### 3.3.4 Organoleptic property

Organoleptic properties of drug Quercetin dihydrate found to be as per literature. The Organoleptic properties of Quercetin dihydrate were found to the given Table 18:

**Table 18: Organoleptic Properties of Quercetin Dihydrate**

| **Odor** | **Odourless** |
|---|---|
| Colour | Yellow powder |

### 3.3.5 Melting point

The melting point of Quercetin dihydrate was carried out to examine the completely melted stage of the drug as shown in Table 19:

**Table 19: Melting Point of Quercetin dihydrate**

| **Drug** | **Observed melting point** | **Reference melting point** |
|---|---|---|
| Quercetin dihydrate | 317.4 °C ±0.873 | 316 - 318 °C |

**Result:** The melting point of Quercetin dihydrate was found to be in range317.4 °C ±0.873 which is of the pure drug. Hence drug sample was free from any type of impurities.

### 3.3.6 HPLC Method

#### 3.3.6.1 Determination of chromatogram of Blank & Standard (Quercetin dihydrate)

On HPLC analysis of Blank and standard solution of Quercetin dihydrate solution of 100µg/ml chromatogram was optimized & analyzed as per the proposed method. HPLC analysis of blank and standard chromatogram was shown in Figure 9&10.

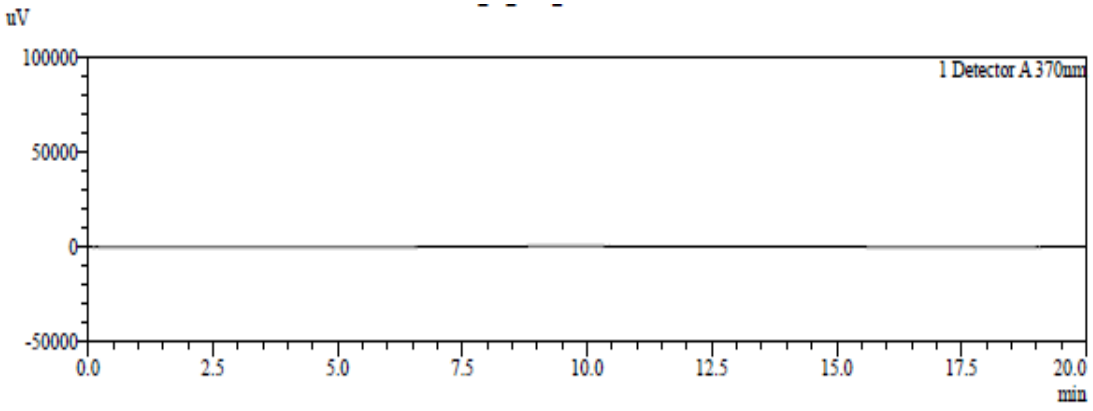



**Figure 9:Chromatogram of Blank**

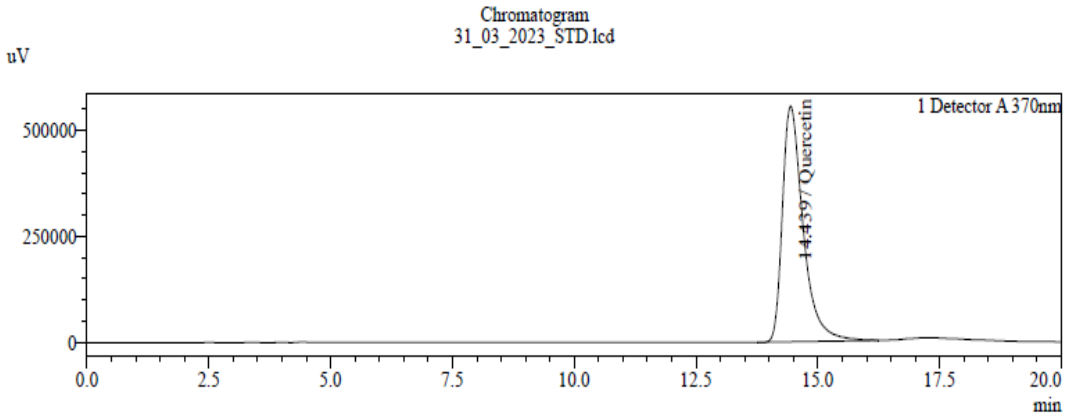



**Figure 10: Chromatogram of standard Quercetin dihydrate of 100 µg/ml.**

**Table 20: Data of Quercetin dihydrate Solution of 100 µg/ml**

| Sr. No. | Compound Name | Retention time | Area |
|---|---|---|---|
| 1 | Quercetin dihydrate | 14.439 | 16474817 |

### 3.3.6.2 Preparation of calibration curve of Quercetin dihydrate

The calibration curve wasprepared by measuring several concentrations creating a linear line as shown in **(Table 21, Figure 11).**

**Table 21: Calibration Curve of Quercetin dihydrate**

| Concentration(µg/ml) | Area |
|---|---|
| 10 | 799076 ±107.983 |
| 20 | 1602525±102.549 |
| 30 | 2413014±1998.502 |
| 40 | 3283191±501.507 |
| 50 | 4058351±1004.5 |

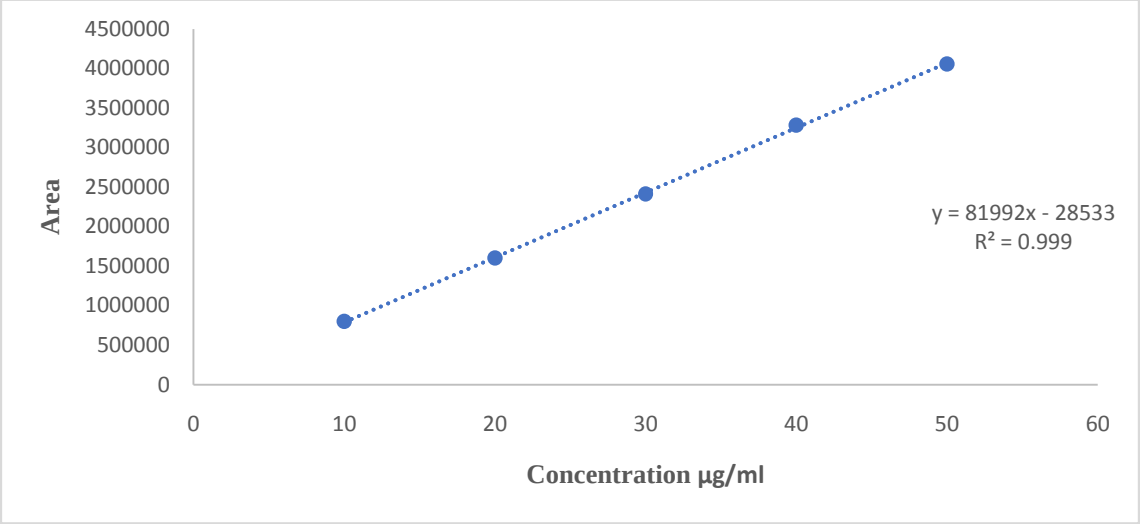



**Figure 11: Calibration curve of Drug Quercetin dihydrate**

**Table 22: Result of regression analysis of estimation of Quercetin dihydrate**

| Statistical parameters | Results |
|---|---|
| Regression equation: y=mx+C | y = 81992x - 28533 |
| Slope (m) | 81992 |
| Intercept (C) | 28533 |
| Correlation coefficient ($r^2$) | 0.999 |

**Result: -** The calibration curve for µg/ml was obtained by using the 10µg/ml to 50µg/ml solution. The calibration curve as shown in the graph indicated the regression equation Y=81992x – 28533and $R^2$ value 0.999 which shows good linearity as shown in Figure 3.

### 3.4 Solubility Study

Solubility of the drug in various solvents were carried out in order to screen for the components to be used for formulation development.

**Table 23: Solubility studies of Quercetin dihydrate for different solvents**

| Sr. no. | Solvent | Solubility (mg/ml) | Inferences |
|---|---|---|---|
| 1 | Methanol | 52.2199±2.13 | Soluble |
| 2 | Ethanol | 55.4777±0.423 | Soluble |
| 3 | PB pH 6.8 | 0.05035±0.0021 | Practically insoluble |
| 4 | Water | 0.10611±1.86 | Insoluble |

* Each value is mean of three independent determinations

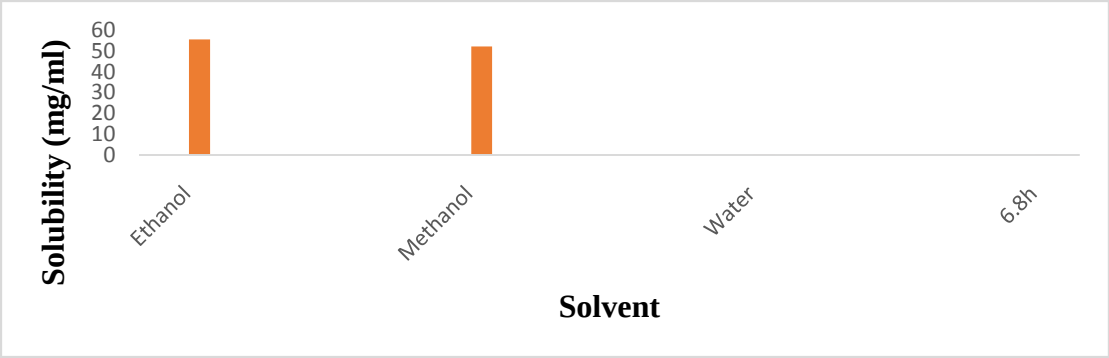



**Figure 4: Solubility study of Drug Quercetin dihydrate**

**Result:** From the above data, it was clearly seen that Quercetin dihydrate is soluble in ethanol and methanol and very slightly soluble in water and practically insoluble in phosphate buffer pH 6.8

### 3.5 FTIR

The FTIR spectrum and its interpretation Quercetin dihydrate is shown in figure 12 and table 24, respectively.

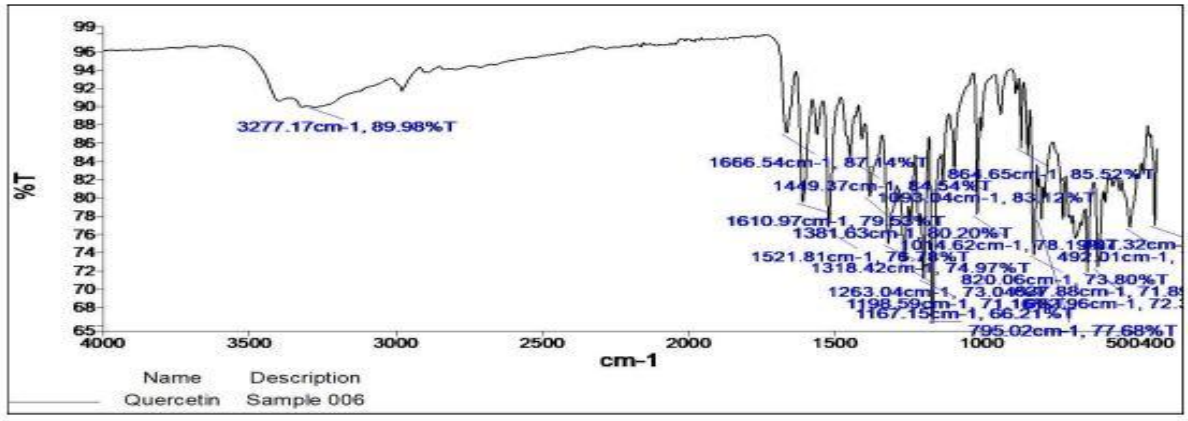



**Figure 12: FTIR spectrum of Quercetin dihydrate**

**Table 24: FTIR spectrum of Drug Quercetin dihydrate**

| Characteristic peaks | Reported ($cm^{-1}$) | Observed ($cm^{-1}$) |
|---|---|---|
| O-H stretch | 3283 | 3277.17 |
| C=O stretch | 1666 | 1666.54 |
| C= C stretch | 1610 | 1610.97 |
| O-H bending of phenol function | 1379 | 1381.63 |
| In-plane and out plane bending | 1317 | 1318.63 |

| | | |
|---|---|---|
| C-O stretch in the aryl ether ring | 1263 | 1263. 04 |
| In-plane and out plane bending | 820 | 820. 06 |

**Result:**

The principal IR absorption peaks of Quercetin dehydrate of O-H stretch 3277.17, C=O stretch at 1666.54, C= C stretch at 1610.97, O-H bending of phenol function at 1381.63, In plane and out plane bending at 1318.63, C-O stretch in the aryl ether ring at 1263. 04, in plane and out plane bending at 820. 06 were all observed in the spectra of Quercetin dehydratewere found to be similar to cited peaks. These observed principal peaks confirmed the purity and Authenticity of the Quercetin dihydrate. The FTIR spectra of Quercetin dihydratewere shown in the and table 24.

**3.6Partition Coefficient of Drug**

The shake flask method was used to carry out the research on partition coefficient determination. Table 25 displays the partition coefficient for the drug Quercetin dihydrate.

**Table 25: Partition coefficient determination of Quercetin dihydrate**

| Drug | Solvent system | Log P Values |
|---|---|---|
| Quercetin dihydrate | n-octanol: water | 1.70± 0.0629 |

**Result:** The partition coefficient of Quercetin dihydrate in n- Octanol: Water was found to be 1.70± 0.0629 this indicates that the drug is lipophilic in nature. This suggests the hydrophilic character of the drug Quercetin dihydrate.

**3.7 Preparation and characterization of silver nanoparticle**

**3.7.1 Evaluation of Silver Nanoparticle**

Evaluation of silver Nanoparticle were determined by colour change, uniformly dispersion to be listed in table 26.

**Table 26: Evaluation of silver Nanoparticle (F1-F5)**

| Sr NO. | Formulation code | Visual appearance |
|---|---|---|
| 1 | F1 | Colour change but dispersion not formed uniformly |
| 2 | F2 | Black Colour Silver nanoparticles crystals formed |
| 3 | F3 | Colour not changed homogenous Dispersion |
| 4 | F4 | Colour not changed homogenous Dispersion |

| | | |
|---|---|---|
| 5 | F5 | Colour not changed homogenous Dispersion |

**Result:** F2 formulation shows the best formulation of silver nanoparticles due to conversion of Ag+ ions to silver nanoparticle from colourless to brown colour and some formulations are not formed uniformly.

### 3.7.1.2 UV Spectroscopy

UV-Vis spectroscopy is one of the best and fastest techniques used for exploring AgNPs. Reduction of silver ions by the plant extract was monitored by colour change, from yellowish-green to brownish-black. The UV-Vis absorbance spectrum showed a sharp peak at 452 nm, indicating AgNPs were able to shift the maximum peak of absorption to the visible light region. The change in colour, after adding the AgNO3 solution, was due to the surface plasmon resonance phenomenon. This occurs due to the combinedvibration of free electrons in the metal nanoparticles resonating with the light waves. Furthermore, the maximum spectrum band observed of F2 at 452nm.

**Table 27: UV–Vis absorbance spectrum of silver nanoparticles (F1-F5)**

| S.No. | Formulation | Wavelength (nm) |
|---|---|---|
| 1 | F1 | Not prepared |
| 2 | F2 | 452nm |
| 3 | F3 | Not prepared |
| 4 | F4 | Not prepared |
| 5 | F5 | Not prepared |

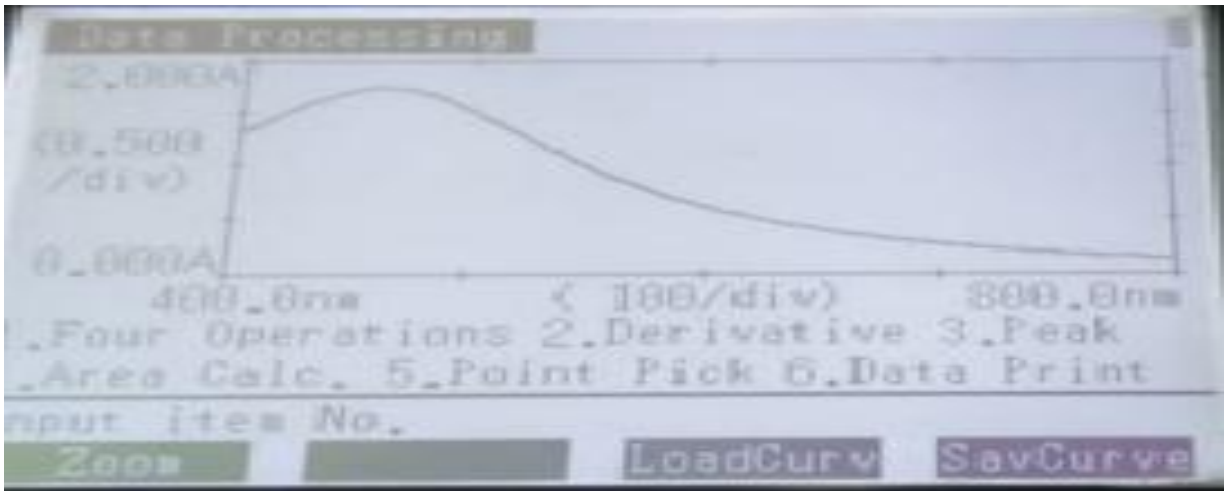



**Figure 13: UV Spectrum of Silver Nanoparticle of F2**

**Result:** In F2 Formulation UV-Vis spectra at 452nm revealed the formation of silver nanoparticles and in some formulations time and temperature variations allow for forming the unstable silver nanoparticle.

**Table 27: Absorption maxima ($\lambda_{max}$) of Silver Nanoparticle water**

| Name Excipient | Absorption maxima ($\lambda_{max}$) | |
|---|---|---|
| | **Observed** | **Reference** |
| Silver Nanoparticle | 452 | 455 |

### 3.7.1.3 FTIR

The FTIR spectrum and its interpretation of silver nanoparticle is shown in figure 14respectively.

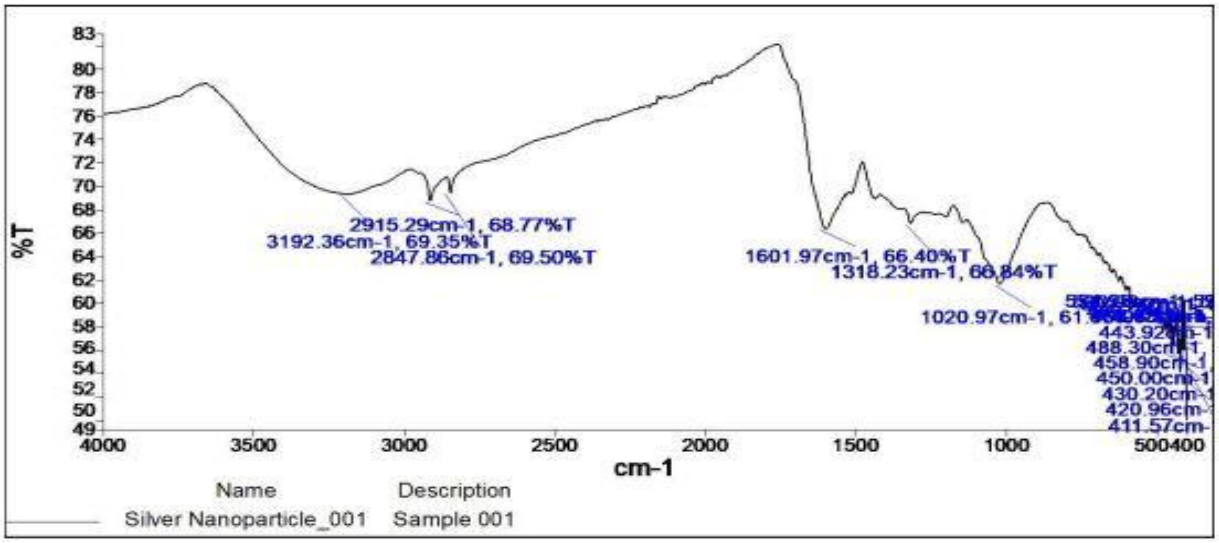
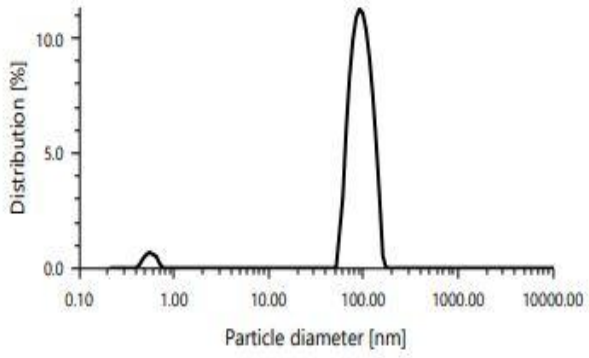



**Figure 14: FTIR of Formulation**

**Result**: The IR spectrum of the AgNPs showed a band at 3192.36 cm−1, which corresponds to stretching of the hydroxyl groups as a functional group of carbohydrates, phenols, flavonoids, and alcohols; the peak at 2915.2 cm−1shows the stretching of the C-H alkaline, and C − H (Alkenyl) groups. The IR spectrum for the same plant extract from India showed bands at 3454, 2083, 1636, and 1113 cm−1, which differed slightly from our observations, suggesting climate can change the plant's phytochemical composition

### 3.7.1.4 Particle size

The prepared F2 Formulation of silver nanoparticle was tested for particle size and polydispersity index, as shown in figure 15.




**Results**

| | | | |
|---|---|---|---|
| **Hydrodynamic diameter** | 107.96 nm | **Mean intensity** | 313.8 kcounts/s |
| **Polydispersity index** | 17.2 % | **Absolute intensity** | 59324.5 kcounts/s |
| **Diffusion coefficient** | 0.1 µm²/s | **Intercept g1²** | 0.7517 |
| **Transmittance** | 0.0 % | **Baseline** | 1.001 |

**Figure 15: Particle size of F2 formulation**

**Result:** The hydrodynamic diameter of the synthesized nano particle was found to be 107.96nm and the polydispersity index was 17.2%.

Based on the above observations the F2 formulation was found to be best suitable for the adsorption of the quercetin dihydrate.

### 3.8 Drug-Loaded Silver Nanoparticle

#### 3.8.1 Visual appearance of Quercetin dihydrate loaded silver nanoparticles

Visual Appearance of formulations of Quercetin dihydrate loaded silver nanoparticle (F1-F5) as shown in Table 28:

**Table 28: Visual Appearance of Quercetin dihydrate loaded silver nanoparticle (F1-F5)**

| Sr. no | Formulation Code | Visual Appearance |
|---|---|---|
| 1 | F2A1 | Yellowish Colour |
| 2 | F2A2 | Light Yellowish Colour |
| 3 | F2A3 | Light Yellowish Colour |
| 4 | F2A4 | Yellowish Colour |
| 5 | F2A5 | Dark yellowish Colour |

### 3.8.2 Percentage yield

Percentage yield of all formulation (F2A1-F2A5) was given in a table 29:

**Table 29: Percentage yield of Quercetin dihydrate loaded silver nanoparticle (F2A1-F2A5)**

| Sr. No. | Formulation Code | % Yield |
|---|---|---|
| 1 | F2A1 | 86.12±1.155 |
| 2 | F2A2 | 90.54±1.091 |
| 3 | F2A3 | 93.10±0.371 |
| 4 | F2A4 | 92.86±1.748 |
| 5 | F2A5 | 95.64±0.539 |

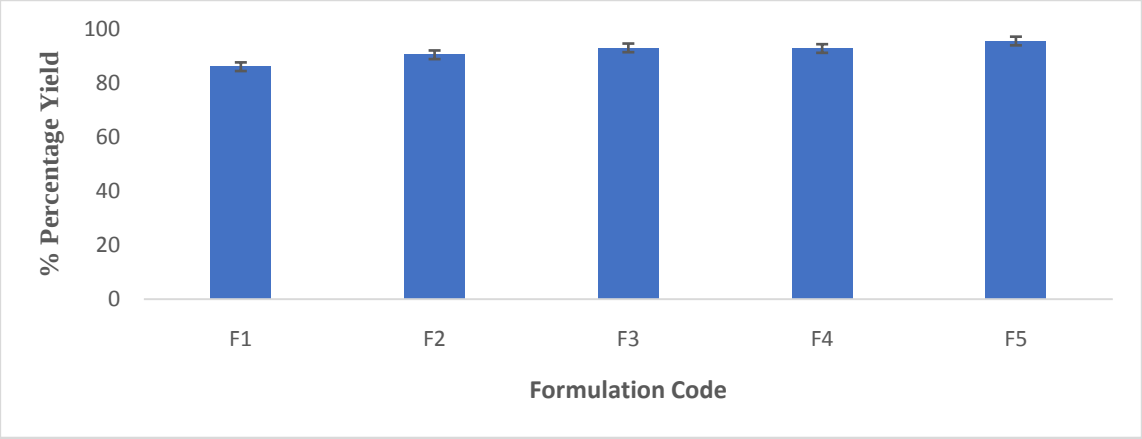



**Figure 16: Percentage yield of Quercetin dihydrate loaded silver nanoparticle (F2A1-F2A5)**

**Result:** The percentage yield of all formulation was found to be in a range of 86.12±1.155 to 95.64±0.539.

### 3.8.3 Drug Entrapment Efficiency

The percentage of Drug Entrapment in all formulations was given in table 30:

**Table 30: Percentage Drug Entrapment of Quercetin dihydrate loaded silver nanoparticle (F2A1-F2A5)**

| Sr. No. | Formulation Code | % EE |
|---|---|---|
| 1 | F2A1 | 76.70±0.13 |

| | | |
|---|---|---|
| 2 | F2A2 | 81.85±0.44 |
| 3 | F2A3 | 85.71±0.22 |
| 4 | F2A4 | 88.54±0.07 |
| 5 | F2A5 | 89.18±0.19 |

**Result:** Drug Entrapment efficiency of Quercetin dihydrate-loaded silver nanoparticles ranges from 76.70±0.13 to 89.18±0.19. The formulation F2A5 has maximum Drug Entrapment of 89.18±0.19.

### 3.8.4 Particle size

The prepared F2A1-F2A5 formulation of Quercetin-dihydrate loaded silver nanoparticle was tested for particle size and polydispersity index, as shown in figure 17.

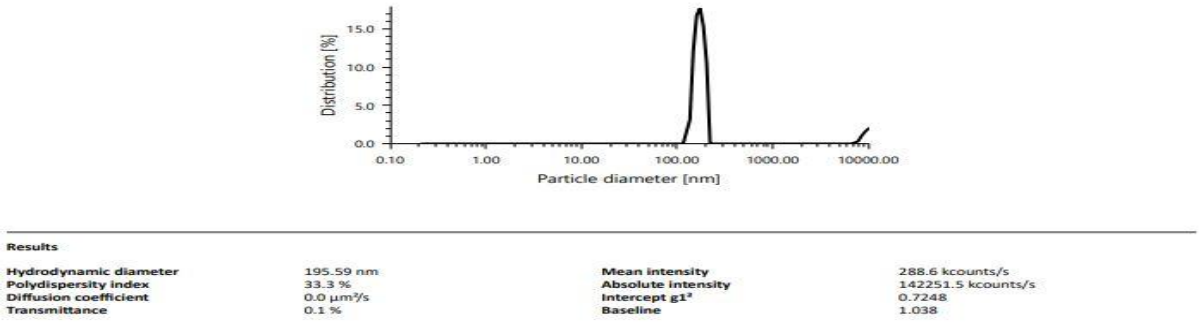



**Figure 17: Particle size of F2A5 formulation**

**Result:** The hydrodynamic diameter of Quercetin dihydrate loaded silver nanoparticle was found to be 95.59 nm and the polydispersity index was 33.3% as shown in figure 18.

### 3.8.5 Transmission electron microscopy (TEM):

Transmission electron microscopy of quercetin-loaded silver Nanoparticles was carried out for structural characteristics as shown in figure 18.

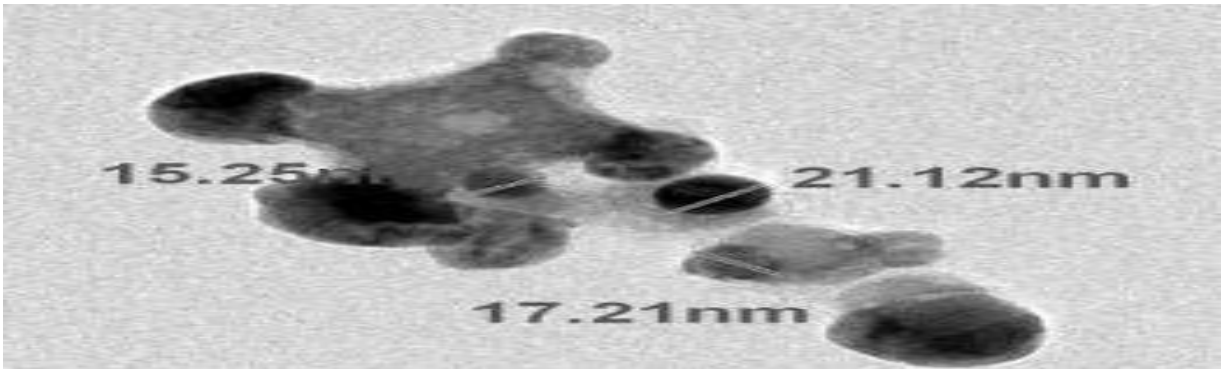



**Figure 18: Transmission electron microscopy of F2A5 formulation.**

### 3.8.6 FTIR

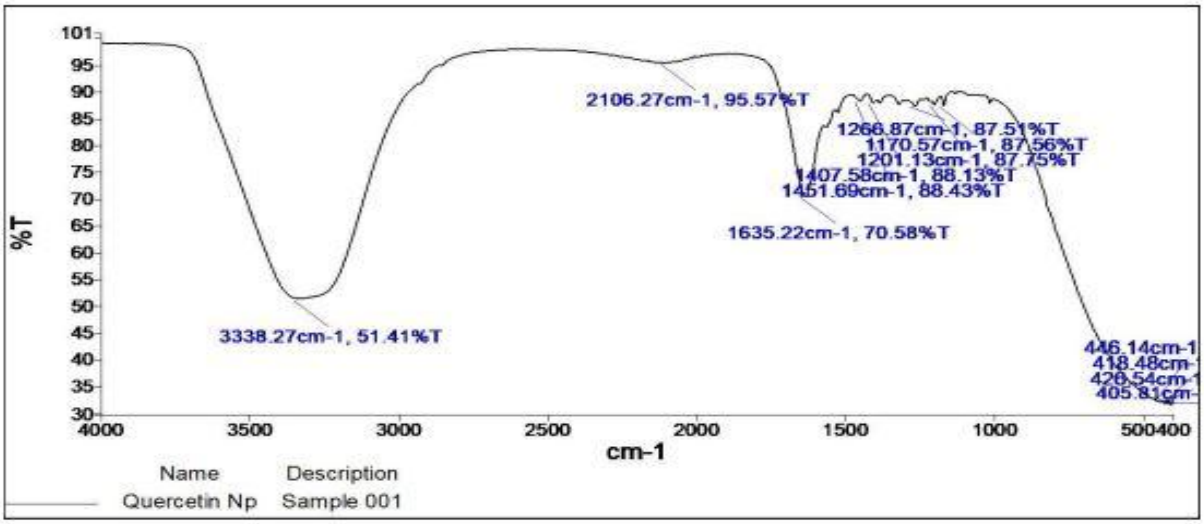



**Figure 19: FTIR interpretation of Quercetin dihydrate loaded Silver Nanoparticle**

**Table 31: FTIR spectrum of Quercetin dihydrate loaded Silver Nanoparticle**

| **Characteristics Peaks** | **Reported ($cm^{-1}$)** | **Observed($cm^{-1}$)** |
|---|---|---|
| O-H stretch | 3283 | 3338.22 |
| C= C stretch | 1610 | 1635.22 |

**Discussion:** The FTIR spectra of Quercetin dihydrate loaded Silver Nanoparticle of *Azadirachta indica* were shown in the table 23. The IR absorption peaks show at 3338.22 cm−1 of O-H stretch and 1635.22 cm-1 of C= C stretch. In Figure 19, the variation of peaks demonstrated the interaction of silver ions and the other functional groups on the edge of Quercetin dihydrate through the formation of coordination bonds.

### 3.8.7 Zeta Potential

Zeta Potential was carried out for the stability of silver nanoparticles as shown in Figure 20.

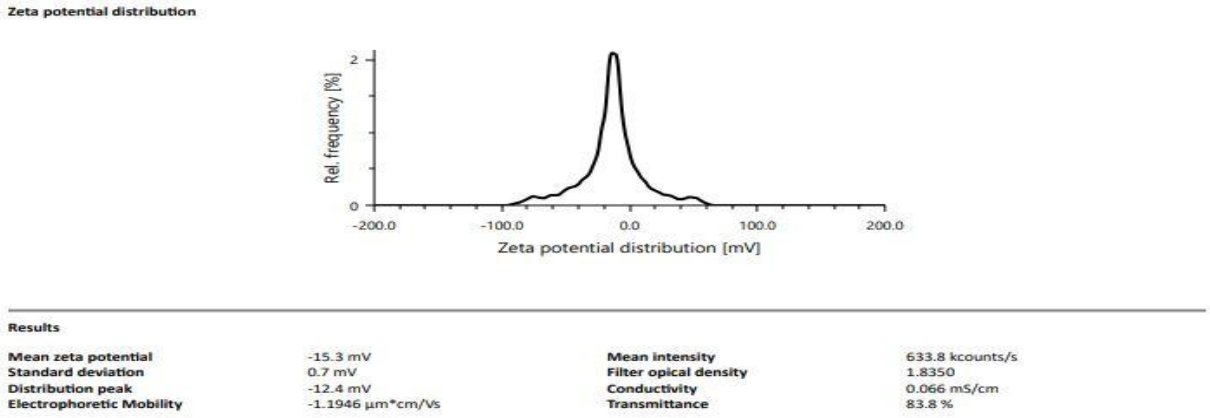



**Figure 20: Zetapotential Quercetin dihydrate loaded Silver Nanoparticle**

**Result:** Zeta potential of formulation F2A5 was -15.3 mV represents the stability of the formulation of Quercetin dihydrate-loaded silver nanoparticles.

### 3.9 Incorporation of Quercetin dihydrate loaded silver nanoparticle into gel

The gel of optimized F2A5B6 formulation was prepared by dispersing the formulation successfully in carbopol 934 different concentrations and then subjected for characterization.

#### 3.9.1 Physical Appearance

Gel visual appearance of formation of Quercetin-dihydrate loaded silver nanoparticles is shown in figure 21.

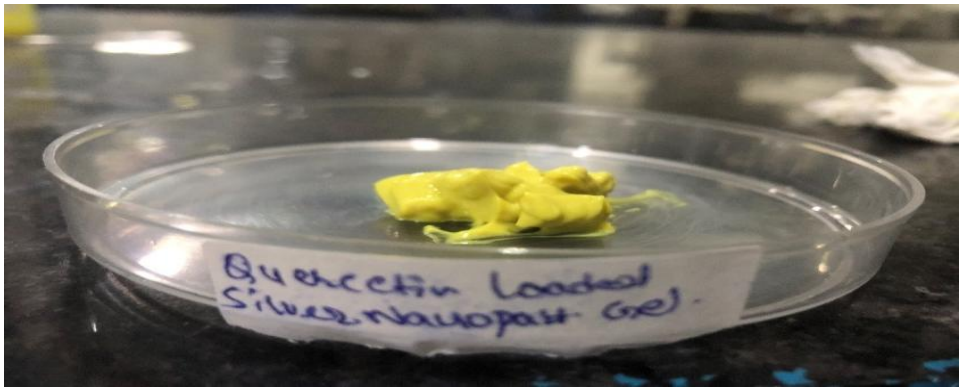



**Figure 21: Visual Appearance of Quercetin dihydrate loaded silver nanoparticle to gel**

**Table 32: Visual Appearance of Quercetin-dihydrate loaded silver nanoparticle to gel**

| Sr. no. | Formulation code | Visual Appearance |
|---|---|---|
| 1 | F2A5B6 | Homogenous, uniform, yellow color gel |
| 2 | F2A5B7 | Homogenous, uniform, yellow color gel |

**Result:** The prepared gel was examined visually for their consistency and found to smooth appearance. Formulation batches F2A5B6 and F2A5B7 were showed good homogeneity with absence of lumps. So those batches were used in further study.

**3.9.2 pH determination**

pH of Quercetin dihydrate loaded silver nanoparticle gel was used to measure the pH of the formulations of F2A5B6 and F2A5B7.

**Table 33: pH study of gel**

| Sr. no. | Formulation code | pH (Mean ± S.D) |
|---|---|---|
| 1 | F2A5B6 | 6.46±0.02 |
| 2 | F2A5B7 | 6.41±0.01 |

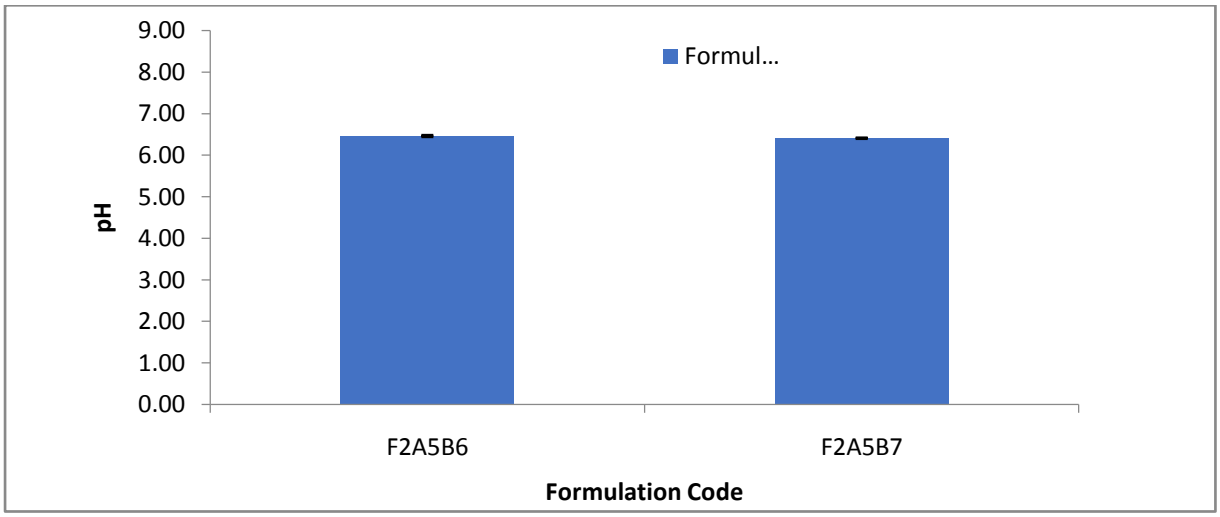



**Figure 22: pH of gel**

**Result:** From the Table 25& fig. 15, it was found that pH of the formulations was found to be in a range 6.46±0.02 to 6.41±0.01.

**3.9.3 Viscosity**

Viscosity of formulated gels(F2A5B6-F2A5B7) was carried out and was rotated at 10 rpm at room temperature in viscometer as shown in figure 23 and table 34.

**Table 34: Viscosity Study of formulations (F2A5B6-F2A5B7)**

| Sr. no. | Formulation code | Viscosity (cps) (Mean ± S.D) |
|---|---|---|
| 1 | F2A5B6 | 41063.00±2.52 |
| 2 | F2A5B7 | 43051.67±1.58 |

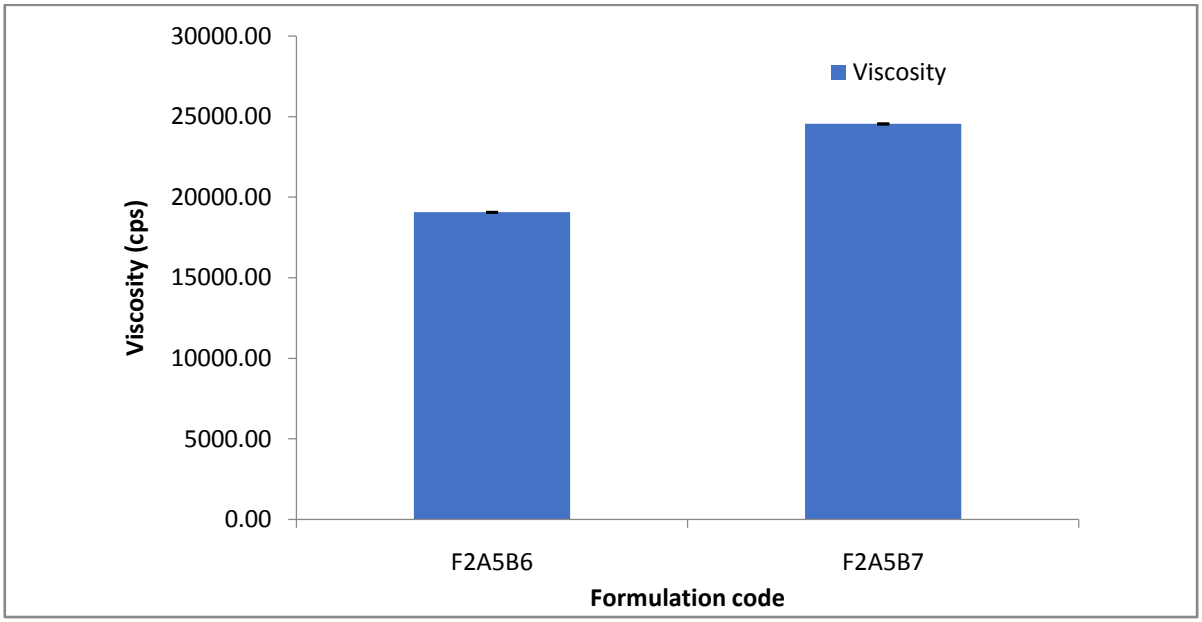



**Figure 23: Viscosity Study of formulations (F2A5B6-F2A5B7)**

**Result:** The viscosity of the formulations was in range of 41063.00±2.52and 43051.67±1.58(**Table 27 and Figure 23)**.

### 3.9.4 Spreadability Study

Spreadability of the formulationF2A5B6 andF2A5B7were carried out in order to signify the gel expension used for formulation development as shown in (**Table 35, Figure 24**)

**Table 35: Spreadability Study of formulations (F2A5B6-F2A5B7)**

| Sr. no. | Formulation code | Spreadability (g.cm/sec) (mean ± SD) |
|---|---|---|
| 1 | F2A5B6 | 5.356±0.05 |
| 2 | F2A5B7 | 4.767±0.03 |

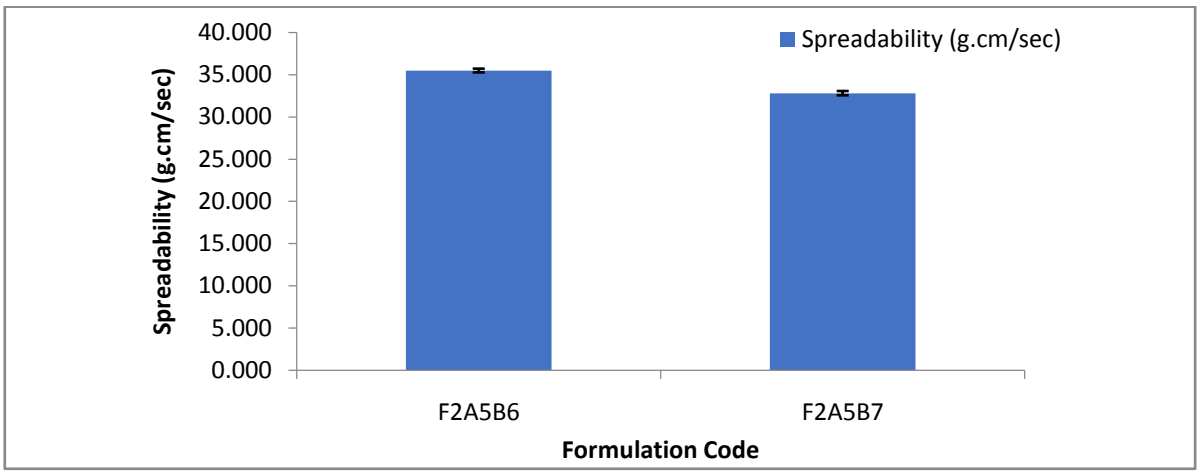



**Figure 24: Spreadability Study of formulations (F2A5B6-F2A5B7)**

**Result:** The Spreadability of the formulations was in range of 5.356±0.05and 4.767±0.03(**Table 35 and Figure 24)**.

### 3.9.5 In Vitro Drug Release Studies

In Vitro, Drug Release Studies were carried out in order to analyze the efficacy and controlled drug release of pure drug and the formulation (F2A5B6-F2A5B7) as shown in **(Table 37, Figure 25)**

**Table 37: In Vitro drug release of Quercetin dihydrate-loaded silver nanoparticle**

| Sr.No. | Time (Hr) | Drug Release of Pure Drug | Drug Release of Formulation F2A5B6 | Drug Release of Formulation F2A5B7 |
|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 0 |
| 2 | 0.5 | 18.605±0.142 | 4.473±0.027 | 3.985±0.072 |
| 3 | 1 | 39.984±0.086 | 9.949±0.068 | 9.166±0.714 |
| 4 | 2 | 59.769±0.511 | 20.648±0.013 | 16.518±2.811 |
| 5 | 4 | 66.062±0.254 | 35.376±0.511 | 31.454±3.038 |
| 6 | 6 | 85.291±0.975 | 53.294±2.668 | 46.438±1.565 |
| 7 | 8 | 95.273±0.251 | 74.575±0.076 | 70.007±1.032 |
| 8 | 12 | | 88.868±2.985 | 85.326±2.260 |
| 9 | 24 | | 97.745±0.543 | 91.607±0.786 |

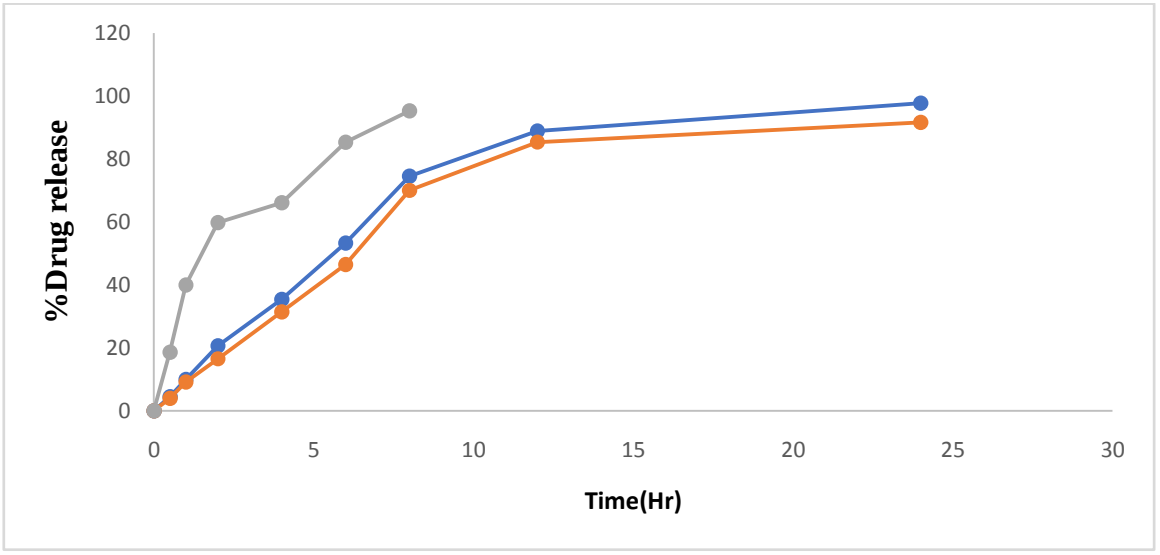



**Figure 25: Percentage drug release of Quercetin dihydrate loaded Silver nanoparticles gel**

### 3.9.5.1 Drug release kinetic study

#### 3.9.5.1.1 Zero order kinetics

Zero-order kinetics of formulation F2A5B6 were plotted out in order to analyze constant drug release as shown in **Figure 26**

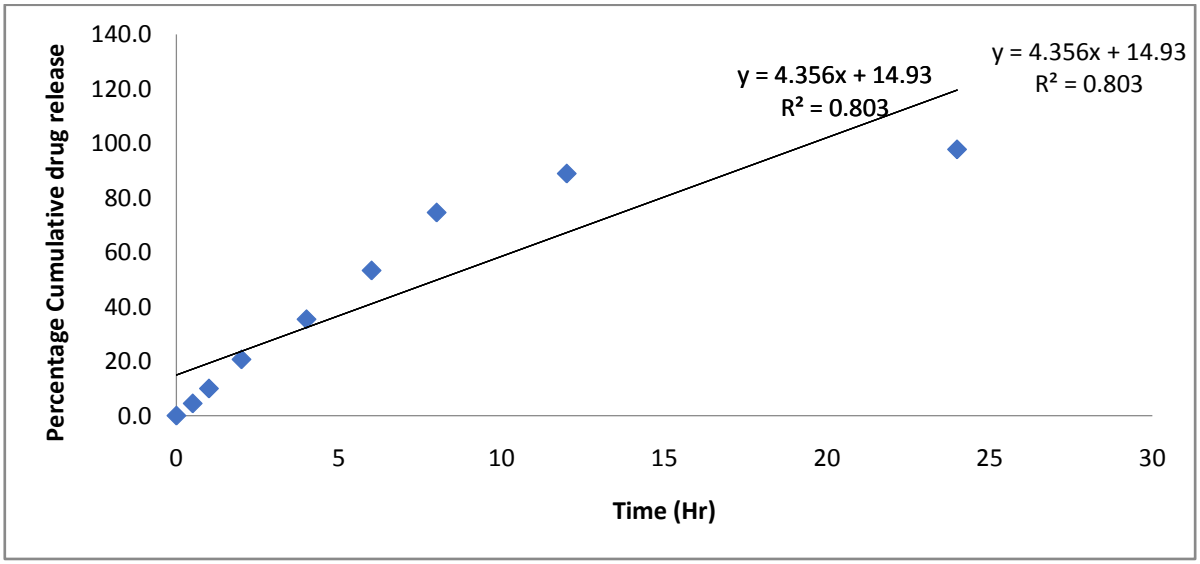



**Figure 26: Zero-order graph of formulation F2A5B6**

#### 3.9.5.1.2 First-order kinetics

First-order kinetics formulation F2A5B6 was plotted out in order to analyze the release of the system as shown in **Figure 27**

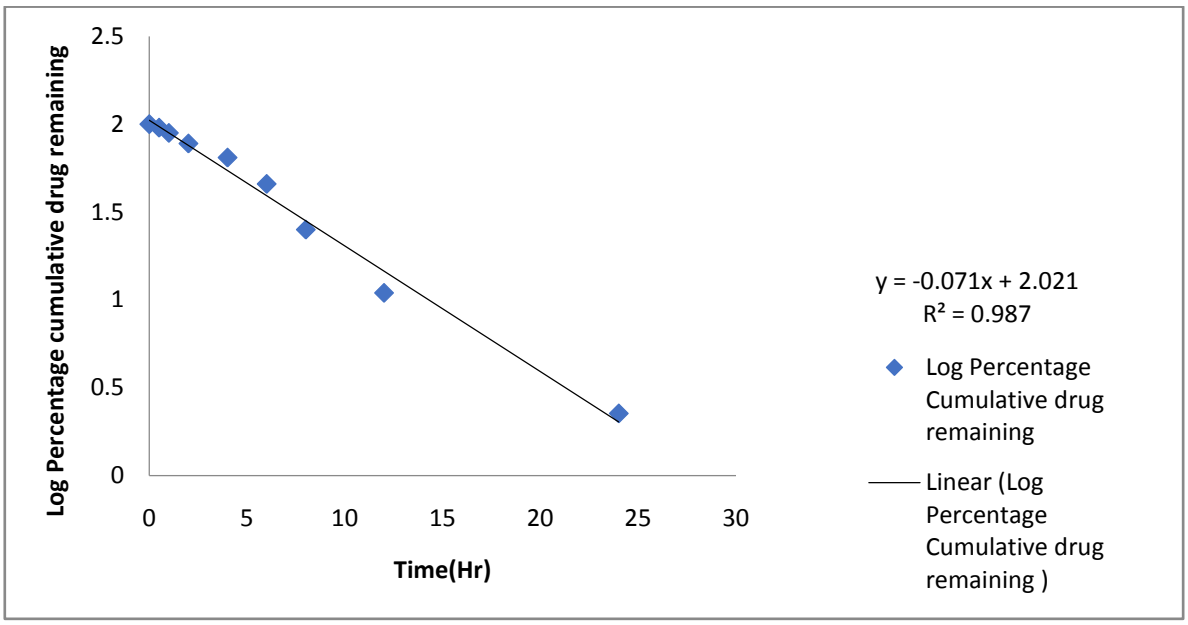



**Figure 27: First order graph of formulationF2A5B6**

### 3.9.5.1.3 Higuchi's model

Higuchi's model of formulation F2A5B6 was plotted out which describes the drug release from the matrix as shown in **Figure 28**

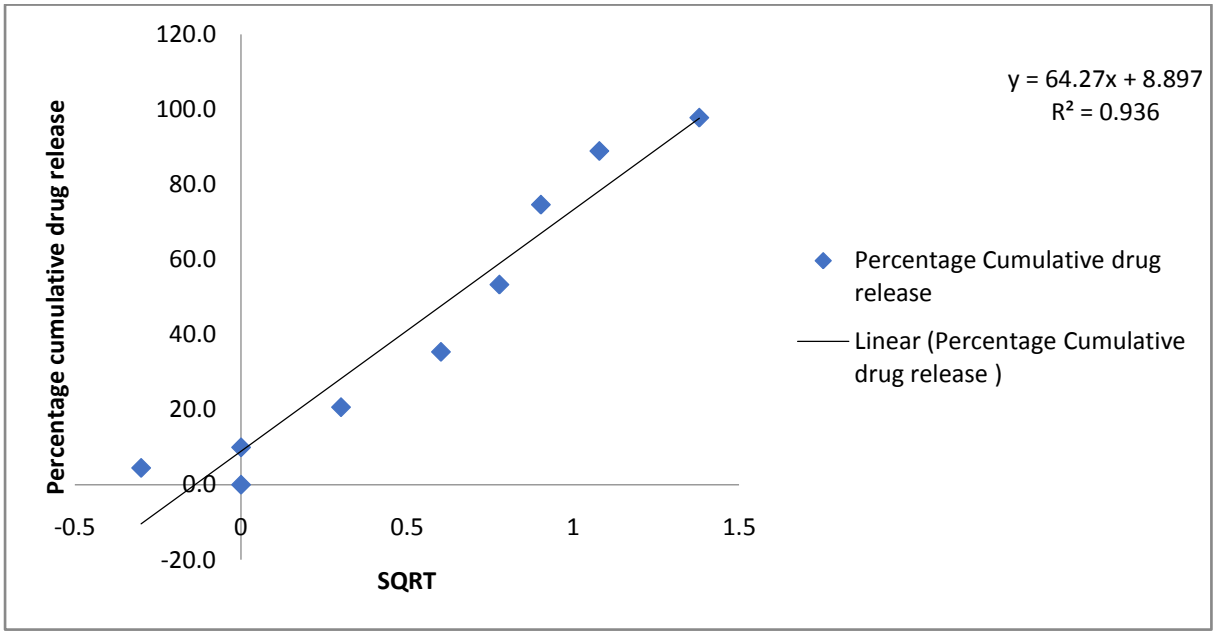



**Figure 28: Higuchi order graph of formulation F2A5B6**

### 3.9.5.1.4 Korsmeyer-Peppas Model

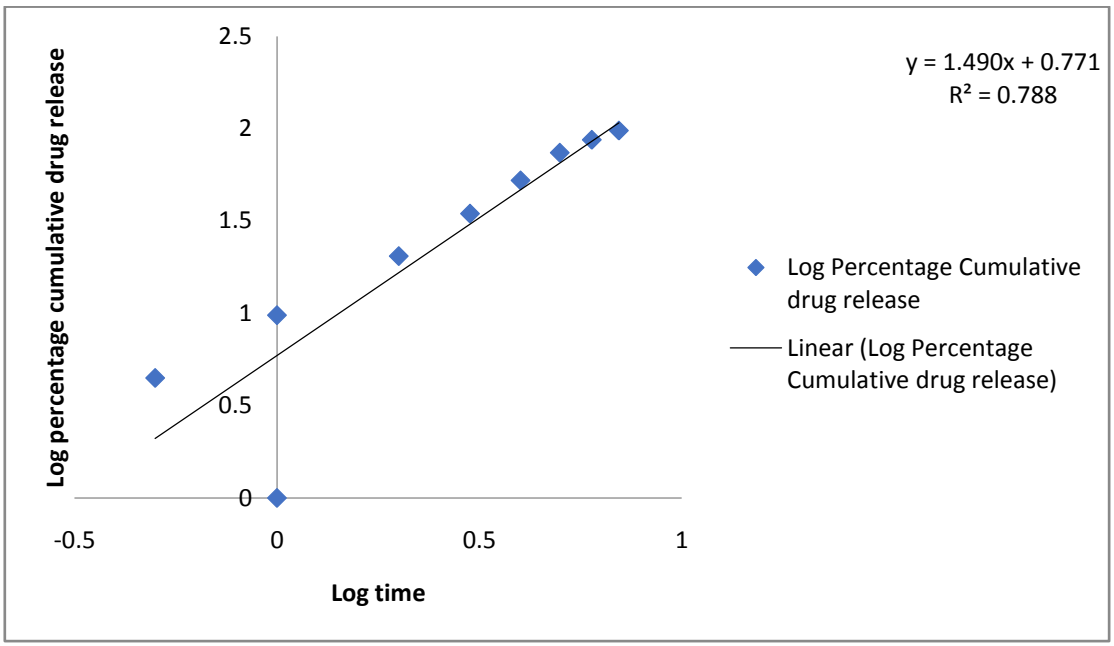



**Figure 29: Korsmeyer-Peppas Model graph for F2A5B6 formulation**

**Table 38: Kinetic equation parameter of F2A5B6 Formulation**

| Formulation Code | Zero-order | | First-order | | Higuchi | | K. Peppas | |
|---|---|---|---|---|---|---|---|---|
| | $K_0$ | $R^2$ | $K_0$ | $R^2$ | $K_0$ | $R^2$ | $K_0$ | $R^2$ |
| **F6** | 4.105 | 0.803 | -0.072 | 0.987 | 25.077 | 0.936 | 0.843 | 0.788 |

Mathematical models are commonly used to predict the release mechanism and compare the release profile. For the optimized formulation, the % drug release vs time (zero order), log percent drug remaining vs time (first order), log percent drug release vs square root of time (Higuchi plot), and log of log % drug release vs. log time (Korsmeyer and Peppas Exponential Equation) were plotted. In each case, the $R^2$ value was calculated from the graph and reported in **Table 38 and Figure 26 to Figure 29.** Considering the determination coefficients, the K Peppas plot model was found ($R^2$=0.788) to fit the release data best. It could be concluded from the results that the drug was released from nanoparticles by a controlled mechanism.

## 4.0 Summary and Conclusion

Quercetin have garnered a lot of interest in showing its antifungal properties. A common flavonoid found in many different kinds of food and plants, quercetin is a flavonoid. The anti-

fungal, anti-inflammatory, cardiovascular, and anticancer properties of quercetin are said to have a variety of positive benefits on human health. It has anti- fungal, anti-cancer, anti-tumor, anti-ulcer, anti-allergy, anti-viral, anti-inflammation, and anti-diabetic properties. Its positive benefits also include gastro-protection, anti-hypertension, immune-modulation, and anti-infection properties. According to research, quercetin inhibits the growth of fungi in a number of fungal infections. Mostly quercetin is found suitable for the treatment of vulvovaginal candidiasis (VVC) and jock itch (caused by *Trychophyton rubrum*).

Physicochemical Parameters and pre-formulation experiments were conducted to characterise the chemical and physical characteristics of the plant part *Azadirachta indica* leavesand drug substance prior to the development of quercetin loaded silver nanoparticles in which *Azadirachta indica* leaves is extremely bitter, unpleasant in odor and green powder in colour. Total ash of *Azadirachta indica* leaf powder was found to have 9.02% ±1.26 and Acid Insoluble ash value of *Azadirachta indica* leaf powder was found to 1.50%±0.29.3. HPLC method of quercetin dihydrate was determined in whichcalibration curve for each µg/ml concentration had regression equation Y=81992x – 28533and $R^2$ value 0.999 which shows good linearity. Solubility of quercetin dihydrate in various solvents were carried out in order to screen for the components to be used for formulation development in which ethanol and methanol was very slightly soluble in water and practically insoluble in phosphate buffer pH 6.8. FT-IR spectra were discovered to be consistent with the reference chemical groups identified in the structure of quercetin dihydrate. The partition coefficient of quercetin in n-Octanol: Water was found to be 1.70± 0.0629 which indicates that the quercetin dihydrate is lipophilic in nature. To prepare silver nanoparticle in various concentrations (1mM, 3mM,4Mm,6mM and 9mM), the best mixture F2 was chosen and the maximum spectrum band observed of F2 was at 452nm. F2 Formulation of silver nanoparticle was tested for particle size and polydispersity index in which hydrodynamic diameter of synthesized nano particle was found to be 107.96nm and the polydispersity index was 17.2%. The percentage yield of all the formulations of quercetin-loaded silver nanoparticlesin a range of 86.12±1.155 to 95.64±0.539. The percentage of Drug Entrapment in all formulationsranges from 76.70±0.13 to 89.18±0.19. The Formulation F2A5 has maximum Drug Entrapment and the hydrodynamic diameter of quercetin-loaded silver nanoparticle was found to be 195.59 nm and the polydispersity index was 33.3%. Zeta Potential was carried out for the stability of silver nanoparticles in which formulation F2A5 was -15.3 mV represents the stability of formulation of quercetin-loaded silver nanoparticles.

To prepare quercetin-loaded silver nanoparticle gel with Carbapol 934P in various concentrations (1% and 2%), the best mixture (F2A5B6) was chosen. The prepared gels' consistency was visibly assessed, and it was discovered that they had a smooth appearance. Batches F2A5B6 and F2A5B7 of the formulation displayed acceptable homogeneity and no lumps. The produced, optimized gels' pH was discovered to be between 6.46 and 6.41. All of the formulations had viscosities between 41063.00±2.52and 43051.67±1.58 cps. All of the formulations had Spreadability between 5.356±0.05and 4.767±0.03. Franz diffusion cell investigations of gel formulation drug release. Comparing formulation F2A5B6to control gel and other gel formulations, it was significantly improved, reaching a high of 97.745% in 24 hours. The in vitro data was fitted to the zero order, first order, Higuchi, and Korsmeyer-Peppas models in order to determine the rate and process of drug release with precision. The findings demonstrated that the F2A5B6 formulation's drug release adhered to the Korsmeyer-Peppas order, which explains the sustained mechanism used by quercetin loaded silver nanoparticle gel. In conclusion, the procedure for making quercetin loaded silver nanoparticle gel was discovered to be straightforward and repeatable.

**Conclusion:** In conclusion, the procedure for making quercetin-loaded silver nanoparticle gel was discovered to be straightforward and repeatable.The findings demonstrated that the F2A5B6 formulation's drug release adhered to the Korsmeyer-Peppas order, which explains the sustained mechanism used by quercetin-loaded silver nanoparticle gel.

**References**

1. Sawant, S. E., &Tajane, M. D.(2016). Formulation and evaluation of herbal ointment containing Neem and Turmeric extract. *Journal of Scientific and Innovative Research*, *5*(4), 149–151. https://doi.org/10.31254/jsir.2016.5410
2. Atawodi, S. E. & Atawodi, J.C. (2009). Azadirachta indica (neem): A plant of multiple biological and pharmacological activities. *Phytochemistry Reviews*, *8*(3), 601–620. https://doi.org/10.1007/s11101-009-9144-6
3. Oli, B., &Gautam, D.(2022). Medicinal value of Azadirachta indica: A review. *Modern Phytomorphology*, *15*(6).
4. Nishan, M., &Subramanian, P.(2014). Pharmacological and non-pharmacological activity of Azadirachta indica (Neem)-A review. *International Journal of Biosciences*, *5*(6), 104–112. https://doi.org/10.12692/ijb/5.6.104-112

5. Shukla, V., Khurshid, M. D., & Kumar, B. (2020). A review on phytochemistry and pharmacological activity of Azadirachta indica (Neem). International Journal of Pharmacy and Biological Sciences, 10, 172–180.
6. Avinash, B., Venu, R., Prasad, T. N. V. K. V., Alpha Raj, M., Srinivasa Rao, K., &Srilatha, C.(2017). Synthesis and characterisation of neem leaf extract, 2, 3- dehydrosalanol and quercetin dihydrate mediated silver Nano particles for therapeutic applications. *IET Nanobiotechnology*, *11*(4), 383–389. https://doi.org/10.1049/iet-nbt.2016.0095
7. Deshmukh, S. G., Thakre, T., Gupta, J., &Waskar, R.(2021). A case study on management of tineacruris with classicalVamanKarma. *Journal of Pharmaceutical Research International*, *33*(33B), 195–202. https://doi.org/10.9734/jpri/2021/v33i33B31811
8. Alzohairy, M. A.(2016). Therapeutics role of Azadirachta indica (Neem) and their active constituents in diseases prevention and treatment. *Evidence-Based Complementary and Alternative Medicine: eCAM*, *2016*, 7382506. https://doi.org/10.1155/2016/7382506
9. Singh, V., Roy, M., Garg, N., Kumar, A., Arora, S., & Malik, D. S. (2021). An insight into the dermatological applications of neem: A review on traditional and modern aspect. *Recent Advances in Anti-Infective Drug Discovery*, *16*(2), 94–121. https://doi.org/10.2174/2772434416666210604105251
10. Ratner, M. A., &Ratner, D.(2003). Nanotechnology: A gentle introduction to the next big idea. *Prentice hallprofessional*.
11. Kango, S., Kalia, S., Celli, A., Njuguna, J., Habibi, Y., &Kumar, R.(2013). Surface modification of inorganic nanoparticles for development of organic–inorganic nanocomposites—A review. *Progress in Polymer Science*, *38*(8), 1232–1261. https://doi.org/10.1016/j.progpolymsci.2013.02.003
12. Yan, W., Herzing, A. A., Kiely, C. J., &Zhang, W. X.(2010). Nanoscale zero-valent iron (nZVI): Aspects of the core-shell structure and reactions with inorganic species in water. *Journal of Contaminant Hydrology*, *118*(3–4), 96–104. https://doi.org/10.1016/j.jconhyd.2010.09.003
13. Singh, A., Madhavi, B. L. R., & Nithin Sagar, M. N. (2021). An overview of green synthesis mediated metal nanoparticles preparation and its scale up opportunities. *Journal of Drug Delivery and Therapeutics*, *11*(6), 304–314. https://doi.org/10.22270/jddt.v11i6.5082

14. V, A., Cutinho, L. I., Mourya, P., Maxwell, A., Thomas, G., &Rajput, B. S.(2020). Approaches for encephalic drug delivery using nanomaterials: The current status. *Brain Research Bulletin*, *155*, 184–190. https://doi.org/10.1016/j.brainresbull.2019.11.017
15. Parashar, U. K., Saxena, P. S., &Srivastava, A.(2009). Bioinspired synthesis of silver nanoparticles. *Digest Journal of Nanomaterials and Biostructures (DJNB)*, *4*(1).
16. Fu, J., Ji, J., Fan, D., &Shen, J.(2006). Construction of antibacterial multilayer films containing nanosilvervialayer- by- layer assembly of heparinandchitosan- silver ions complex, *The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials*. *Journal of Biomedical Materials Research. Part A*, *79*(3), 665–674. https://doi.org/10.1002/jbm.a.30819
17. Yin, I. X., Zhang, J., Zhao, I. S., Mei, M. L., Li, Q., &Chu, C. H.(2020). The antibacterial mechanism of silver nanoparticles and its application in dentistry. *International Journal of Nanomedicine*, 15, 2555–2562. https://doi.org/10.2147/IJN.S246764
18. Feldberg, R. S., Chang, S. C., Kotik, A. N., Nadler, M., Neuwirth, Z., Sundstrom, D. C., &Thompson, N. H.(1988). In vitro mechanism of inhibition of bacterial cell growth by allicin. *Antimicrobial Agents and Chemotherapy*, *32*(12), 1763–1768. https://doi.org/10.1128/AAC.32.12.1763
19. Fröhlich, E.(2013). Cellular targets and mechanisms in the cytotoxic action of non-biodegradable engineered nanoparticles. *Current DrugMetabolism*, *14*(9), 976–988. https://doi.org/10.2174/1389200211314090004
20. Yasuda, K., Kiyama, M., Umemura, S. I., &Takeda, K.(1996). Deoxyribonucleic acid concentration using acoustic radiation force. *Journal of the Acoustical Society of America*, *99*(2), 1248–1251. https://doi.org/10.1121/1.414635
21. Ravindran, Aswathy, Chandran, P., &Sudheer Khan, S.(2013). Biofunctionalized silver nanoparticles: Advances and prospects. *Colloids and Surfaces, Part B: Biointerfaces*, *105*, 342–352.
22. Karimzadeh, Z., Mahmoudpour, M., Rahimpour, E., &Jouyban, A.(2022). Nanomaterial based PVA nanocomposite hydrogels for biomedical sensing: Advances toward designing the ideal flexible/wearable Nanoprobes. *Advances in Colloid and Interface Science*, 305, 102705. https://doi.org/10.1016/j.cis.2022.102705

23. Mauri, E., Perale, G., &Rossi, F.(2018). Nanogel functionalization: A versatile approach to meet the challenges of drug and gene delivery. *ACS Applied Nano Materials*, *1*(12), 6525–6541. https://doi.org/10.1021/acsanm.8b01686
24. Keskin, D., Zu, G., Forson, A. M., Tromp, L., Sjollema, J., & van Rijn, P. (2021). Nanogels: A novel approach in antimicrobial delivery systems and antimicrobial coatings. *Bioactive Materials*, *6*(10), 3634–3657. https://doi.org/10.1016/j.bioactmat.2021.03.004
25. Bhinge, S. D., Bhutkar, M. A., Randive, D. S., Wadkar, G. H., Kamble, S. Y., Kalel, P. D., &Kadam, S. S.(2019). Formulation and evaluation of polyherbal gel containing extracts of Azadirachta indica, Adhatoda vasica, Piper betle, Ocimum tenuiflorum and Pongamia pinnata. *Journal of Research in Pharmacy*, *23*(1).
26. Ogbonna, O. J., &Umedum, C. U.(2022). Antifungalactivity of Curcuma longa and Azadirachta indicaextracts on dematiaceousfungiisolated from environmental samples at Ulicommunity, Anambra State Nigeria. *Journal of Advances in Microbiology*, *22*(12), 83–90. https://doi.org/10.9734/jamb/2022/v22i12696
27. Tippayawat, P., Phromviyo, N., Boueroy, P., &Chompoosor, A.(2016). Green synthesis of silver nanoparticles in aloe vera plant extract prepared by a hydrothermal method and their synergistic antibacterial activity. PeerJ, 4, e2589. https://doi.org/10.7717/peerj.2589
28. Kellie, A. E.(1966). The extraction and hydrolysis of steroid monoglucuronides. *Biochemical Journal*, *100*(3), 631–635. https://doi.org/10.1042/bj1000631
29. Pekić, B., Kovač, V., Alonso, E., &Revilla, E.(1998). Study of the extraction of proanthocyanidins from grape seeds. *Food Chemistry*, *61*(1–2), 201–206. https://doi.org/10.1016/S0308-8146(97)00128-3
30. Asghar, M. T., Yusof, Y. A., Mokhtar, M. N., Yaacob, M. E., Ghazali, H. M., Varith, J., Chang, L. S., &Manaf, Y. N.(2020). Processing of coconut sap into sugar syrup using rotary evaporation, microwave, andopen- heat evaporation techniques. *Journal of the Science of Food and Agriculture*, *100*(10), 4012–4019. https://doi.org/10.1002/jsfa.10446
31. Dai, J., Yaylayan, V. A., Raghavan, G. S., &Parè, J. R.(1999). Extraction and colorimetric determination of azadirachtin-related limonoids in neem seed kernel. *Journal of Agricultural and FoodChemistry*, *47*(9), 3738–3742. https://doi.org/10.1021/jf990227h

32. Susmitha, S., Vidyamol, K. K., Ranganayaki, P., &Vijayaragavan, R.(2013). Phytochemical extraction and antimicrobial properties of Azadirachta indica (Neem). *Global Journal of Pharmacology*, *7*(3), 316–320.
33. Paul, S., Mukherjee, S., &Banerjee, P.(2023). Medicinal bioactivity: Anticancerous and anti-HIV activity of medicinal plants. *Phytochemicals in Medicinal Plants*, *153*.
34. Ding, F., Wang, D., Song, S., &Xu, S.(2011). Preliminary investigation of sintered plates bonded or end-capped with phenyl for planar electrochromatography. *JPC – Journal of Planar Chromatography – Modern TLC*, *24*, 10–15.
35. Hagel, L.(1998). Gel-filtration chromatography. *Current Protocols in MolecularBiology*, *44*(1), 10–19.
36. Caboni, P., Sarais, G., Angioni, A., Garcia, A. J., Lai, F., Dedola, F., &Cabras, P.(2006). Residues and persistence of neem formulations on strawberry after field treatment. *Journal of Agricultural and Food Chemistry*, *54*(26), 10026–10032. https://doi.org/10.1021/jf062461v
37. Neves Costa, F. D., &Leitão, G. G.(2010). Strategies of solvent system selection for the isolation of flavonoids by countercurrent chromatography. *Journal of Separation Science*, *33*(3), 336–347.
38. Schäfer, S., Salcher, S., Seiter, M., Ranninger, C., Möst, M., Obexer, P., Huber, C. G., Ausserlechner, M. J., Schwaiger, S., &Stuppner, H.(2016). Characterization of the XIAP-inhibiting proanthocyanidin fraction of the aerial parts of Ephedra sinica. *Planta Medica*, *82*(11–12), 973–985. https://doi.org/10.1055/s-0042-107253
39. Aral, H., Aral, T., Ziyadanoğulları, B., &Ziyadanoğulları, R.(2013). Development of a novel amide-silica stationary phase for the reversed-phase HPLC separation of different classes of phytohormones. *Talanta*, *116*, 155–163.
40. Joshi, B. C., Prakash, A., &Kalia, A. N.(2015). Hepatoprotective potential of antioxidant potent fraction from Urtica dioicaLinn. (whole plant) in CCl4 challenged rats. *Toxicology Reports*, *2*, 1101–1110. https://doi.org/10.1016/j.toxrep.2015.07.020
41. Zelles, L., &Bai, Q. Y.(1993). Fractionation of fatty acids derived from soil lipids by solid phase extraction and their quantitative analysis by GC-MS. *Soil Biology and Biochemistry*, *25*(4), 495–507.
42. Velavan, S.(2015). Phytochemical techniques-a review. *World Journal of Science and Research*, *1*(2), 80–91.
43. Rajput, N., Karole, S., Chakraborty, A. K., &Loksh, K. R.(2022). Quantitative analysis of quercetin in various extracts of Curcuma amada by high-performance

liquid chromatography. *World Journal of Biology Pharmacy and Health Sciences*, *12*(2), 205–214.

44. Roman, M. C., Betz, J. M., &Hildreth, J.(2007). Determination of synephrine in bitter orange raw materials, extracts, and dietary supplements by liquid chromatography with ultraviolet detection: Single-laboratory validation. *Journal of AOAC International*, *90*(1), 68–81.
45. Mayee, R., Rawat, S., Thosar, A., Atre, K., &Mane, P.(2011). Development and validation of HPLCmethod for determination of diclofenac sodium by tapestrippingmethod. *Asian Journal of Pharmaceutical & Biological Research (AJPBR)*, *1*(3).
46. Aldosary, S. K., &El-Rahman, S. N.(2019). Green synthesis and antibacterial properties of silver nanoparticles of Lawsonia inermis, rhamnus Frangula, Camellia sinensis and thymus vulgaris extracts. *Journal of Pure and AppliedMicrobiology*, *13*(2), 1279–1284. https://doi.org/10.22207/JPAM.13.2.71
47. Hossain, M. D., Sarwar, M. S., Dewan, S. M. R., Hossain, M. S., Shahid-Ud-Daula, A., &Islam, M. S.(2014)Investigation of total phenolic content and antioxidant activities of Azadirachta indica roots. *Avicenna Journal of Phytomedicine*, *4*(2), 97–102.
48. Devi, N., &Maji, T. K.(2009). A novel microencapsulation of neem (Azadirachta indicaA. Juss.) seed oil (NSO) in polyelectrolyte complexofκ- carrageenan and chitosan. *Journal of Applied Polymer Science*, *113*(3), 1576–1583. https://doi.org/10.1002/app.30038
49. Bose, P., Priyam, A., Kar, R., &Pattanayak, S. P.(2020). Quercetin loaded folate targeted plasmonic silver nanoparticles for light activated chemo-photothermal therapy of DMBA induced breast cancer in Sprague Dawley rats. RSC Advances, 10(53), 31961–31978. https://doi.org/10.1039/d0ra05793b
50. Abdelmalak, N. S., &El-Menshawe, S. F. (2012). A new topical fluconazole microsponge loaded hydrogel: Preparation and characterization. InternationalJournal of Pharmacy and Pharmaceutical Sciences, 4, 460–469.
51. Padhi, S., &Behera, A.(2022). Biosynthesis of Silver Nanoparticles: Synthesis, mechanism, and characterization. In *Agric-waste and microbes for production of sustainablenanomaterials* (pp. 397–440). Elsevier.

52. Alqarni, M. H., Foudah, A. I., Alam, A., Salkini, M. A., Muharram, M. M., Labrou, N. E., &Kumar, P.(2022). Development of gum-acacia-stabilizedsilvernanoparticlesgel of Rutin against Candida albicans. *Gels*, *8*(8), 472. https://doi.org/10.3390/gels8080472

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## Authors' Contribution

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