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DESIGN AND CHARACTERIZATION OF TOPICAL ANTIBACTERIAL FORMULATION CONTAINING EXTRACT OF *QUERCUS INFECTORIA* GALLS

Akshada Rajesh Shendge * and Krishnamurthy Apparao Kamalapurkar

Department of Pharmaceutics, D. S. T. S. Mandal's College of Pharmacy, Solapur - 413004, Maharashtra, India.

Keywords:

Quercus infectoria gall, Antibacterial activity, Silver nanoparticles, Thermosensitive mucoadhesive gel, Mouth ulcer

Correspondence to Author:
Akshada Rajesh Shendge

Research Scholar,
Department of Pharmaceutics,
D. S. T. S. Mandal's College of Pharmacy, Solapur - 413004,
Maharashtra, India.

E-mail: arshendge25@gmail.com

ABSTRACT: Presently, the surge in the consumption of herbal formulations is encouraged by several factors; the main factor is that they are relatively safe and effective. Plant extracts are considered an alternative source of synthetic drugs and are expected to produce promising results for treating several diseases. The present study aimed to formulate and evaluate antibacterial thermosensitive mucoadhesive gel containing *Quercus infectoria* gall extract and synthesize and characterize the silver nanoparticles by using *Quercus infectoria* gall extract. For extraction, water and ethanol were used as a solvent. The major constituents of the extract are tannic acid, gallic acid, ellagic acid, and these three constituted showed synergistic antibacterial activity. Formulated gel delivered the drug for more than 4 hr. The batch F5 optimized with good viscosity, spreadability and extrudability, mucoadhesive strength, release of drug is more, and the gel is stable; hence it is used for further evaluation like *in-vitro* skin irritation study and *in-vitro* antibacterial test. The formulated gel was not irritant and showed antibacterial activity greater than the marketed gel formulation containing tannic acid as an active pharmaceutical ingredient to treat mouth ulcers and gum disorders. The successful synthesis of silver nanoparticles from the extract of *Quercus infectoria* gall act as a reducing and capping agent. AgNPs exhibit significant antibacterial activity.

INTRODUCTION: Presently, the surge of consumption of herbal formulations is encouraged because they are relatively safe and effective. Plant extracts are considered an alternative source for synthetic drugs and are expected to produce promising results for treating several diseases. Herbal medicines are always considered as primary and emergency treatment from ancient times. For example, when finger cuts during chopping vegetables, turmeric powder is applied, which acts as antibacterial, anti-inflammatory and has shown lots of medicinal uses.

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Traditionally, powders, juices or extracts of herbal plant part are used directly for treatment. Nowadays, a stable, effective herbal formulation is a growing need of society. Herbal medicinal plants were used for the treatment of many diseases. The chemical constituents present in medicinal plants have various pharmacological activities. Such compounds are separated and isolated using various techniques.

For extraction of a chemical constituent from the herbal plant, we have to select a solvent depending upon the class of chemical constituent to be separated from the extract following the isolation of a particular chemical constituent using chromatographic or recrystallization techniques and further characterize it using quantitative and qualitative techniques. Natural products represent an important source of new lead compounds in drug discovery. Several drugs currently used as

therapeutic agents are developed from natural sources; plant sources are extensively preferred. In the past few decades, pharmaceutical companies demonstrated significant attention towards natural product drug discovery, mainly due to its intrinsic complexity [1]. The goal of any drug delivery system is to provide a therapeutic amount of drug to the desired site in the body promptly to achieve and maintain the desired drug concentration. The route of administration has a significant impact on the therapeutic outcome of a drug. Skin is one of the most readily accessible organs in the human body for topical administration and is the main route for the topical drug delivery system. External topical is spread, sprayed, or otherwise dispersed to cutaneous tissues to cover the affected area. Internal topicals that are applied to the mucous membrane orally, vaginally or on anorectal tissues for local activity [2].

Because it can be easily sampled and is significantly linked to serious oral infectious disorders such mouth ulcers, tooth decay (dental caries), and gum disease, the human oral microbiome is the most researched component of the human microflora. In the human oral cavity, 600 common bacterial species have been found [3].

The human oral cavity is a miniature ecosystem of the tongue's dorsal and ventral sides, the buccal epithelium, the hard and soft palates, and supra-gingival plaque on the tooth surfaces. These areas are home to various microorganisms, including fungi, viruses, and bacterial fungi. The Human Mouth Microbiome Database contains information on 1100 distinct taxa found in the oral cavity [4].

Mouth ulcers represent a very common unpleasant oral mucosal disease that can reduce a patient's quality of life, due to a painful stinging sensation that worsens during daily activities like speaking, eating or even drinking. Its causes include physical trauma, radiation, chemical injury, and microbial infection (bacterial, viral, and fungal). However, some ulcerations, such as recurrent aphthous stomatitis (RAS), commonly known as aphthae or canker sores, have uncertain etiology and causes of RAS are not fully elucidated.

RAS can be classified phenotypically into 3 different types, although their classification varies slightly in terms of size and healing time between different authors:

Minor RAS is the most frequently observed (80%) small, round, clearly defined below 5 mm in diameter (2–3 mm in average), but painful ulcers typically heal between 7 and 14 days without scaring. Major RAS that account for about 10% of all RAS are larger (diameter exceeds 10 mm), deep, can last for 6 weeks or longer, affecting both mucosa and keratinized tissues, and frequently heal with scaring. Herpetiform RAS, may look 'herpetic' in nature but do not have a viral etiology, are mostly present as multiple small clusters of pinpoint lesions that form large irregular ulcers, and similar to minor RAS, heal within 14 days without scaring [5].

In particular, saliva, in association with swallowing, chewing, and phonation, washes away most of the drug from the site of application, resulting in a short retention time of the dosage forms and, consequently, low therapeutic efficacy.

The *Quercus infectoria* Olivier is a plant belonging Fagaceae family. It is a shrub about 2 meters in height with lots of spreading branches. It is found in Turkey, Syria, Peris, Cyprus, and Greece.[6] The bark of the plant is slightly grey in color. Leaves are 4 to 6 meters long and rigid. Flowers are unisexual and male flowers are tangled into hanging axillary catkins with 6-8 tapaled perigone and 6-10 stamens; female flowers are single in leaf axils of dropping stipules. The perigone is 6-tipped with an inferior 3-chambered ovary surrounded by an initially inconspicuous cupula and then later cup-shaped. The galls are globular in shape and form, 10 to 25 mm in diameter. The basal stalk is short, and numerous projections around the surface. The galls have a very astringent taste. The galls are collected before the insect emerges [7, 8].

Synonyms: Oak galls, Turkish galls, Nut galls, Mecca galls, Aleppo galls [9].

Botanical Name: *Quercus infectoria* Olivier.

Vernaculars of *Quercus infectoria:*

Marathi: Mayphal

Hindi: Mazuphal

Sanskrit: Majuphal

Kannada: Machikani

Classification [9]:

Kingdom: Plantae

Sub-kingdom: Tracheobionta

Super division: Spermatophyta

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Hamamelidae

Order: Fagales

Family: Fagaceae

Genus: Quercus

Species: *Quercus infectoria* Olivier

FIG. 1: *QUERCUS INFECTORIA* OLIVIER GALLS

The galls of *Quercus infectoria* contain tannic acid 50 to 70%, gallic acid, ellagic acid, starch hexagalloyl glucose, amentoflavone, β-sitosterol, calcium oxalate, methyl oleanolate [7, 8, 9]. According to different studies, the gall extract showed antibacterial, antioxidant, anti-inflammatory, antifungal, anti-viral, anti-protozoal, anti-amoebic, antiulcer, larvicidal, tonic to teeth and gum, antipyretic, anticancer and wound healing activities [7, 8]. Gels are hydrocolloid suspensions containing either small inorganic molecules or large organic molecules dispersed in a suitable liquid [10]. According to IP, gels are homogeneous, semi-solid preparations usually consisting of solutions or dispersions of one or more medicaments in suitable hydrophilic or hydrophobic bases [11].

Temperature Sensitive Poloxamer-based Hydrogels: To enable the localized, sustained and prolonged release of a drug, hence reducing the number of administrations, preventing side effects, and adhering to low doses, hydrogels have been employed as drug-controlled delivery systems. Temperature-sensitive hydrogels, in which physical entanglements, hydrogen bonds, and hydrophobic interactions are the major components that comprise the crosslinks, are the environmentally responsive systems that have been investigated the most. Existing thermosensitive hydrogels can either gel by heating beyond the lower critical gelation temperature (LCGT) or by chilling below the upper critical gelation temperature (UCGT). Since, they enable encapsulation in mild settings (temperature 37 °C), hydrogels with LCGT behaviour and sol-to-gel transition at 37 °C have drawn increasing attention in the biomedical area as carriers for cells, medicines and biomolecules.

Water-soluble non-ionic triblock copolymers known as poloxamers or Pluronics® are made up of polar (polyethylene oxide) and non-polar (poly propylene oxide) blocks that give the polymers amphiphilic and surface-active characteristics. Additionally, the cohabitation of hydrophilic and hydrophobic monomers into block copolymers enables the creation of ordered structures in solution, the most prevalent of which are micelles. Their aqueous solutions go through a sol-to-gel transition when the temperature is raised above an LCGT.

The formation of micelles in solution is a reversible and dynamic process useful for encapsulating hydrophobic drugs and delivering them into an aqueous environment. Due to their ability to alter their structure in response to pH, temperature, and salt concentration, these polymers can be regarded as smart due to their stimuli-sensitive characteristics. Since, poloxamers are non-toxic and non-irritating, they can be used as solubilizers, emulsifiers, stabilisers and delivered via oral, parenteral, or topical methods. They are FDA-approved and included in the US and European Pharmacopoeia. They are helpful in ointments, suppository bases, and gels as wetting agents [12]. In order to create these hydrogels, various polymers are typically used, including hydroxyl propyl methyl cellulose (HPMC), *N*-isopropylacrylamide copolymers (pNIPAM), poly(ethylene oxide) (PEO), poly(propylene oxide) (PPO) (poloxamers,

Tetronics® or Pluronics®), poly (acrylic acid) (PAA), and poly(acrylamide) (PAAm). The use of poloxamers in creating and describing thermosensitive hydrogels will be the main topic of the current work [13].

Silver Nanoparticles: Nanotechnology has been emerging as a rapidly growing field with various applications in science and technology for new material production; it is defined as the utilization of structures with at least 1 nm size dimension in the construction of materials, devices, or systems with improved properties [14]. Nanoparticles (NP) are natural, incidental, or manufactured materials containing particles, where 50% or more of the particles lie in the size range 1–100 nm [15]. Silver nanoparticles have been developed as a potent antibacterial, antifungal, antiviral, and anti-inflammatory agent. Silver nanoparticles are an antimicrobial gel formulation for conventional topical antimicrobial agents, especially for burn treatment [16].

Green synthesis of nanoparticles is progressively emerging as a key branch of nanotechnology. The nanoparticles are produced with the help of biological entities like microorganisms, plant extracts, or plant biomass. They could be a substitute for chemical and physical methods in an eco-friendly manner. Green synthesis is preferred over physical and chemical methods as the former is environment-friendly, cost-effective, and easily scalable to large-scale syntheses. There is no need to use high temperatures, energy and toxic chemicals [15].

Silver nanoparticles act by getting attached to the cell membrane's surface, interrupting the cell's permeability and metabolic pathways. Silver nanoparticles not only interact with the surface of the membrane, but can also penetrate into the bacterial cell membrane. In addition, silver nanoparticles can bind to the DNA inside bacterial cells, preventing its replication or interaction with the bacterial ribosome. It has been discovered that silver nanoparticles can damage the structure of the bacterial cell membrane and reduce the activity of some membranous enzymes, which cause *E. coli* bacteria to die eventually [16]. The present study is to formulate a thermosensitive antibacterial gel of *Quercus infectoria* aqueous extract as well as nanoparticles of *Quercus infectoria* aqueous extract for the treatment of mouth ulcer and gum disorder and to investigate the anti-bacterial synergistic activity of tannic acid, gallic acid, ellagic acid present in the extract and compare the antibacterial activity of formulated gel with the marketed formulation.

MATERIALS AND METHODS:

Materials: The galls of *Quercus infectoria* Olivier were obtained from a local supplier of Solapur. The tannic acid from Milton chemicals, Mumbai; gallic acid from Modern chemical cooperation, Mumbai; Ellagic acid and Pluronic F68 from Ozone International, Mumbai; Pluronic F127 from Chemsworth, Mumbai; Carbopol 971P and Carbopol 974P from Maruti chemicals and silver nitrate were purchase from Vikas pharma and drug, Mumbai.

Methods:

Collection and Authentication of *Quercus infectoria* Oliver: Identification and authentication of *Quercus infectoria* Oliver, a member of the Fagaceae family, were correctly identified by referring to herbarium by PG Department of Botany and Research Center. Prof. Dr. M. N. Jagtap and Dr. Randive S. D. had catalogue No. 720a Kotshy K.G.T 23/05/1862 & catalogue No. 2 Kotshy K.G.T, Turkey, 23/05/1862.

Method of Extraction: After botanical evaluation, the shade-dried plant material was subjected to size reduction to get the coarse powder and then passed through a different sieve to get uniform powder. The uniform powder galls of *Quercus infectoria* Oliver were extracted using water and 50% v/v ethanol.

The galls of *Quercus infectoria* were washed, dried, and powdered in a mortar. 10 g powdered material was soaked in 50 ml of 50% v/v ethanol at the temperature of 70 ºC for 10 min and then kept for continuous stirring for 10 hr. The extract was filtered through Whatman filter no. 1. The filtrate was collected, dried, and stored in an airtight container [17].

Physicochemical Evaluation of Extract: The dried extract was subjected to standardization with different parameters.

Qualitative Analysis: The aqueous extract galls of *Quercus infectoria* were taken and then subjected to a qualitative test to identify the plant constituent. Phytochemical analysis was done to determine constituents such as carbohydrates, amino acids, proteins, saponins, alkaloids, glycosides, flavonoids, phenolic compounds and tannins [18].

Detection of Phenolic Compounds and Tannins: 100 mg of the extract was boiled with 10 ml of distilled water and filtered. To 2 ml of filtrate, 2 ml of 1% w/v ferric chloride solution was added. Bluish-green color indicates the presence of phenolic compounds and tannins.

Test for Alkaloids: The extracts were treated with a few drops of concentrated hydrochloric acid and filtered. The filtrate was tested carefully with various alkaloidal reagents Maver's reagent (cream precipitate) Dragendorff's reagent (orange-brown precipitate). Hager's reagent (yellow precipitates) and Wagner's (reddish brown precipitate).

Detection of Carbohydrates: Small quantities (50 mg) of alcoholic and aqueous extracts were dissolved separately in 5 ml of distilled water and filtered. The filtrate may be subjected to Molisch's test to detect the presence of carbohydrates.

Detection of Saponins: About 1 ml of alcoholic and aqueous extracts were diluted separately with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes. One cm layer of foam indicates the presence of saponins. The test solution may be subjected to a test for haemolysis.

Detection of Proteins and Free Amino Acids: To the extract, 0.25% w/v of Ninhydrin reagent was added and boiled for a few minutes. A purple color indicated the presence of proteins.

Detection of Flavonoids: The extract was treated with concentrated sulphuric acid. A yellowish to orange colour indicates the presence of flavonoids,

Test for Glycosides: The extracts were hydrolyzed with hydrochloric acid for few hours in a water bath and the hydrolyzate was subjected to various tests.

Solubility Study of *Quercus infectoria* Oliver Gall Extract: The solubility study of *Quercus infectoria* Oliver gall extract was carried out in solvents such as distilled water, ethanol, methanol, acetone, dimethyl sulfoxide, phosphate Buffer pH 6.8.

Calibration Curve of *Quercus infectoria* Oliver Gall Extract: Accurately weighed 10mg of extract was dissolved in a sufficient amount of phosphate buffer 6.8 to make up the volume 10 ml with the same solvent in a graduated volumetric flask. 1 ml of this solution was taken and diluted with phosphate buffer 6.8 in 10 ml graduated volumetric flask. From this stock solution, take out 0.5ml, 1ml, 1.5ml, 2ml, and 2.5ml solutions again dilute up to mark on 10 ml graduated volumetric flask with phosphate buffer pH 6.8. The absorbance of the sample was measured on UV Visible Spectrophotometer at 275 nm.

Quantitative Analysis:

High-Performance Liquid Chromatography analysis of *Q. infectoria* Oliver Gall Extract: The *Quercus infectoria* galls contain 60 to 70% tannins in the extract. The determination of active constituents from the extracted compound was examined using high-performance liquid chromatography (HPLC). To evaluate the quantity of tannic acid, gallic acid, ellagic acid from the Quercus infectoria Oliver galls extract was analyzed using reverse-phase High-Performance Liquid Chromatography. Method development and validation for simultaneous estimation of Tannic acid, Gallic acid, and Ellagic acid by reversed-phase high-performance liquid chromatography from the *Quercus infectoria* galls extract was performed by reversed-phase HPLC on a column-Agilent Zorbax Bonus RP (250×4.6mm,5µ) by using binary gradient elution with consisting methanol and buffer (40-60% v/v). Solvent A methanol (99.9%) and solvent B buffer (2.4gm of sodium dihydrogen Phosphate) were pumped at 1ml/min. The column temperature was maintained at 30 ºC. The peaks were identified by comparing them with the standard solution of tannic acid, gallic acid, ellagic acid at 275nm. The percentages of tannic acid, gallic acid, ellagic acid were calculated using the appropriate calibration curve.

Standard Preparation:

Gallic Acid Standard Stock Solution-I (GSSS-I): Initially Prepare a Standard Stock Solution (SSS-I) of by adding 10 mg of Gallic Acid in 10 ml

volumetric flask & add 5 ml diluent, mix for 2 minutes and make the volume to 10 ml with diluent. (conc. of Gallic acid = 1000 µg/ml).

Tannic acid Standard Stock Solution-I (TSSS-I): Then prepare a Standard Stock Solution (SSS-II) of Tannic acid by adding 10 mg in 10 ml volumetric flask & add 5 ml diluent, mix for 2 minutes and make the volume to 10 ml with diluent. (conc. of Tannic acid = 1000 µg/ml).

Ellagic acid Standard Stock Solution-I (ESSS-I): Then prepare a Standard Stock Solution (SSS-II) of Ellagic acid by adding 10 mg in 10 ml volumetric flask & add 5 ml diluent, mix for 2 minutes and make the volume to 10 ml with diluent. (conc. of Ellagic acid = 1000 µg/ml). Then add 1.0 ml of GSSS-I, 1.0 ml TSSS-I and 1.0 ml of ESSS-I in 10 ml volumetric flask and add 5 ml diluent and vortex and make up the volume with diluent. (Conc. of Gallic Acid = 100 µg/ml, Tannic Acid = 100 µg/ml & Ellagic Acid = 100 µg/ml).

Extract Sample Preparation for Assay: 10 mg of extract was accurately weighed and sonicated for 10 minutes and made the volume to 10 ml with diluent. (conc. of extract = 1000 µg/ml). 1 ml of above solution was further transferred in a 10 ml volumetric flask and the volume to 10 ml with diluent. (conc. of extract = 100 µg/ml).

Minimum Inhibitory Concentration (MIC) of *Quercus infectoria* Extract: The MIC values obtained for *Quercus infectoria* Oliver galls extract by serial dilutions of the antimicrobial agent were made in nutrient broth and were kept in a test tube. MIC against the *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* were found. The last tube was kept free of antibiotic and served as growth control. Arranged all the test tube in racks and standardized the suspension of the microorganisms to be tested in inoculated into the tubes. Tubes were incubated at 35-37 °C for 24 hrs.

Bacterial Cultures and Growth Conditions: Clinical isolates of (gram-positive) *Staphylococcus aureus*, (gram-negative) *Pseudomonas aeruginosa*, *Escherichia coli* were obtained from the Department of Microbiology. All the test strains maintained Nutrient agar slants and subcultured onto Nutrient agar for 24 hours before testing. These bacteria served as test pathogens for antibacterial activity assay.

Antibacterial Activity of Extract: Antibacterial activity of *Quercus infectoria* Oliver extracts was determined by agar well diffusion method according to National Committee for Clinical Laboratory Standards (NCCLS). Inoculums containing bacterial culture to be tested spread on nutrient agar plates with a sterile swab moistened with the bacterial suspension. Subsequently, wells of 6 mm diameter were punched into the agar medium, filled with plant extract, and allowed to diffuse at room temperature for 2 h. The plates were then incubated upright at 37°C for 24 hrs. Wells containing the same volume of DMSO (10%), as negative controls, while standard antibiotic discs of streptomycin (10 µg) were used as the positive controls. After incubation, the diameters of the growth inhibition zones were measured in mm.

Formulation of Gel:

Formulation of Thermosensitive Mucoadhesive Gel: Plain and medicated *in situ* gel were prepared using the cold method. The plain gel was formulated using different concentrations of Pluronic F127 and Pluronic F68 and to give gelation at the oral cavity temperature, i.e., 33- 37 ºC. Poloxamers were added to cold distilled water with continuous agitation and kept overnight at 6 to 8 ºC until a clear solution was obtained. *Quercus infectoria* gall extract and mucoadhesive polymer (Formulation **Tables 1** and **2**) were added to the Poloxamer solution with constant stirring [19].

TABLE 1: FORMULATION OF THERMOSENSITIVE MUCOADHESIVE GEL

Sr. no.	Formulation code	Extract(%W/V)	Pluronic F127 (%)	Pluronic F68 (%)	Carbopol 971P (%)	Carbopol 974P (%)
1	F1	2	20	-	-	-
2	F2	2	25	-	-	-
3	F3	2	30	-	-	-
4	F4	2	20	1	-	-
5	F5	2	25	2	-	-

6	F6	2	30	3	-	-
7	F7	2	20	-	1	-
8	F8	2	25	-	1	-
9	F9	2	30	-	1	-
10	F10	2	20	-	-	1
11	F11	2	25	-	-	1
12	F12	2	30	-	-	1

Formulation of Tannic, Gallic, Ellagic Acid and Nanoparticle Gel: For formulation 10g of Tannic, Gallic, Ellagic acid and *Quercus infectoria* silver nanoparticle (QI. AgNP) gel weighed the specific amount of chemical constituents given in **Table 2** and mucoadhesive polymer were added with poloxamer solution with constant stirring.

TABLE 2: FORMULATION OF TANNIC, GALLIC, ELLAGIC ACID, QI. SILVER NANOPARTICLES

Sr. no.	Chemical constituents of gel	Concentration	Pluronic F127	Pluronic F68
1	Extract of *Quercusinfectoria* galls	200mg	2.5gm	0.2gm
2	Tannic acid	80mg	2.5gm	0.2gm
3	Gallic acid	50mg	2.5gm	0.2gm
4	Ellagic acid	20mg	2.5gm	0.2gm
5	*Quercus infectoria* silver nanoparticles	5mg	2.5gm	0.2gm

Evaluation Test for Gel [2, 17-27]:

Physical Appearance: Physical appearance of the formulation is checked by visual method. The appearance of the formulation was observed, which included clarity and transparency was determined visually.

Color: The color was observed visually against a black background.

Odor: The odor of gel was checked by dissolving some amount of gel in water and smelling it.

Homogeneity: All developed gel formulations were tested for homogeneity by visual inspection after the gels had been set into the container. They were tested for their presence and appearance of any aggregates.

Taste: A small amount of gel was placed on the tongue, and its taste was sensed.

pH: The pH was determined at 35ºC using Systronics pH meter. 5 g gel was taken in 45 ml of distilled water. pH of this solution is determined by calibrated pH meter.

Gelation Temperature: The gelation temperature was determined using the test-tube inverting method. A volume of 2ml of formed gel batches was placed in a test tube, which was then immersed in a water bath at 15 °C. The water bath temperature was then gradually increased, samples were examined every 2 minutes and the gelation temperature was recorded when the gel stopped flowing upon test tube inversion at 90°. The readings were taken for an average of 3 samples.

Syringeability Study: The ability of the prepared formulations to flow easily through a syringe of a 21-gauge needle. One ml of the cold gel was filled in a 21-gauge needle syringe, and the ability of the gel to flow under normal handling pressure was assessed.

Extrudability: A closed collapsible tube containing about 10 g of gel was pressed firmly at the crimped end, and a clamp was applied to prevent rollback. The cap was removed, and the gel was extruded. The amount of the extruded gel was collected and weighed. The percentage of the extruded gel was calculated.

Viscosity: The viscosity of the cream was determined by using Brookfield DV-EL Viscometer with helipath stand was used for viscosity studies. The reading was taken by using type s64 spindle at 10 rpm at 37 ºC. Three readings were taken and then average reading was recorded.

Spreadability Test: 1 g of sample was applied between two glass slides applied some uniform weight.

The diameter of circle after spreading of formulation was measured and determine time required for spreadability and calculate the spreadability by following formula-

$$\text{Spreadability} = M \times L / t$$

Where, M = weight tied to upper side, L = length moved on the glass slide, T = time taken.

Gelling Capacity: The gelling capacity of formed gel was determined using visual inspection and different grades were allotted as per gel consistency and rate of gelation.

Drug Content Assay: The weighed amount of gel equivalent to 1 g of drug was accurately taken and dissolved in phosphate buffer (pH 6.8). The product content was measured against phosphate buffer at 275 nm (pH 6.8) using UV Visible Spectrophotometer (Systronic 2202) and determined from the calibration curve.

***In-vitro* Drug Release Studies:** The study of *Quercus infectoria* Oliver extract release from the gel formulations was conducted using cellulose acetate membrane (0.45µm/0.47mm diameter) for a period of 4 hr on Franz diffusion apparatus. Phosphate buffer was the dissolution medium of pH 6.8. Tied to one end of a glass cylinder was the cellulose acetate membrane, previously soaked overnight in the Phosphate buffer. Then 1 ml of the formulated formulation was wrapped in cellulose acetate membrane and placed in phosphate buffer. The dissolution medium was stirred with a magnetic stirrer at 50 rpm. The sample was collected at regular intervals and replaced by a receptor medium volume similar to that. At the time interval pre-determined, 4 ml of the sample was taken and spectrophotometrically analyzed at 275 nm.

Mucoadhesive Strength: *Ex-vivo* mucoadhesive strength was determined using fresh sheep small intestinal mucosa. The mucosal membrane was separated by removing the underlying fat and loose tissues. The balance was equilibrated on both sides by placing one side beaker on the right pan and a weight (5 g) on the opposite pan. The sheep intestinal mucosa was cleaved into 4 to 5 cm^2 and tide with help of non-absorbable tread to upper and lower part of left side. A thin film of the prepared gel (1 g) was spread on the lower surface of the left side pan. The left pan was lowered and was spread with gel by removing the beaker from the right pan. The pan was left undisturbed for 2 min to ensure proper contact between the intestinal mucosa and the gel. Following this, water was slowly added to the left pan using a burette until the intestinal mucosa was separated from lower surface. Take a weight, the mucoadhesive force was calculated by determining the weight required to separate the mucosa. The total weight (gm) required to detach two vial was taken as measure mucoadhesive strength. From this mucoadhesive strength mucoadhesive fore was calculated by

$$\text{Fore of adhesion} = \text{Mucoadhesive strength} \times 9.81/100$$

Stability Study: The gel formulations were prepared are subjected to stability studies at 40º C/75% RH for a period of 3 month as per ICH Guidelines. Samples were withdrawn at 1-month time intervals and evaluated for physical appearance, pH, rheological properties, spreadability and extrudability.

Optimization of Batch: The batches were optimized by checking, and by studying physical evaluation to their pH, viscosity, spreadability, extrudability, syringability, gelation temperature, drug content assay, *in-vitro* drug released study, mucoadhesive strength and stability study the formulation gel.

Bacterial Cultures and Growth Conditions: Clinical isolates of (Gram positive) *Staphylococcus aureus*, (Gram negative) *Pseudomonas aeruginosa*, *Escherichia coli* were obtained from the Department of Microbiology. All the test strains were maintained on Nutrient agar slants and subculture on Nutrient agar for 24 hour prior to testing. These bacteria served as test pathogens for antibacterial activity assay.

Antibacterial Activity for Formulation of Gel: Antibacterial activity of *Quercus infectoria* Oliver formulated gels of optimum batch was determined by agar well diffusion method according to National Committee for Clinical Laboratory Standards (NCCLS). Inoculums containing bacterial culture to be tested were spread on nutrient agar plates with a sterile swab moistened with the bacterial suspension. Subsequently, wells of 6 mm diameter were punched into the agar medium and filled with plant extract and allowed to diffuse at room temperature for 2 h. The plates were then incubated in the upright position at 37°C for 24 hrs. Wells containing the same volume of

DMSO (10%), as negative controls while standard antibiotic discs of and streptomycin (30 µg) were used as the positive controls. After incubation, the diameters of the growth inhibition zones were measured in mm.

FT-IR of Formulated Gel and Extract of Quercus *infectoria* Oliver Galls: The potential for drug-polymer interaction was assessed using FTIR (Bruker alpha2 Germany) formulation, and extract was scanned 4000cm^{-1} to 500 cm^{-1} wavenumbers, and characteristic peaks were observed.

Hen's Egg Test (HET-CAM) Test Method [2]

Source of Chicken Eggs: Fertile White Leghorn chicken eggs should be obtained from commercial sources. Fresh (not older than seven days), fertile, clean eggs weighing between 50 and 60 grams should be used.

Test Substance Preparation: All test substances should be evaluated undiluted unless dilution is justified. If dilution is justified, then 0.9% w/v NaCl or olive oil should be used as the diluent, depending on substance solubility. Use of a different solvent should be justified. Dilutions should be prepared on the same day as the test. Paste, particulate, or granular test substances or formulations should be evaluated without dilution.

Controls:

Negative Control: A 0.9% w/v NaCl negative control included in each experiment in order to provide a baseline for the assay endpoints and to ensure that the assay conditions do not inappropriately result in an irritant response.

Positive Control: A known irritant should be included in each experiment to verify that an appropriate response is induced. The selection of positive control test substances should be based on the availability of best quality *in-vivo* data. Positive control should be the substances 1% w/v NaOH that induce sever response in HET-CAM test.

CAM Preparation: Select fresh (not older than 7 days), clean, fertile 50-60 g White Leghorn chicken eggs. Candled the eggs and discarded any eggs that were nonviable or defective. Placed the eggs in an incubator with a rotating tray. Incubated eggs at 38.3 ±0.2 °C and 58±2% relative humidity when incubating in a still-air incubator or at 37.8± 0.3 °C and 58±2% relative humidity when incubating in a forced-air incubator. Candled the eggs on incubation day 8 and removed any nonviable or defective eggs. Eggs were returned to the incubator (without hand rotation) with the large end of the eggs upwards for an additional day. Mark the air cell of the egg. Cut the section marked as the air cell with a rotating dentist saw blade and then pare it off. Care should be taken when removing the eggshell to ensure that the inner membrane is not injured. Moistened the inner membrane with 0.9% NaCl. A disposable glass pipette used to apply the solution. Placed the egg into the incubator for a maximum of 30 minutes. The egg removed from the incubator, prior to its use in the assay, and decanted the 0.9% NaCl solution. Carefully removed the inner membrane with forceps, ensuring that the inner membrane is not injured. Moisten the inner membrane with 0.9% NaCl. A disposable glass pipette can be used to apply the solution. Place the egg into the incubator for a maximum of 30 minutes. Remove the egg from the incubator, prior to its use in the assay, and decant the 0.9% NaCl solution. Carefully remove the inner membrane with forceps, ensuring that the inner membrane is not injured.

Observations: Observe the reactions on the CAM over a period of 300 seconds. The time for the appearance of each of the noted endpoints should be monitored and recorded in seconds. Endpoints that should be observed are:

Hemorrhage (bleeding from the vessels), Vascular lysis (blood vessel disintegration), Coagulation (intra- and extra-vascular protein denaturation), Hemorrhage time = observed start (in seconds) of hemorrhage reactions on CAM, Lysis time = observed start (in seconds) of vessel lysis on CAM, Coagulation time = observed start (in seconds) of coagulation formation on CAM, Collection of additional information and data may be useful in further analyses and conducting retrospective studies. To maximize the likelihood of obtaining reproducible results, reference photographs for all endpoints should be available.

Evaluation of Test Results: Each of the three HET-CAM endpoints at fixed time intervals of 0.5, 2, and 5 minutes. The time-dependent numerical scores for lysis, hemorrhage, and coagulation

Table 3 are summed to give a single numerical value indicating the irritation potential of the test substance on a scale with a maximum value of 21.

TABLE 3: SCORING SCHEME FOR IRRITATION OF (HET -CAM) TEST

Effect	Score		
	0.5 min	2 min	5min
Lysis	5	3	1
Haemorrhage	7	5	3
Coagulation	9	7	5

Criteria for an Acceptable Test: A test is considered acceptable if the negative and positive controls each induce a response that falls within the classification of non-irritating and severely irritating. Historical control studies indicate that using 0.9% NaCl as a negative control, the value was 0.0. Historical control studies indicate that using 1% SDS and 0.1 N NaOH as positive controls, the IS values ranged between 10 and 19, respectively.

Synthesis of Silver Nanoparticle: Take 250 ml round bottom flask add 45ml of 1 mM aqueous solution of silver nitrate, and later add 5ml of 5 mg/ml aqueous solution extract was placed in an ultrasonication for 10 min so that the yellow-colored solution turned into brown color which indicated bioreduction of silver ion in the solution and this color changes shows that formation of silver nanoparticles than the collect the silver nanoparticle of *Quercus infectoria* by centrifugation at 3000 rpm for 10 min collected nanoparticles washed with water. Dry the nanoparticles and store in airtight container at cool conditions [17, 26].

Evaluation of Synthesis Silver Nanoparticle of Galls Extract of *Q. infectoria* Galls:

Characterization of Silver Nanoparticles: The formation of silver nanoparticles is monitored by measuring the UV- vis spectra of the reaction mixture at a wavelength of 300- 500nm.

XRD of Silver Nanoparticles: The crystalline nature of the nanoparticles was analyzed by powder X-ray diffractometer (Rigaku Japan).

The silver nanoparticle is evaluated by X-ray diffraction (XRD) technique using Cu radiation at 40 kV, and 30mA, and data were collected in the range of 2θ= 0–80°

Particle size and zeta Potential: Zeta potential analysis was performed to study the surface characteristics, stability, and charge of the nanoparticles using Microtrac Particle Analyser, and particle size and its distribution also analysis.

FTIR of Silver Nanoparticles: The functional groups were analysed by Fourier-transform infrared spectroscopy (FTIR). Using FTIR (Bruker alpha 2 Germany) synthesis, silver nanoparticle was scanned at 4000 cm^{-1} to 500 cm^{-1} wavenumbers, and characteristic peaks were observed.

Differential Scanning Calorimetry (DSC): Differential scanning calorimeter (DSC) (Mettler-Toledo) was used to assess the stability of drug excipients and also to detect further phase shift, such as glass transition temperature, amorphous crystallization shape, and polymer.

RESULTS AND DISCUSSION:

Collection and Authentication *Quercus infectoria* Oliver: The extraction yield of galls of *Quercus infectoria* Olivier with ethanol (50 %v/v) obtained was 40 %.

Physicochemical Evaluation of Extract: The extract was fine and crystalline, passed from sieve number 120 and evaluations of the extract of galls were carried as per stated procedure which revealed the presence of tannins, carbohydrates, amino acids and proteins.

TABLE 4: PHYTOCHEMICAL INVESTIGATION OF EXTRACT

Sr. no.	Phytochemical Constituent	Result
1	Phenolic compounds and tannins	+
2	Alkaloids	-
3	Carbohydrates	+
4	Proteins and amino acids	+
5	Saponins	-
6	Flavonoids	-
7	Glycosides	-

Solubility Study of *Quercus infectoria* Galls Extract: The solubility study of Quercus *infectoria* galls were study in different solvent listed in Table

TABLE 5: SOLUBILITY OF EXTRACT IN VARIOUS SOLVENTS

Medium	Result
Distilled Water	Soluble
Ethanol	Soluble
Methanol	Soluble
Acetone	Soluble
Dimethyl sulfoxide	Soluble
Phosphate Buffer pH 6.8	Soluble

Calibration Curve of *Quercus infectoria* Oliver Extract: The concentration range was 5 to 25 µ/ml at 275nm. The analytical Parameter linearity was found to be linear. The regression coefficient was found to be 0.996.

TABLE 6: LINEARITY RESULT

Concentration(µ/mL)	Absorbance
0	0
5	0.231
10	0.512
15	0.756
20	1.122
25	1.374

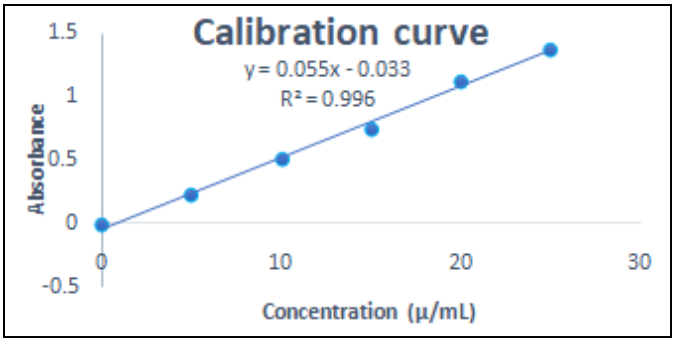

FIG. 2: CALIBRATION CURVE OF *QUERCUS INFECTORIA* OLIVER EXTRACT

High-Performance Liquid Chromatography (HPLC) analysis of *Quercus infectoria* Oliver Extract: The *Quercus infectoria* Oliver galls extract contain a large amount of tannic acid and a small amount of Gallic and ellagic acid.

The results revealed that tannic acid is a major bioactive constituent in *Quercus infectoria* Oliver extract due to the high concentration of tannic acid.

Most of the pharmacological activities exhibited by galls extract can be attributed to tannic acid. The concentration of tannic acid, gallic acid, and ellagic acid present in the extract is listed in **Table 7.**

TABLE 7: CONCENTRATION OF TANNIC, GALLIC, ELLAGIC ACID IN QUERCUS INFECTORIA GALLS

Sr. no.	Chemical constituents	Concentration
1	Tannic acid	4.03 mg/10mg extract of QI
2	Gallic acid	2.91 mg/10mg extract of QI
3	Ellagic acid	1.31 mg/10mg extract of QI

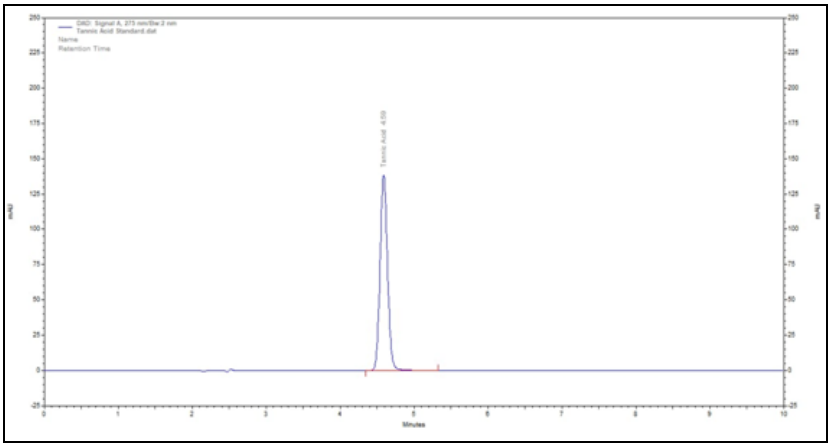

FIG. 3: HPLC CHROMATOGRAM FOR STANDARD TANNIC ACID

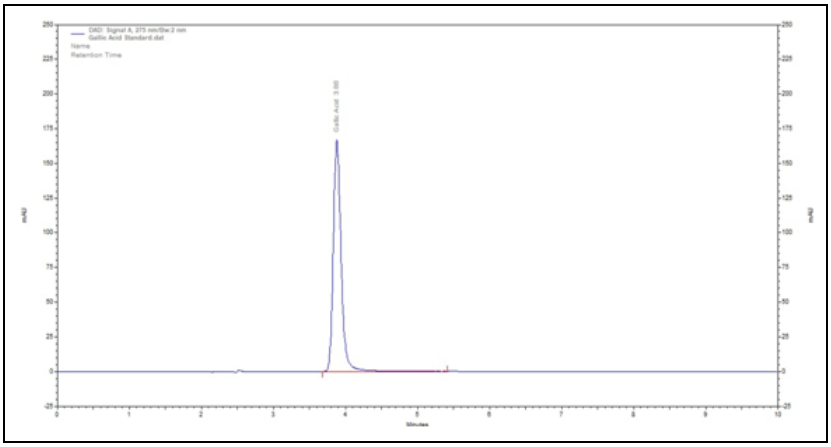

FIG. 4: HPLC CHROMATOGRAM FOR STANDARD GALLIC ACID

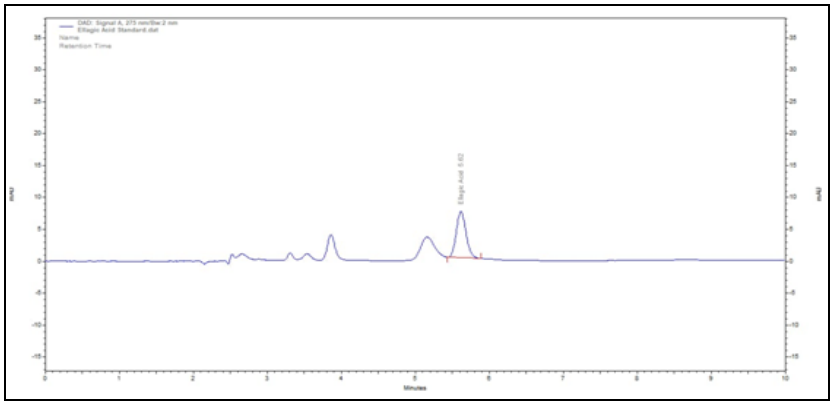

FIG. 5: HPLC CHROMATOGRAM FOR STANDARD ELLAGIC ACID

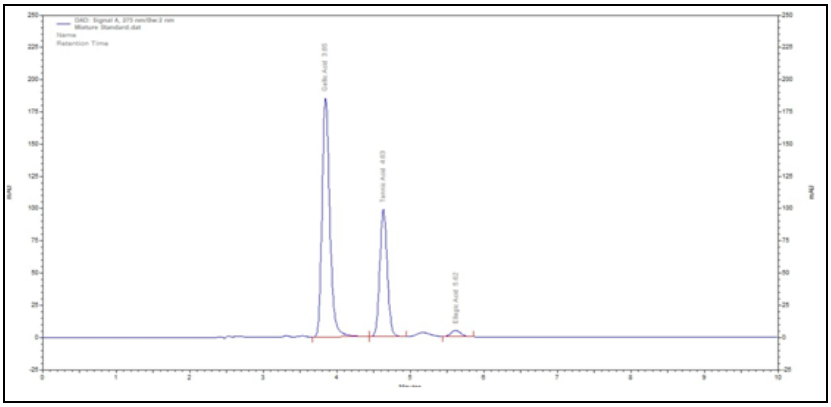

FIG. 6: HPLC CHROMATOGRAM OF MIXTURE OF STANDARD (TANNIC, GALLIC, ELLAGIC ACID).

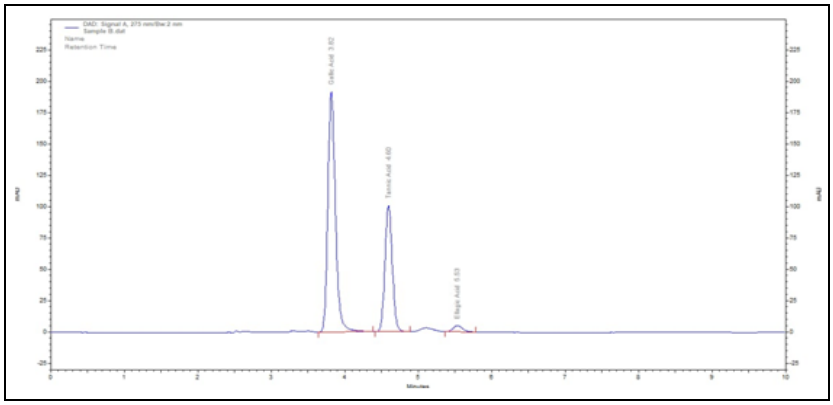

FIG. 7: HPLC CHROMATOGRAM FOR TANNIC, GALLIC, ELLAGIC ACID IN *QUERCUS INFECTORIA* OLIVER EXTRACT

Minimum Inhibitory Concentration (MIC) of *Quercus infectoria* Extract: The extract of *Quercus infectoria* Oliver galls showed activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*.

The extract showed significant activity against Gram-positive and Gram-negative bacteria. The MIC was found to be 40 µg/ml for *Pseudomonas aeruginosa* and 20 µg/ml for *Staphylococcus aureus*. For *Escherichia coli* it was found to be 60 µg/ml.

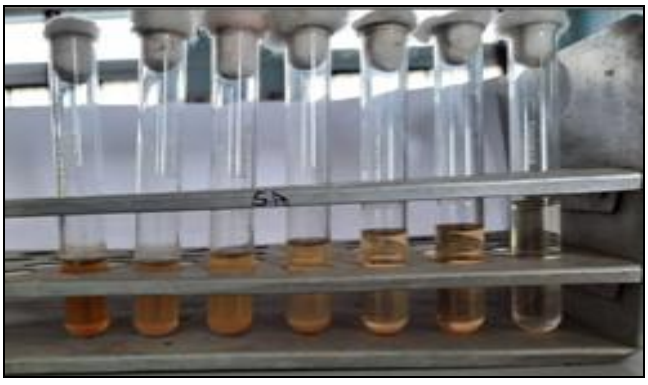

FIG. 8: MIC OF EXTRACT AGAINST *STAPHYLOCOCCUS AUREUS*

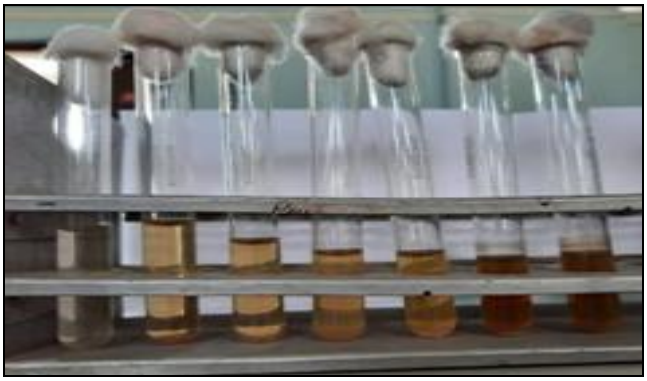

FIG. 9: MIC OF EXTRACT AGAINST *PSEUDOMONAS AERUGINOSA*

FIG. 10: MIC OF EXTRACT AGAINST *ESCHERICHIA COLI*

Antibacterial Activity of Extract of *Quercus infectoria* Oliver: The galls showed activity against *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Escherichia coli*. The extract showed significant activity against Gram-positive and Gram negative bacteria. The solvent used in activity DMSO does not show any activity; the standard antibiotic streptomycin shows the zone of inhibition is 22 mm. *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Escherichia coli* showed 30 mm, 26 mm, 24 mm respectively. The extract showed better antibacterial activity at 10 mg/ml concentration; hence this concentration was chosen for formulation.

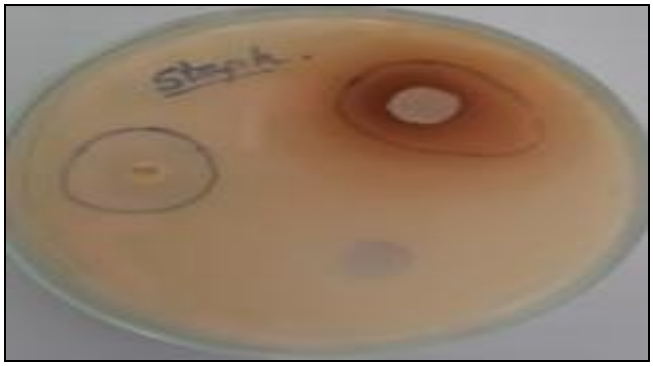

FIG. 11: ANTI-MICROBIAL ACTIVITY OF EXTRACT AGAINST *STAPHYLOCOCCUS AUREUS*

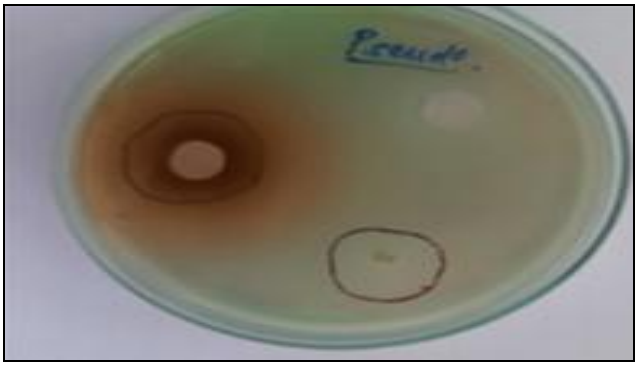

FIG. 12: ANTI-MICROBIAL ACTIVITY OF EXTRACT AGAINST *PSEUDOMONAS AERUGINOSA*

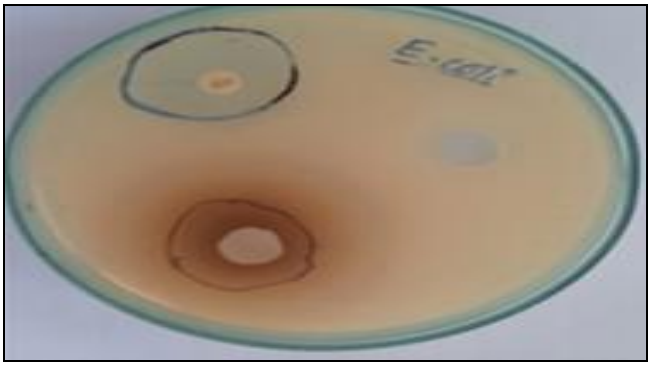

FIG. 13: ANTI-MICROBIAL ACTIVITY OF EXTRACT AGAINST *ESCHERICHIA COLI*

Evaluation Test for Formulation of Gel Containing *Quercus infectoria* Oliver Galls Extract: The present work aimed to formulate stable gel and increase gel antibacterial activity. Twelve batches of formulation were prepared with different concentrations of Pluronic F127, Pluronic F68, and Carbopol 971P, Carbopol 974P as gelling agents. The prepared formulations were characterized for Physical appearance, Color, Odor, pH, Viscosity, Spreadability, Homogeneity, Syringeability, Extrudability, Gelation Temperature, *in-vitro* anti-bacterial study, Mucoadhesive strength, and *in-vitro* skin irritation study.

TABLE 8 EVALUATION PARAMETERS OF THERMOSENSITIVE GEL CONTAINING EXTRACT OF *QUERCUS INFECTORIA* OLIVER GALLS (BATCH NO. F1 TO F6)

Sr. no.	Parameters	F1	F2	F3	F4	F5	F6
1	Color	Faint Brown	Faint Brown	Faint Brown	Faint Brown	Faint Brown	Faint Brown
2	Odor	Fruity	Fruity	Fruity	Fruity	Fruity	Fruity
3	pH	6.7	6.8	6.8	6.8	6.7	6.8
4	Viscosity (cps)	4800	4980	5200	5490	5640	6450
5	Spreadability (gm.cm/sec)	38.46	35.23	33.68	22.36	24.33	21.34
6	Extrudability (%)	98	95	95	94	95	93
7	Gelation Temperature (ºC)	34.4	34.6	34.4	35.34	36.2	36.1
8	Mucoadhesive strength	28.5	28.65	29.8	33.4	34.6	33.9

TABLE 9 EVALUATION PARAMETERS OF THERMOSENSITIVE GEL CONTAINING EXTRACT OF *QUERCUS INFECTORIA* OLIVER GALLS (BATCH NO. F7 TO F12)

Sr. no.	Parameters	F7	F8	F9	F10	F11	F12
1	Color	Faint Brown	Faint Brown	Faint Brown	Faint Brown	Faint Brown	Faint Brown
2	Odor	Fruity	Fruity	Fruity	Fruity	Fruity	Fruity
3	pH	6.6	6.7	6.7	6.8	6.7	6.7
4	Viscosity(cps)	8860	8980	9090	9500	9590	9890
5	Spreadability (gm.cm/sec)	19.2	17.12	15.33	18.2	24.33	21.34
6	Extrudability (%)	92.2	90.6	88.78	93.6	91.2	88.3
7	Gelation Temperature (ºC)	36.9	37.2	37.4	37.1	36.1	37.3
8	Mucoadhesive strength	36.2	38.40	35.45	38.4	37.6	36.9

Evaluation Parameters for Gel:

Physical Appearance: Out of twelve batches, F1 to F6 gels were clear and transparent whereas, F7 to F12 batches were semi-transparent.

Color: Color all batches was Faint Brown.

Odor: All the batches had fruity smell.

Homogeneity: All batches of formulation were homogeneous.

Taste: The formulated gel was tasteless.

Ph: The pH of all prepared formulations ranges from 6 to 7, which is considered acceptable to avoid the risk of skin irritation upon application to skin and mucus membranes.

Gelation Temperature: At higher temperatures, Polyoxyethylene (POE) and Poly-oxy-propylene (POP) fragments of Poloxamer get cross-linked to yield a gel-like structure. The proportion of Pluronic F68 and Pluronic F127 in the formulation batches was optimized to show the gelation temperature at about 33-37 °C. It was observed that the addition of mucoadhesive polymer increases the gelation temperature. Hence, the prepared formulations ensure the sol-to-gel transformation in the oral cavity and during applications on the skin.

Syringeability: The syringeablity of formulated gel was determined using a 21-gauge needle syringe; the formulated gel from F1 to F6 had easy flow through syringe under normal handling pressure. For F6 to F12, batches required more force.

Extrudability: Among all the gel formulations, F1 to F12 showed more than 90% extrudability, indicating the gel has excellent extrudability.

Viscosity: The observed viscosity of formulations is given in **Tables 8** and **9**. The gel formulation showed increased viscosity with an increase in polymer concentration.

Spreadability: The spreadability of formulated gel increases with increasing concentration of polymer as well as the addition of polymers.

The observed spreadability of all batches is given in **Tables 8** and **9**. The spreadability of F1 to F6 batches is higher than the rest of the batches.

Gelling Capacity: The gelling capacity of formulated gel was determined by visual inspection. The gel shows good gelling capacity. As the concentration of mucoadhesive polymer increases, the gelling capacity increases.

Drug Content Assay: The drug content assay indicated the amount of drug present in the formulation. The range of drug content in formulated gel was found to be 95.5 to 98.8.

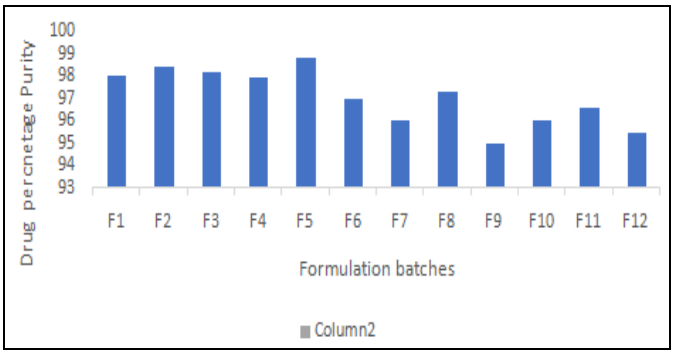

FIG. 14: ASSAY OF FORMULATION

***In-vitro* Drug Released Studies:** The *in-vitro* drug release profile of formulation gel containing extract *Quercus infectoria* Oliver galls was studied. It was found that the release of extract from gel started from 15 min and extended-release up to 4 hr by most of the formulations. This ensures that, as the concentration of polymer increases, the release of extract decreases from gel, as shown in **Fig. 15**. The maximum release was found in F1 and F4, F5 batches.

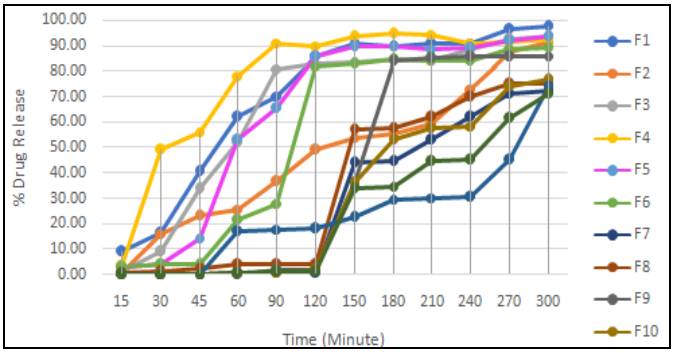

FIG. 15: *IN-VITRO* PERCENTAGE DRUG RELEASE STUDIES

Mucoadhesive Strength: The mucoadhesive strength was performed on the modified physical balance to measure the mucoadhesive strength required to detach the gel form the intestine. The mucoadhesive strength of formulated batches was found to be in range of 28 to 37.

From the mucoadhesive strength, the mucoadhesive force was calculated and found to be in the range of 2.7 to 3.6. From mucoadhesive force, it revealed that the gel has good mucoadhesive properties. The mucoadhesive strength was given in **Table 8** and **9**.

Stability Study: Stability testing aims to provide evidence on how the drug product varies with time under the influence of temperature and relative humidity. The stability study was performed by evaluating its appearance, color, pH, and spreadability. The F1 to F6 batches were found to be stable from the twelve batches.

Optimization of Batch: After analysis of all batches of formulations for their evaluation parameters like pH, viscosity, spreadability, extrudability, syringability, gelation temperature, drug content assay, *in-vitro* drug release study, mucoadhesive strength, and stability study, the formulation gel, batch F5 from the extract of galls of *Quercus infectoria* Olivier showed good results. The batch F5 optimized with the good viscosity, spreadability, and Extrudability, mucoadhesive strength, release of drug is more, and the gel is stable hence it is used for further evaluation like *in-vitro* skin irritation studies and *in-vitro* antibacterial test. The polymer used in F5 formulation was used for formulation gel of tannic acid, gallic acid, ellagic acid, and silver nanoparticles of extract of *Quercus infectoria* Olivier galls.

Antibacterial Activity of Formulated Gel: The *in-vitro* antibacterial activity of formulated gel was studied against the *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* microbes and the zone of inhibition was shown in **Table 10.**

The zone of inhibition observed for an extract of galls of *Quercus infectoria* was 24 mm, 37 mm, 34 mm against *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Escherichia coli,* respectively. The zone of inhibition observed for tannic acid gel, gallic acid gel and ellagic acid gel concluded that these three constituents of the extract showed antibacterial activity and synergistic antibacterial activity. The synthesized silver nanoparticles of galls extract *Quercus infectoria* (QI AgNP) were evaluated against *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Escherichia coli* and

showed zone of inhibition 16 mm, 19 mm and 20 mm respectively. The QI AgNP in gel was 5 mg and much less than the extract concentration in F5 batch.

The zone of inhibition of marketed gel containing tannic acid were also studied.

Marketed gel 1-Orasep LA Gel-contain 5% w/w of tannic acid along with lignocaine 2%. Marketed gel 2-Orasep Gel contains 5% w/w of tannic acid, choline salicylate 8%, and Benzalkonium chloride 0.02%.

The marketed Gel 1 showed the zone of inhibition is 25 mm, 23 mm, 40 mm, and marketed gel 2 showed 45 mm, 22 mm, 37 mm against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli.*

TABLE 10: ZONE OF INHIBITION OF FORMULATED GEL

Sr. no.	Formulation	Zone of inhibition (mm)		
		E. coli	***S. aureus***	***P. Aeruginosa***
1	Blank gel	-	-	-
2	Streptomycin	26	29	20
3	QI. Extract gel (F5 Batch)	34	24	37
4	Tannic acid Gel	22	24	27
5	Gallic acid Gel	41	37	34
6	Ellagic acid Gel	21	18	18
7	QI AgNP gel	20	16	19
8	Marketed gel 1	40	25	23
9	Marketed gel 2	37	45	22

FIG. 16: ANTI-BACTERIAL ACTIVITY OF NINE BATCHES AGAINST *STAPHYLOCOCCUS AUREUS*

FIG. 17: ANTIBACTERIAL ACTIVITY OF NINE BATCHES AGAINST *PSEUDOMONAS AERUGINOSA*

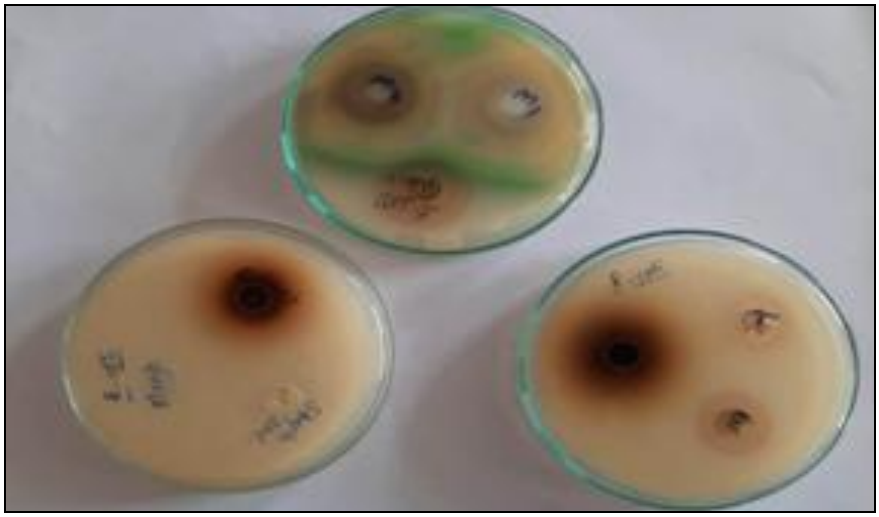

FIG. 18: ANTIBACTERIAL ACTIVITY OF NINE BATCHES AGAINST *ESCHERICHIA COLI*

FT-IR of Formulated Gel Containing Extract of *Quercus infectoria* Galls: The FT-IR studies of *Quercus infectoria* extract and its *in-situ* gel were done. The spectrum of *Quercus infectoria* extract showed peaks at 3284 cm^{-1} (H-bonded -OH stretching), 1687 cm^{-1} (carbonyl group), 1445, 1553 and 1607 cm^{-1} (aromatic C=C) and 1186 cm^{-1} (C-O stretching).

Characteristic peaks in both extract and gel are 3284 cm^{-1}(H-bonded -OH stretching), 1687 cm^{-1} (conjugated carbonyl group), and 1186 cm^{-1} (C-O stretching) which suggests the presence of carboxylic acid or ester functional groups. Thus, the spectra of the gel don't show any additional peaks suggesting that there is no interaction between the drug and excipient.

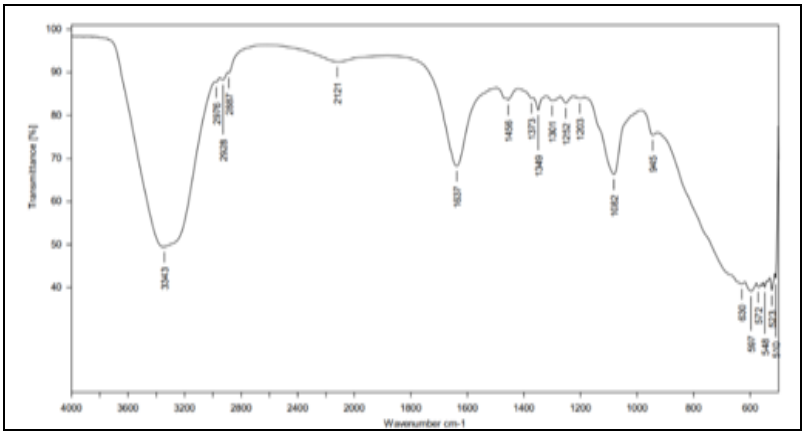

FIG. 19: FT-IR SPECTRUM OF FORMULATED GEL CONTAINING EXTRACT OF *QUERCUS INFECTORIA* GALL

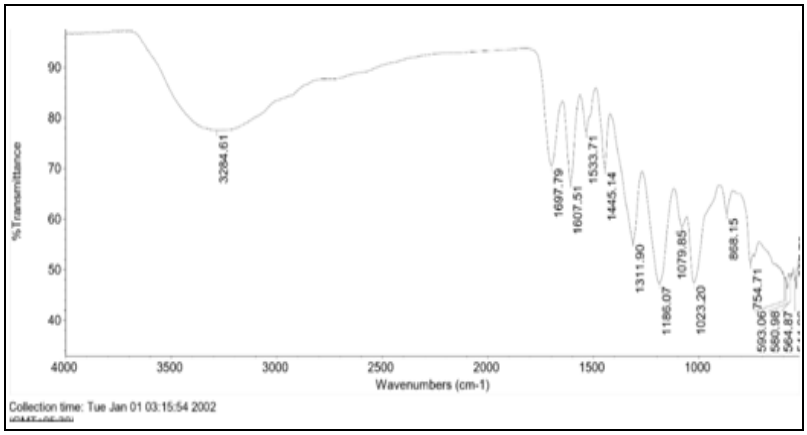

FIG. 20: FTIR GRAPH OF EXTRACT OF *QUERCUS INFECTORIA* GALLS

Skin Irritation Test (HET -CAM) Test Method: Patients' absence of skin irritation in gel formulation is acceptable. Skin irritation test was performed on optimized gel using *in-vitro* (HET -CAM). Hen's Egg Test -Chorioallantois Membrane (HET -CAM) Test with a stand-by time more than 5 min with 0 irritancy score was observed. According to the result, the formulated gel was non-irritant to skin and soft tissue. All gel formulations were found to be free from irritation.

TABLE 11: SCORING SCHEME FOR IRRITATION OF (HET -CAM) TEST

Effect	Score		
	0.5 min	2 min	5min
Lysis	-	-	-
Hemorrhage	-	-	-
Coagulation	-	-	-

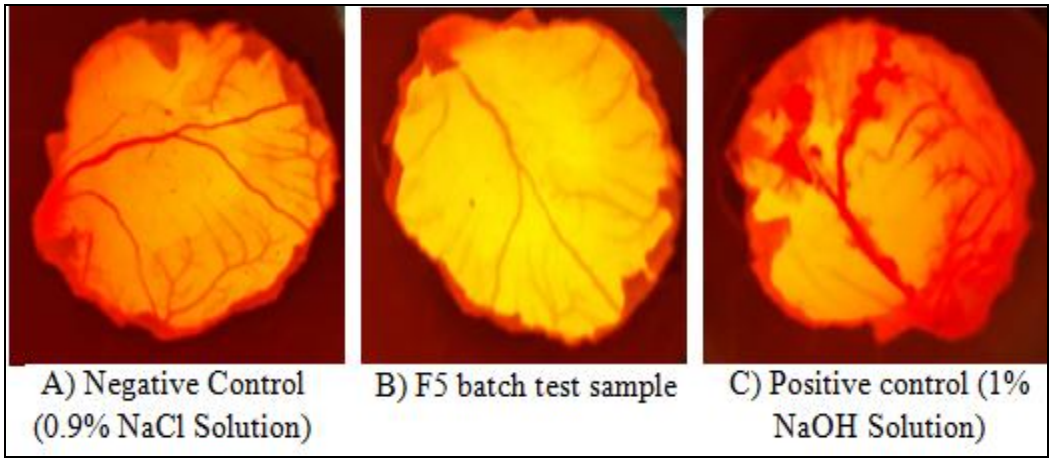

FIG. 21: (HET -CAM) TEST RESULT

Evaluation of Synthesis Silver Nanoparticle of Galls extract of *Quercus infectoria:* The synthesis of Silver nanoparticle of galls extract *Quercus infectoria* (QI. AgNP) with ultrasonic irradiation was rapid and successful reaction.

In this reaction, the constituent present in extract act as capping and reducing agent, reducing Ag^{+} into silver nanoparticle.

The colour change from yellow to brownish gray indicates that the silver nanoparticle was formed.

UV Visible Spectra: Fig. 22 shows UV-Visible spectra' absorption as a time function. The QI. AgNP showed maximum absorption at 432 nm. Results revealed that Surface Plasmon Resonance (SPR) intensities of Ag nanoparticles steadily increase with the passage of time. Surface Plasmon Resonance occurs when the electromagnetic wave is resonant with the collective oscillation of the conduction-free electrons, then produces strong absorption bands at around 432 nm.

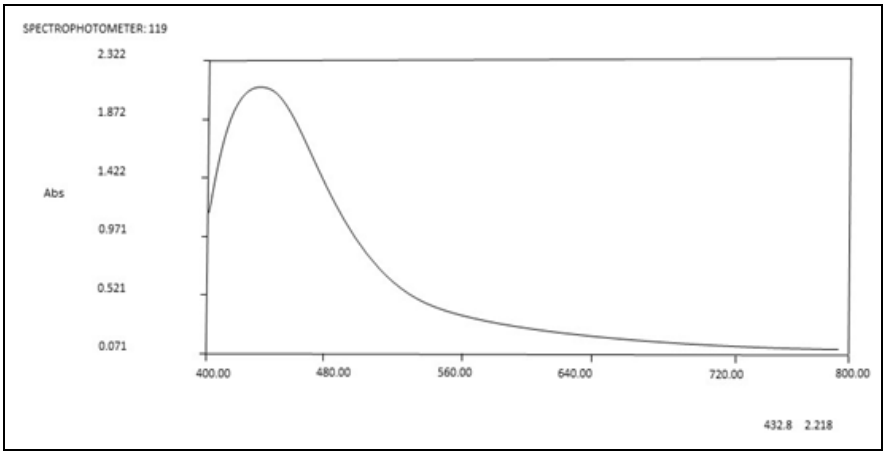

FIG. 22: UV-VISIBLE SPECTRA OF QI.AGNP

XRD of Silver Nanoparticles: Fig. 23 depicts X-ray diffraction pattern of AgNPs. The diffraction peaks were located at 2θ=38.14, 44.22, 64.64, and 77.50, which represents the (111), (200), (220) and (311) Bragg's reflections of the face-centered cubic (fcc) structure of silver nanocrystals. Furthermore, the diffraction peaks were sharp, indicating Ag nanoparticles with large dimensions. The average crystal size of AgNPs can be estimated according to the diffraction reflections using the Debye–Scherrer formula D=0.9λ/b.cos θ, where D is the average crystalline size, k is the wavelength of Cu Kα (0.15405 nm), b is the full width at half maximum (FWHM) of the diffraction peaks, and θ is the diffraction angle. The crystal size was estimated to be 72 nm. Apart from the assigned peaks for silver nanoparticles, the appearance of two assigned peaks may be due to the presence of crystallized bioorganic compounds on the nanoparticles.

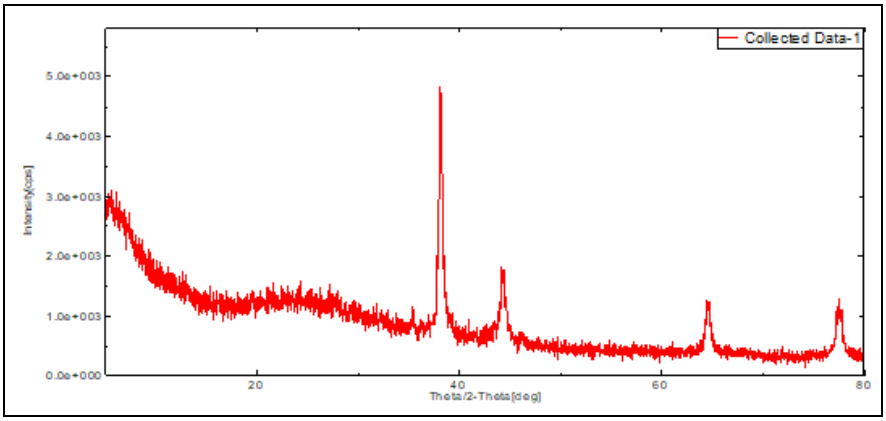

FIG. 23: XRD SPECTRA OF SILVER NANOPARTICLES OF EXTRACT OF *Q. INFECTORIA*

Particle size Analysis and Zeta Potential: The analysis of silver nanoparticle of extract of *Quercus infectoria* was done using Microtrac instruments, and the particle size was estimated as 72 nm, and size distribution was plotted as in **Fig. 24**. Zeta potential was found to be -5.9 Mv.

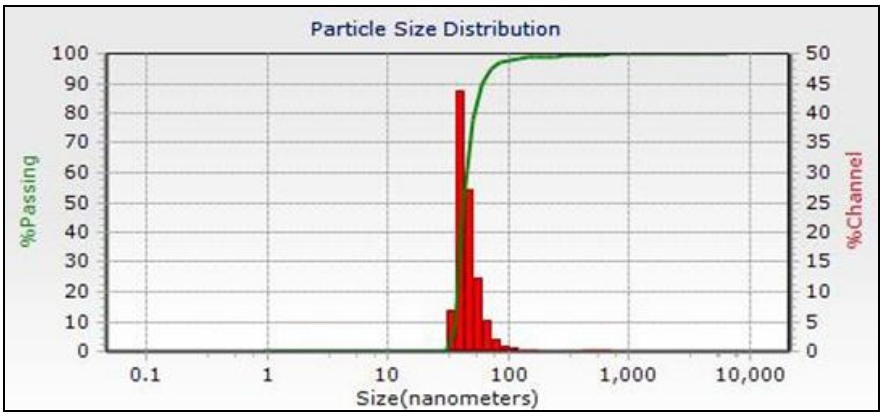

FIG. 24: PARTICLE SIZE DISTRIBUTION GRAPH OF SILVER NANOPARTICLES OF *Q. INFECTORIA*

FTIR of Silver Nanoparticles: FTIR of extract and nanoparticles was evaluated to understand metal interaction with compounds present in the extract and its nanoparticles. The spectra of FTIR of extract of *Quercus infectoria* and synthesis silver nanoparticles are given in **Fig. 20** and **Fig. 25.** After reaction of galls extract with $AgNO_3$, there was a shift in the peaks of 3284 cm^{-1} (–OH "hydrogen bonded alcohols and phenols"), 1533 cm^{-1} (C=C), 1319 cm^{-1} (C—C), 1186 cm^{-1} (stretching C–O "alcohols , ethers, carboxylic acid and ester"), 1025 cm^{-1} (C–O–C) and 754 cm^{-1} (C–H "aromatic compounds"). These shifts in the functional groups indicate that AgNPs are bounded to the bio-molecules. The FT-IR analysis of the galls extract of *Quercus infectoria* demonstrated the presence of alkenes, aromatic compounds, alkanes, hydrogen-bonded alcohols, and phenols. The functional groups may be involved in reducing the Ag^+ to Ag^0 and making it highly stable.

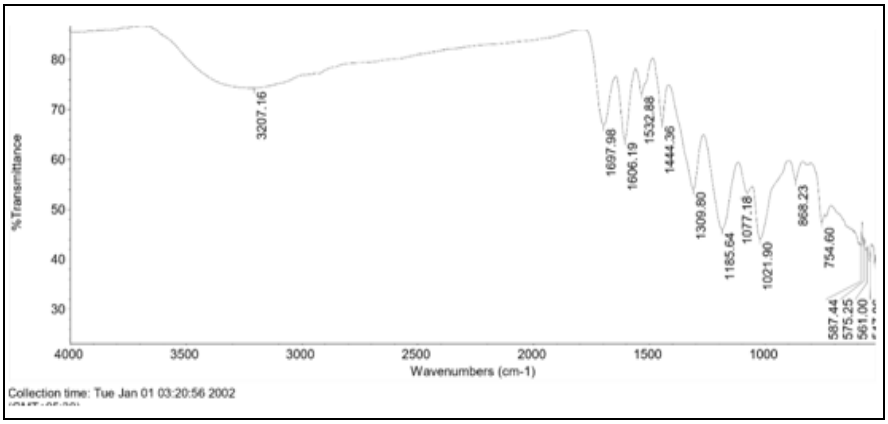

FIG. 25: FTIR OF SYNTHESIS SILVER NANOPARTICLES OF EXTRACT OF *Q. INFECTORIA*

DSC Curve of Silver Nanoparticle of Extract of *Q. infectoria*: DSC (Mettler Toledo) was performed of aqueous extract of *Quercus infectoria,* which provided the melting point of gallic acid (250-252 °C), ellagic acid (290-292 °C) and tannic acid (208-210 °C) as compared with the literature.

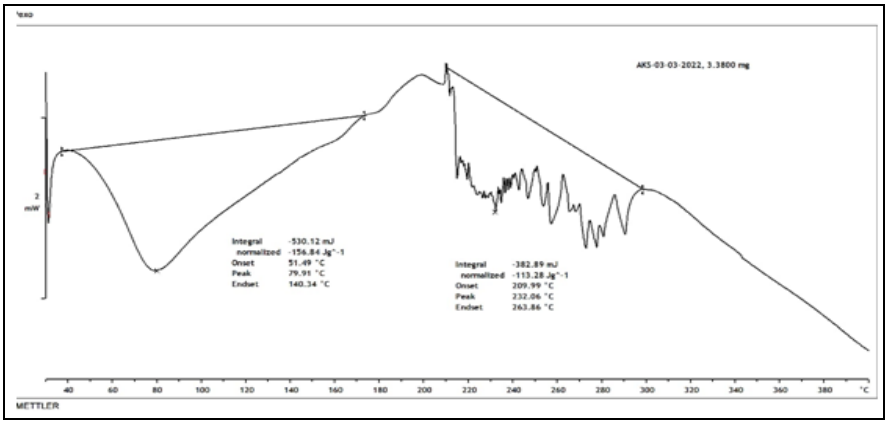

FIG. 26: DSC CURVE OF SILVER NANOPARTICLE OF EXTRACT OF *Q. INFECTORIA*

CONCLUSION: Gel-based drug delivery methods have recently been used to improve the therapeutic effectiveness of topical medicines. *Quercus infectoria* galls have been used for a long period against bacterial diseases. From ancient times *Quercus infectoria* galls powder was used for dental problems. The topical and buccal paths of a containing *Quercus infectoria* were chosen to maximize drug concentration at the site of action.

The objective of the research work was to design an antibacterial dosage form using *Quercus infectoria* Olivier. Ethanolic extract was used for the formulation of gel and silver nanoparticles. From the HPLC analysis, the active constituent present in *Quercus infectoria* Olivier galls in 10 g of extract contain 4.03 mg of Tannic acid, 2.9 mg of Gallic acid and 1.31 mg of Ellagic acid. From the *in vitro* antibacterial study, these three active constituents present in the extract show synergistic antibacterial activity. Mucoadhesive thermosensitive sol-gel systems were formulated using Pluronic F127, Pluronic F68, and Carbopol 971P, Carbopol 974P. From the *in vitro* release study, it was observed that, as the concentration of polymer increases, the rate of release of the drug decreases. Due to more cross-linking between polymer, retarded rate of diffusion resulting in fewer paths for the water drags and slower penetration. Formulated gel delivered the drug in more than 4 h.

The batch F5 optimized has good viscosity, spreadability, extrudability, mucoadhesive strength, the release of drug was and the gel was stable; hence it is used for further evaluation like in vitro skin irritation study and in vitro antibacterial test. The skin irritancy test was done by the HET-CAM test, which also shows no irritancy after the stated period. The antibacterial activity of formulated

thermosensitive mucoadhesive gel containing extract of *Quercus infectoria* galls compared with the two marketed formulations. Marketed formulations contain tannic acid as an active pharmaceutical ingredient used to treat mouth ulcers and gum disorders. The in-vitro antibacterial activity formulated F5 batch results were comparable with the marketed formulation. And F5 batch shows more antibacterial activity than the marked formulation. The mouth ulcer is painful oval sores form in the mouth of the cheek area, near gums, and tongue areas. They are mostly caused by nutrition deficiency, poor oral hygiene, and infection. The formulated gel was used to treat mouth ulcer and gum disorders.

Silver nanoparticles were effectively produced using an extract of *Quercus infectoria* galls and ultrasonic irradiation. Interestingly, the proposed approach for making silver nanoparticles is simple, quick, cost-effective, and environmentally benign. Silver nanoparticles derived from *Quercus infectoria* galls extract, and ultrasonic irradiations had a spherical form and a size of 72 nm.

Silver nanoparticles and *Quercus infectoria* galls extract have a strong potential for antibacterial activity against Gram-positive and Gram-negative bacteria. AgNPs were found to have higher antibacterial activity than gall extract. It also suggests that it might be employed in a variety of biomedical applications and improves people's lives.

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CONFLICTS OF INTEREST: None

REFERENCES:

1. Najmi A, Javed SA, Bratty MA and Alhazmi HA: Modern Approaches in the Discovery and Development of Plant-Based Natural Products and Their Analogues as Potential Therapeutic Agents. Molecules 2022; 27: 349.
2. Jamadar MJ: Preparation and Evaluation of Herbal Gel Formulation. SGVU Journal of Pharmaceutical Research & Education 2017; 1: 201-224.
3. Chen T, Yu WH, Izard J and Baranova O: The Human Oral Microbiome Database: a web accessible resource for investigating oral microbe taxonomic and genomic information. Database 2010; 2010: 1-10.
4. Alghamdi S: Isolation and identification of the oral bacteria and their characterization for bacteriocin production in the oral cavity. Saudi Journal of Biological Sciences 2022; 9: 318–323.
5. Dalessandri D, Zotti F, Laffranchi L, Migliorati M and Isola G: Treatment of recurrent aphthous stomatitis (RAS; aphthae; canker sores) with a barrier forming mouth rinse or topical gel formulation containing hyaluronic acid: a retrospective clinical study. BMC Oral Health 2019; 19: 153.
6. William CE: Trease and Evans Pharmacognosy. Edinburgh Saunders Elsevier 16th Edition 2009.
7. Ahmad W: Mazu (*Quercus infectoria*, Oliv)- An overview. Indian Journal of Unani Medicine 2011; 4: 17-22.
8. Ansari SAH: Ethno-Pharmacology of *Quercus infectoria* Olivier galls: A review. Hippocratic Journal of Unani Medicine 2016; 2(3): 105-118.
9. Misba N: *Quercus infectoria* (Mazu): Review. World Journal of Pharmaceutical Research 2017; 6: 178-185.
10. Rathod H: A Review on pharmaceutical gel. Acta Scientifica International Journal of Pharmaceutical Science 2015; 1: 33-47.
11. Ministry of Health and Family and Welfare, the Indian Pharmacopoeia Commission Ghaziabad, Indian Pharmacopoeia. 10th edition, 2010, Volume 2: 725-726.
12. 12. Russo E, Carla V: Poloxamer Hydrogels for Biomedical Applications. Pharmaceutics 2019; 11: 671.
13. Ruel E and Gariepy JC: Leroux: *In-situ* forming hydrogels review of temperature-sensitive systems. European Journal of Pharmaceutics and Biopharmaceutics 2004; 58: 409–426.
14. Rezazadeh F, Nazhvani AD, Abolmaali SS, Nazhvani FD and Bonyani A: Efficacy of silver nanoparticle gel on healing of traumatic oral ulcers compared with triamcinolone oral paste: An experimental study on rats. Dent Res J 2021; 18: 33.
15. Mathur P, Jha S, Ramteke S and Jain NK: Pharmaceutical aspects of silver nanoparticles, Artificial Cells, Nanomedicine and Biotechnology 2018; 46: 115-126.
16. Chen M, Yang Z and Hongmei W: Antimicrobial activity and the mechanism of silver nanoparticle thermosensitive gel. International J of Nanomedicine 2011; 6: 2873–2877.
17. Khatamifar M and Fatemi SJ: Green and eco-friendly synthesis of silver nanoparticles by *Quercus infectoria* galls extract: thermal behavior, antibacterial, antioxidant and anticancer properties. Particulate Science and Technology 2021; 2021: 1-6.
18. Kokate CK and Purohit AP: Gokhale: Textbook of Pharmacognosy. Nirali Prakashan Edition 2015; 51.
19. Thorat SY, Sarvagod AM, Kulkarni S and Hosmani A: Treatment of Mouth Ulcer by Curcumin Loaded Thermoreversible Mucoadhesive Gel: A Technical Note. Int J Pharm Science 2015; 7: 399-402.
20. Zachariah SM and Kumar NM: Phytochemical Screening, Formulation and Evaluation of Dried Galls of *Quercus infectoria* Oliv. Int J Pharm Sci Rev Reserch 2014; 26: 125-130.
21. Suzilla WY: Formulation and evaluation of antimicrobial herbosomal gel from *Quercus infectoria* extract. IOP Conf. Serres: Mater Sci Engineering 2020; 736: 022030.
22. Sanjana A and Mohammed GA: Preparation and evaluation of *in-situ* gels containing hydrocortisone for the treatment of aphthous ulcer. Journal of Oral Biology and Craniofacial Research 2021; 11: 269–276.

23. Jain HK: Formulation and Evaluation of an Antimicrobial Mucoadhesive Dental Gel of *Azadirachta indica* and *Glycyrrhiza Glabra*. International Journal of Applied Pharmaceutics 2019; 11: 176-184.
24. Aslani A: Design, Formulation and Evaluation of an Oral Gel from *Punica granatum* Flower Extract for the Treatment of Recurrent Aphthous Stomatitis. Advanced Pharmaceutical Bulletin 2016; 6: 391-398.
25. Pandey A and Jagtap JV: Formulation and Evaluation of *In-vitro* Antimicrobial Activity of Gel Containing Essential Oils and Effect of Polymer on Their Antimicrobial Activity. Int J Pharm Sci 2010; 3: 234-37.
26. Majeed RM and Hussei NN: Detection the Antimicrobial Activity of AGNPs Synthesized by *Quercus infectoria* Plant. Plant Archives 2020; 20: 579-584.
27. Sharma T, Gupta SK, Dwivedi S and Dubey R: Formulation and evaluation of in situ herbal gel containing aqueous and methanolic extract of fruits of *Quercus infectoria* Oliv. for vaginal application. Journal of Drug Delivery & Therapeutics 2018; 8: 495-503.

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