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Formulation and evaluation of *in situ* herbal gel containing aqueous and methanolic extract of fruits of *Quercus infectoria* Oliv. for vaginal application

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ABSTRACT

Conventional vaginal dosage forms frequently produce leakages and drip. There is a need for the development of innovative vaginal formulation technology that fulfills certain criteria such as desirable product dispersion throughout the vagina, retention for intended intervals, and adequate release of drug. These features can be achieved by the use of bioadhesive based novel delivery systems. *In-situ* gelation is a process of gel formation at the site of application after the composition or formulation has been applied the site. Formulation and evaluation of one such bioadhesive based novel drug delivery system for an effective and patient friendly use of an antifungal drug to formulated In-situ gel. *Quercus infectoria* is medicinally important plant grown wildly in India and is useful in the treatment of fungal and microbial infection by tribal's of India. The plant is used by tribal women to treat vaginal infection as mentioned in folk-lore. Therefore, the present plant as selected to formulate *in-situ* herbal gel using *Quercus infectoria* as active ingredients for the treatment of vaginal infection.

Keywords: Herbal Gels, *Quercus infectoria*, bioadhesive, vaginal drug delivery

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INTRODUCTION

The conventional dosage forms such as preformed gel and solutions have limitations that they do not remain for long time at the site of application and needs frequent dosing. Direct application of gels onto the infected sites of the vagina might be difficult, inconvenient as well as have frequent dosing because the conventional gels do not remain for long time at the site of application. A new and recent approach is to try to combine advantages of both gels and solution so that an accurate dose can be administered with ease of administration. These formulations remain to a solution state before administration but transforms to gel after administration in to vaginal cavity.[1-2] *In situ* gel has broad drug absorption peak and a longer drug residence time as compared to conventional dosage form. For a better therapeutic efficacy and patient compliance, mucoadhesive, thermosensitive and prolonged release vaginal gel was formulated for the treatment of vaginitis.

Nowadays, *in situ*-gelling liquids have also proved as more convenient dosage forms for local applications because they are easy to administer into desired body cavities. To achieve desirable therapeutic effect, vaginal delivery systems need to reside at the sites of infection for a prolonged period. The conventional formulations such as solutions, suspensions, ointments, etc., shows some constraints such as increased elimination, high variability in efficiency which reduces their bioavailability. *In situ* activated gel forming systems are

liquid upon instillation and undergo phase transition in the vagina to form a viscoelastic gel in response to environmental changes such as change in temperature and pH.[3-4] Hence, it offers higher efficacy and bioavailability as compare to other conventional dosage form.

Plant Profile

Botanical name*: Quercus infectoria* Oliv.

Synonyms: *Quercus carpinea* Kotschy ex A.DC., *Quercus grosseserrata* Kotschy ex Wenz., *Quercus puberula* O.Schwarz, *Quercus thirkeana* K.Koch

Local name: Oak, Majuphal

Family: Fagaceae

Description[5-7]

Quercus infectoria or locally known as Manjakani in Malaysia is a small tree native of Greece and Asia Minor, with four to six feet in height. The stems are crooked, shrubby looking with smooth and bright-green leaves borne on short petioles of 1 to 1.5 inches long. The leaves are bluntly mucronate, rounded, smooth, unequal at the base and shiny on the upper side. Meanwhile, Quercus infectoria galls are corrugated and can be used as a thickener in stews or mixed with cereals for making bread.

Chemical constituents[8-9]

The galls from *Quercus infectoria* contain the highest naturally occurring level of tannin, approx. 50–70%, syringic acid, β-sitosterol, amentoflavone, hexamethyl ether, isocryptomerin, methyl betulate, methyl oleanate and hexagalloyl glucose. They also contain 2-4% each of gallic and ellagic acid that are polymerized to make tannins. Tannins have been used for hundreds of years for medical purposes and are currently indispensable in dermatology and have been used for tanning of leather.

Tannins comprise a large group of natural products widely distributed in the plant kingdom. They have a great structural diversity, but are usually divided into two basic groups: the hydrolyzable type and the condensed type. Hydrolyzable tannins include the commonly occurring gallic and ellagic acid contained in the nutgalls.

Hydrolyzable tannins are present in many different plant species but are found in particularly high concentrations in nutgalls growing on Rhus semialata (Chinese and Korean gallotannins) and Quercus infectoria (Turkish and Chinese gallotannins), the seedpods of Caesalpinia spinosa (Tara tannins), and the fruits of Terminalia chebula. The gallic and ellagic acid hydrolyzable tannins react with proteins to produce typical tanning effects; medicinally, this is important to treat inflamed or ulcerated tissues. They also contribute to most of the astringent property of manjakani and are therefore great for vaginal tightening.

Although both types of tannin have been used to treat diseases in traditional medicine, the hydrolyzable tannins have long been considered official medicinal agents in Europe and North America. They have been included in many pharmacopoeias, in the older editions in particular, and are specifically referred to as tannic acid. These were recommended for treatment of inflammation and ulceration, including topical application for skin diseases and internal use for intestinal ulceration and diarrhea. Now, the condensed tannins also have important medicinal roles, such as stable and potent antioxidants. In China, tannin-containing substances, such as galls, pomegranate rinds, and terminalia fruits, are used in several medicinal preparations.

Pharmacology[10-14]

The galls of *Quercus infectoria* have also been pharmacologically documented to possess astringent, antidiabetic, antitremorine, local anaesthetic, antiviral, antibacterial, antifungal, larvicidal and anti-inflammatory activities. The main constituents found in the galls of Quercus infectoria are tannin (50-70%) and small amount of free gallic acid and ellagic acid.

The wide range of pharmacological activities of this plant might support the efficacy of extract preparation of *Quercus infectoria* that are widely used in Malaysia for treating many kinds of health problems since many decades ago. The nutgalls have been pharmacologically documented on their antiamoebic, anticariogenic and anti-inflammatory activities, to treat skin infections and gastrointestinal disorders.

Traditional Uses[15]

- It has been used as dental powder and in the treatment of toothache and gingivitis.
- Traditionally dried fruit powder used in the treatment of women disorders
- Whole plant is useful in the treatment of microbial infection

Figure 1: *Quercus infectoria* Oliv. Fresh Fruits

Figure 2: *Quercus infectoria* Oliv. Dried Fruits

MATERIAL AND METHODS

Selection, Collection and authentication of Plant material

Quercus infectoria Oliv. commonly known as Majuphal (H) and Nutgalls (E) is medicinal herb having a pivotal importance in traditional system of medicine. The plant has been reported to be used in fungal infection. Keeping this view the present plant was selected to formulate effective herbal preparation. The dried fruits of *Quercus infectoria* Oliv. was purchased from local market of Indore district of Madhya Pradesh and was authenticated by Dr. Neeta Singh, Professor & Head, Botany Department, Govt. College, Bhopal, (M.P.), A voucher specimen No. QF/F/189 was coded for further reference.

Extraction of Plant material

The methanolic extract was prepared by immersing 100 g of the *Quercus infectoria* Oliv. fruit powder in 500 ml of absolute methanol (Merck) for 72 h in 50°C water bath. The mixture was then filtered using Whatman filter paper No 1. The filtrates were concentrated under reduced pressure using a rotary evaporator at temperature of 55°C. The resulting pellet was finally pounded to dryness at 50°C for 48 h to produce a powdered and brown crude extract. The aqueous extract was prepared by immersing 100 g of *Quercus infectoria* Oliv. fruit powder in 500 ml of sterile distilled water for 72 h in 50°C water bath. The mixture was then pre-filtered using a coffee filter and then filtered using Whatmann filter paper No 1. The filtrates were concentrated under reduced pressure using a rotary evaporator at a temperature of 80°C. The resulting pellet was freeze-dried at −50°C under vacuum until the pellet produce a fine crystal-like crude extract. The crude extracts were stored in airtight jars at 4°C.[16]

Formulation of *In situ* Herbal gel

Poloxamer (PLX) 407 & 188 was dissolved slowly with stirring in 60 mL of demineralized water for 1 h to avoid agglomeration Then disodium edetate and triethanolamine were dissolved in 10 mL of demineralized water separately and stirred for 10 min. Mixed HPMC K 100M in 12 mL of demineralized water with stirring for 10 min. Disodium edetate and triethanolamine solution were added to PLX solution and the pH was then adjusted to 7.4 by stirring the solution for 10 min. Then HPMC K 100 M solutions were added with stirring for 10 min until a clear consistent gel base was obtained. At last weighed quantity of extract was added with continuous stirring and volume was adjusted with DM water. Twelve *in situ* herbal gel formulations was prepared using AEQIF (aqueous fruit extract of *Quercus infectoria* Oliv.*)* and MEQIF (methanol fruit extract of *Quercus infectoria* Oliv.*)* as per drug formulation manual[17] as mentioned in Table 1.

Table 1: Formulation of in-situ herbal gel

Formulation Code	AEQIF (g)	MEQIF (g)	PLX 407 (g)	PLX 188 (g)	Triethanol amine (g)	Disodium EDTA (g)	HPMC K100M (g)	D.M. water (100 g)
ISG-1	0.5	-	5	30	1.5	0.005	0.25	100
ISG-2	1.0	-	10	25	1.5	0.005	0.25	100
ISG-3	1.5	-	15	20	1.5	0.005	0.50	100
ISG-4	2.0	-	20	5	1.5	0.005	0.50	100
ISG-5	2.5	-	25	10	1.5	0.005	0.75	100
ISG-6	3.0	-	30	5	1.5	0.005	0.75	100
ISG-7	-	0.5	30	5	1.5	0.005	0.25	100
ISG-8	-	1.0	25	10	1.5	0.005	0.25	100
ISG-9	-	1.5	20	15	1.5	0.005	0.50	100
ISG-10	-	2.0	5	20	1.5	0.005	0.50	100
ISG-11	-	2.5	10	25	1.5	0.005	0.75	100
ISG-12	-	3.0	5	30	1.5	0.005	0.75	100

Abbr.: AEQIF: Aqueous extract *Quercus infectoria* Oliv. fruit; MEQIF: Methanolic extract of *Quercus infectoria* Oliv. fruit: Poloxamer (PLX); HPMC: Hydoxyl prpyl methyl cellulose; DM: De-mineralized water

Evaluation of *In situ* Herbal gel[17-19]

Physical evaluation

The appearance of the formulation was observed which included clarity and transparency was determined visually.

Determination of pH

The pH of the gel was determined using a calibrated pH meter at 4 °C. The readings were taken for an average of 3 samples.

Gelling capacity

The gelling capacity was measured by visual method. 100µl sample was placed in a vial containing 2 ml of artificial tear fluid freshly prepared and equilibrated at 35 ^{0}C and then visually assessing the gel formation and noting the time taken for gel formation.

Gelation temperature

The gelation temperature was determined using the test-tube-inverting method. A volume of 2 ml of the *in-situ* gel was placed in a test tube, which was then immersed

in a water bath at 15 °C. The temperature of the water bath was then gradually increased, samples were examined every 2 minutes, and the gelation temperature was recorded when the gel stops fowing upon test tube inversion at 90°. The readings were taken for an average of 3 samples

Viscosity

Viscosity of sols was measured using Brookfield viscometer (model DVII, Engineering Laboratories, Middleboro, MA) spindle no 01 at 20 r.p.m. at temperature 4 °C and 37 °C. The experiment was carried out in triplicate.

Syringeability study

The ability of the prepared formulations to fow easily through a syringe of 21 gauge needle was assessed using the method employed by Maheshwari. One ml of the cold gel was filled in 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 20 g of gel was pressed firmly at the crimped end and a clamp was applied to prevent any roll back. 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.

Spreadability

Two sets of glass slides of standard dimensions were taken. The herbal gel formulation was placed over one of the slides. The other slide was placed on the top of the gel, such that the gel was sandwiched between the two slides in an area occupied by a distance of 7.5 cm along the slides. Hundred g weight of gel was placed on the upper slides so that the gel was between the two slides was pressed uniformly to form a thin layer. The weight was removed and the excess of gel adhering to the slides was scrapped off. The two slides in position were fixed to a stand without slightest disturbance and in such a way that only upper slides to slip off freely by the force of weight tied on it. A 20 g weight was tied to the upper slide carefully. The time taken for the upper slide to travel the distance of 7.5 cm and separated away from the lower slide under the influence of the weight was noted. The experiment was repeated for three times and the mean time was taken for calculation.

Spreadability was calculated by using the following formula:

$$S = m \times l/t$$

where, S= spreadability, m-weight tied to upper slides (20 g), l- length of the glass slide (7.5 cm), t- time taken in sec.

Drug content

Each formulation (1 g) was taken in a 50 mL volumetric flask and made up to volume with methanol and shaken well to dissolve the active constituents in methanol. The solution was filtered through Whatman filter paper and 0.1 mL of the filtrate was pipetted out and diluted to 10 mL with methanol. The content of active constituents was estimated spectro photometrically by using standard curve plotted at 280 nm.

***In-Vitro* release studies**

A sample of 1 ml of gel was placed into a dialysis membrane 7 cm long. Bags were then suspended in 50 ml of (ethanol: water 1:1) preheated at 37± 0.5 °C in shaking water bath at 37 °C and 25 strokes per min. At predetermined time intervals, one milliliter sample was withdrawn and replaced with an equal volume of fresh medium. The whole release media were changed and replaced with fresh media every day (24 h) during the release studies duration (up to one week). Samples were diluted and analyzed using an UV spectrophotometer for tannins concentration at λ 280 nm. The cumulative amount of drug released was calculated based on a calibration curve. All experiments were done in triplicate.

Stability testing of *In situ* Herbal gel[20]

The main objective of the stability testing is to provide evidence on how the quality of the drug product varies with time under the influence of temperature and humidity. The stability study for the insitu herbal gel formulation was done as per ICH guidelines in a stability chamber for a period of 6 months. The selected *in-situ* herbal gel formulation of extract was loaded in a humidity chamber at 25°C ± 2°C/60% RH ± 5% RH, 32°C ± 2°C/60% RH ± 5% RH and 40°C ± 2°C/75% RH ± 5% RH.

Antifungal activity (Anti-*Candida* activity)[21]

Fungal strain

Culture Collection (ATCC) strains of *Candida albicans* was used in this study.

Screening by disc-diffusion method

The standardized test inoculum was spread in three directions onto the surface of the Mueller Hinton agar using a sterile cotton swab. The optimized herbal formulation ISG-3 & ISG-9 discs were placed on the inoculated agar surface along with negative and positive control within 15 min of inoculation. Disc impregnated with sterile distilled water was used as negative control, while amphotericin B disc (10 µg) was used as positive control. The test was done in triplicate. All plates were incubated at 35°C for 24 h. The anti-*Candida* activity was observed from the size of the inhibition zone diameter surrounding the disc measured in millimeters (mm).

Statistical analysis

All the values were statistically analyzed by one-way analysis of variance (ANOVA) followed by student t-test. Comparison between positive control (standard) and treated were considered to be significant (*P<0.001). All values are expressed as mean ± SEM.

RESULTS AND DISCUSSION

The fruits of plant were collected; dried and powdered plant material was extracted with methanol and water. The percentage yield of aqueous extract was found to

be 12.23 % w/w is more than the methanolic extract 9.87% w/w. Twelve different batches of formulation were prepared using aqueous and methanolic extract of *Quercus infectoria* fruit.

ISG-1 to ISG-6 was formulated using different concentration (0.5, 1.0, 1.5, 2.0, 2.5 & 3.0) of aqueous extract of *Quercus infectoria* Oliv, while ISG-7 to ISG-12 was formulated using different concentration (0.5, 1.0, 1.5, 2.0, 2.5 & 3.0) of methanolic extract of *Quercus infectoria* Oliv. In above mentioned both the extract different proportion (5 to 30 g) of PLX 407 & 188 was added along with three different concentrations (0.25, 0.50 & 0.75) of HPMC K 100 M.

Various evaluation parameters such as physical appearance, pH, gelling capacity, gelation temperature, viscosity, Syringeability study, Extrudability, Spreadability and drug content were performed to evaluate the formulated *in-situ* herbal gel.

From the results observed it was concluded that all the prepared herbal gel has good clarity and transparency. The pH so obtained was within the limit as for most of the preparation indented to be used for mucosal vaginal membrane. The pH values of all the formulations were in the close range of neutral pH (7.42-7.88) and hence it caused no skin irritation.

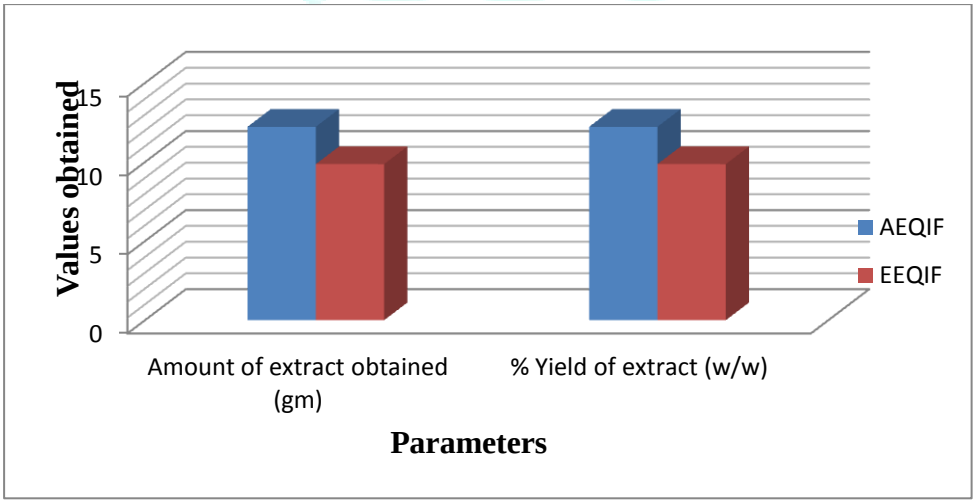
The gelling capacity and gelation temperature were found within the limit. Polymers were included in the designed topical formulations in order to provide a prompt release of drug and to achieve as well as to maintain the drug concentration within the therapeutically effective range. As the concentration of the polymer was 0.25, 0.50 & 0.75 in all gel formulations no major variation in viscosity was observed. Further the value between 0.38 and 0.39 poise was reported to be an ideal viscosity value for topical *in-situ* gel formulation developed using HPMC polymers. Values of the spreadability indicated that the gel formulations are easily spreadable. Among the gel formulations ISG-1 to ISG-12, more than 90% of the contents were extrudable indicating they have excellent extrudability (>90% extrudability: excellent, >80% extrudability: good, >70% extrudability: fair).

The drug content was found to be maximum of 99.87 (ISG-3) followed by 99.78 (ISG-5), 99.43(ISG-11) & 99.27 (ISG-9). From the data obtained it was concluded that the insitu herbal gel formulated using aqueous extract of *Quercus infectoria* Oliv. fruit was found to be more potent and efficacious than methanolic extract of *Quercus infectoria* Oliv. fruit.

Further the same conclusion has been confirmed by the results of *in-vitro* drug release studies. All the four formulation i.e., ISG-3, ISG-5, ISG-9 & ISG-11 was further tested for stability testing for a period of one month at 25°C ± 2°C/60% RH ± 5% RH, 32°C ± 2°C/60% RH ± 5% & 40°C ± 2°C/75% RH ± 5% RH. From the results it was concluded that the formulation code ISG-3 (1.5% AEQIF) is best formulation and shows optimum efficacy at 25°C ± 2°C/60% RH ± 5% RH.

Table 2: Percentage yield of Extract

Sample Code	Weight of crude drug taken (gm)	Amount of extract obtained (gm)	% Yield of extract (w/w)
AEQIF	100	12.23	12.23
MEQIF	100	9.87	9.87



Graph 1: Percentage yield of Extract

Table 3: Evaluation parameters of *in-situ* herbal gel containing aqueous extract of *Quercus infectoria* Oliv.

Evaluation Parameters	Formulation Code					
	ISG-1	ISG-2	ISG-3	ISG-4	ISG-5	ISG-6
Clarity	C	C	C	C	C	C
Transparency	T	T	T	T	T	T
pH	7.68	7.52	7.23	7.44	7.28	7.50
Gelling capacity	-	+	++++	+++	++++	+
Gelation temperature	25.2	28.9	30.1	38.1	32.8	42.4
Viscosity (Poise)	0.3812	0.3882	0.3921	0.3810	0.3914	0.3841
Syringeability study	E	E	E	E	E	E
Extrudability (%)	94.28	97.46	99.88	97.58	99.15	95.42
Spreadability (gcm/sec)	59.13	67.28	74.39	68.49	72.43	60.37
Drug content (%)	97.49	97.48	99.87	98.47	99.78	98.68

Abbr: - : No gelation, + : Gel forms after some time, ++ : Gel forms immediately, +++: Immediate gelation remains for 8 hrs, ++++ : Immediate gelation remains for more than 10hrs. T : Translucent, C: Clear, E: Easily easily syringeable through 21-gauge needle at cold temperature.

Table 4: Evaluation parameters of *in-situ* herbal gel containing methanolic extract of *Quercus infectoria* Oliv.

Evaluation Parameters	Formulation Code					
	ISG-7	ISG-8	ISG-9	ISG-10	ISG-11	ISG-12
Clarity	C	C	C	C	C	C
Transparency	T	T	T	T	T	T
pH	7.61	7.48	7.22	7.51	7.18	7.52
Gelling capacity	-	+	++++	+++	++++	+
Gelation temperature	24.8	29.2	32.7	40.5	35.4	40.8
Viscosity (Poise)	0.3842	0.3871	0.3979	0.3715	0.3998	0.3748
Syringeability study	E	E	E	E	E	E
Extrudability (%)	93.22	97.45	99.65	97.02	99.42	94.28
Spreadability (gcm/sec)	60.21	66.25	75.28	69.18	70.43	59.81
Drug content (%)	95.45	96.28	99.27	97.25	99.43	97.42

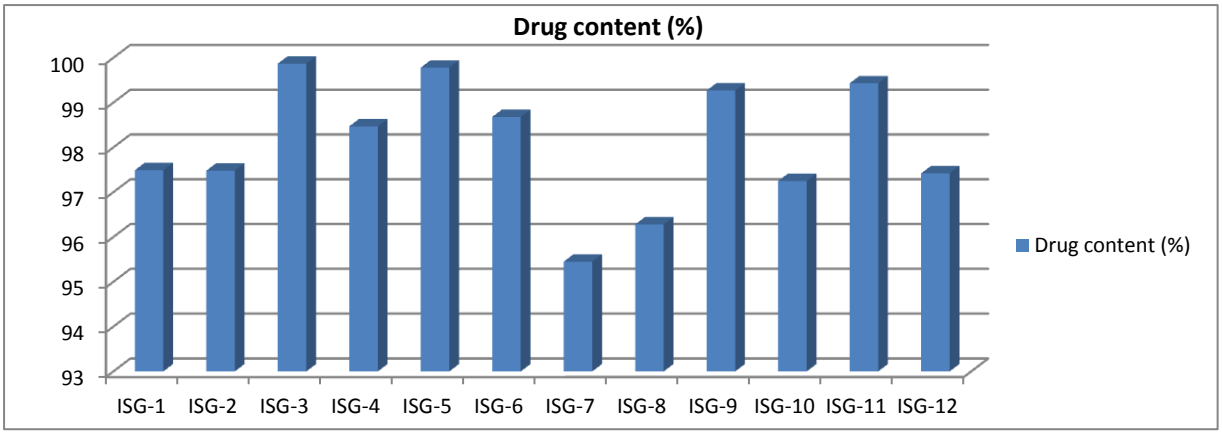
Abbr: - : No gelation, + : Gel forms after some time, ++ : Gel forms immediately, +++: Immediate gelation remains for 8 hrs, ++++ : Immediate gelation remains for more than 10hrs. T : Translucent, C: Clear, E: Easily easily syringeable through 21-gauge needle at cold temperature.

Table 5: *In-vitro* drug release of *in-situ* herbal gel containing aqueous extract of *Quercus infectoria* Oliv.

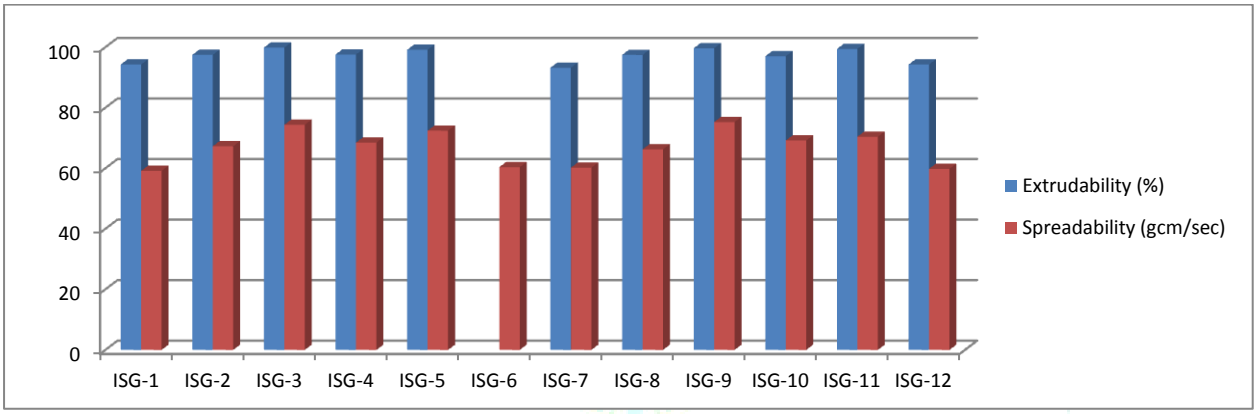
Time (hrs)	Formulation Code					
	ISG-1	ISG-2	ISG-3	ISG-4	ISG-5	ISG-6
0	0	0	0	0	0	0
2	23.49	25.46	32.18	27.34	30.27	24.14
4	47.28	51.27	58.29	55.19	56.19	46.09
6	79.82	81.90	88.49	84.94	86.27	77.46
8	88.38	90.16	98.11	94.15	97.43	89.10

Table 6: *In-vitro* drug release of *in-situ* herbal gel containing methanolic extract of *Quercus infectoria* Oliv.

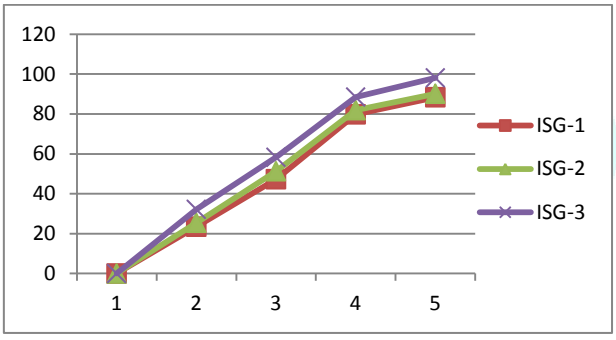
Time (hrs)	Formulation Code					
	ISG-7	ISG-8	ISG-9	ISG-10	ISG-11	ISG-12
0	0	0	0	0	0	0
2	20.62	24.78	30.64	25.81	28.99	22.82
4	45.94	49.53	56.44	54.80	54.29	45.16
6	68.26	78.97	85.03	83.18	84.11	75.20
8	85.81	88.15	97.98	92.09	97.22	80.83



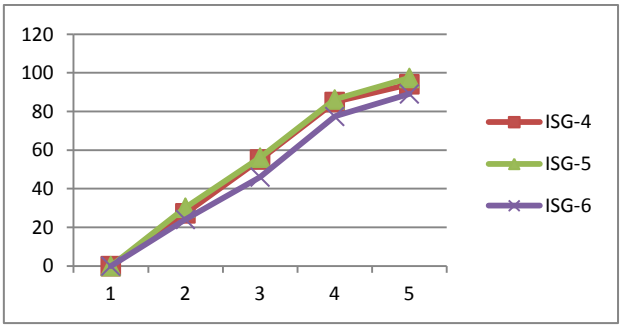
Graph 2: Drug content of *in-situ* herbal gel containing aqueous & methanolic extract of *Quercus infectoria* Oliv.



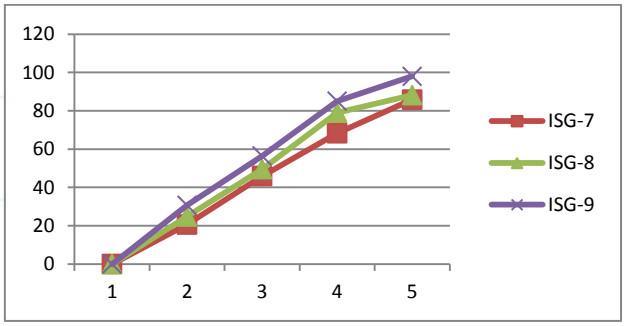
Graph 3: Extrudability and spreadbility of *in-situ* herbal gel containing aqueous & methanolic extract of *Quercus infectoria* Oliv.



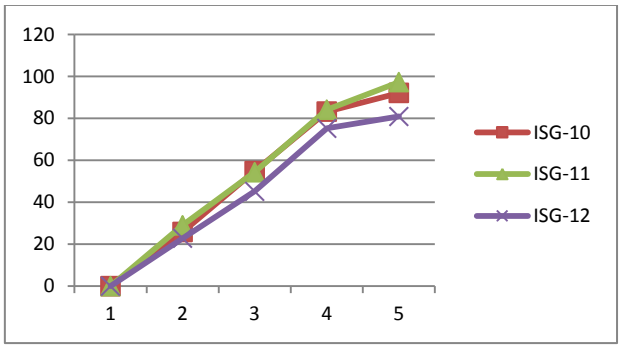
Graph 4: *In-vitro* drug release of *in-situ* herbal gel containing aqueous extract of *Quercus infectoria* Oliv. (ISG-1, ISG-2 & ISG-3)



Graph 5: *In-vitro* drug release of *in-situ* herbal gel containing aqueous extract of *Quercus infectoria* Oliv. (ISG-4, ISG-5 & ISG-6)



Graph 6: *In-vitro* drug release of *in-situ* herbal gel containing methanolic extract of *Quercus infectoria* Oliv. (ISG-1, ISG-2 & ISG-3)



Graph 7: *In-vitro* drug release of *in-situ* herbal gel containing methanolic extract of *Quercus infectoria* Oliv. (ISG-4, ISG-5 & ISG-6)

Table 7: Stability testing of *in-situ* herbal gel containing aqueous & methanolic extract of *Quercus infectoria* Oliv. at 25°C ± 2°C/60% RH ± 5% RH

Evaluation Parameters	Formulation Code							
	ISG-3		ISG-5		ISG-9		ISG-11	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Clarity	C	C	C	C	C	C	C	C
Transparency	T	T	T	T	T	T	T	T
pH	7.23	7.23	7.28	7.26	7.22	7.20	7.18	7.13
Gelling capacity	++++	++++	++++	++++	++++	++++	++++	++++
Gelation temperature	30.1	30.0	32.8	31.9	32.7	32.5	35.4	35.1
Viscosity (Poise)	0.3921	0.3920	0.3914	0.3911	0.3979	0.3978	0.3998	0.3996
Syringeability study	E	E	E	E	E	E	E	E
Extrudability (%)	99.88	99.87	99.15	99.11	99.65	99.63	99.42	99.40
Spreadability (gcm/sec)	74.39	74.36	72.43	72.41	75.28	75.61	70.43	70.42
Drug content (%)	99.87	99.13	99.78	99.03	99.27	99.00	99.43	98.98

Table 8: Stability testing of *in-situ* herbal gel containing aqueous & methanolic extract of *Quercus infectoria* Oliv. at 32°C ± 2°C/60% RH ± 5%

Evaluation Parameters	Formulation Code							
	ISG-3		ISG-5		ISG-9		ISG-11	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Clarity	C	C	C	C	C	C	C	C
Transparency	T	T	T	T	T	T	T	T
pH	7.23	7.21	7.28	7.24	7.22	7.21	7.18	7.15
Gelling capacity	++++	++++	++++	++++	++++	++++	++++	++++
Gelation temperature	30.1	30.0	32.8	31.4	32.7	32.1	35.4	35.0
Viscosity (Poise)	0.3921	0.3918	0.3914	0.3889	0.3979	0.3974	0.3998	0.3990
Syringeability study	E	E	E	E	E	E	E	E
Extrudability (%)	99.88	99.85	99.15	98.79	99.65	98.61	99.42	98.14
Spreadability (gcm/sec)	74.39	74.30	72.43	72.33	75.28	74.58	70.43	69.10
Drug content (%)	99.87	99.10	99.78	98.10	99.27	98.12	99.43	97.82

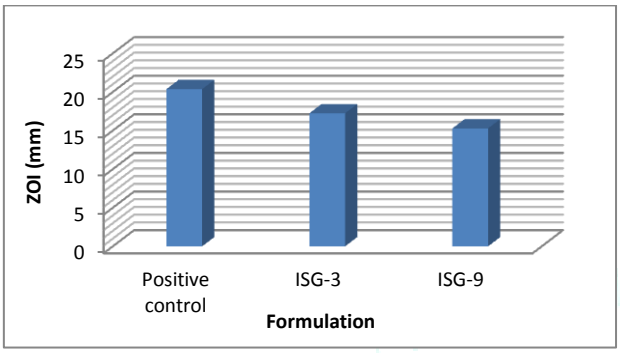
Table 9: Stability testing of *in-situ* herbal gel containing aqueous & methanolic extract of *Quercus infectoria* Oliv. 40°C ± 2°C/75% RH ± 5% RH.

Evaluation Parameters	Formulation Code							
	ISG-3		ISG-5		ISG-9		ISG-11	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Clarity	C	C	C	C	C	C	C	C
Transparency	T	T	T	T	T	T	T	T
pH	7.23	7.20	7.28	7.21	7.22	7.20	7.18	7.14
Gelling capacity	++++	++++	++++	+++	++++	+++	++++	+++
Gelation temperature	30.1	30.0	32.8	30.4	32.7	31.6	35.4	34.8
Viscosity (Poise)	0.3921	0.3910	0.3914	0.3878	0.3979	0.3971	0.3998	0.3983
Syringeability study	E	E	E	E	E	E	E	E
Extrudability (%)	99.88	99.82	99.15	98.71	99.65	98.58	99.42	98.11
Spreadability (gcm/sec)	74.39	74.31	72.43	72.29	75.28	74.48	70.43	69.08
Drug content (%)	99.87	98.49	99.78	97.08	99.27	97.84	99.43	97.43

Table 10: Anti-*Candida* activity of herbal gel conating aqueous and methanolic extract of fruit of *Quercus infectoria* Oliv.

Formulation Code	Zone of Inhibition (mm; Mean±SEM)
Positive control (10 µg/disc)	20.37±0.51
ISG-3 (5 mg/disc)	17.24±0.35*
ISG-9 (5 mg/disc)	15.28±0.28*

Abbr.: All values are mean±SEM (n=3). The values are statistically significant (student t-test) when compared from standard (Positive control) p<0.001



Graph 8: Anti-*Candida* activity of herbal gel containing aqueous and methanolic extract of fruit of *Quercus infectoria* Oliv.

CONCLUSION

From the results of stability testing it was found that the formulation code ISG-3 and ISG-9 was found to have satisfactory results, therefore further these two formulations was screened for anti-*Candida* activity. The results indicate that herbal gel formulation ISG-3 i.e., AEQIF is more potent when compared with ISG-9 i.e., MEQIF.

Hence, it was concluded from the present investigation that ISG-3 i.e., AEQIF is beneficial in the treatment of vaginal infection in the form of herbal gel. Moreover, further detail clinical trial may be carried in respect of its safety and efficacy profile.

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