Research Article

Design and Standardization of Novel Muco-Adhesive Vaginal Herbal Tablet for Treatment of Leucorrhea Using In-vitro & In-situ Methods

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ABSTRACT

The study aims to standardize a novel vaginal herbal muco-adhesive formulation containing aqueous extract of *Ficus glomerata* Roxb. and *Symplocos racemosa* Roxb. stem barks using standard parameters like hardness, friability, disintegration, surface pH, swelling index, muco-adhesion strength, in-vitro dissolution release and in-situ kinetic drug release pattern. Three formulations namely F-XI, F-XII & F-XIII having 500 mg research drug, fixed Lactose monohydrate, Talc and Magnesium stearate, and different concentrations of Carbopol 934P, Hydroxy-Propyl-Methyl Cellulose K4M and Micro-Crystalline Cellulose polymers were selected. Formulation F-XI having high concentrations of Carbopol and HPMC and low concentration of Microcrystalline cellulose resulted in high hardness (8.0 ±4.52), low swelling index at 24 hour (65.26 ±0.62 % w/w) and low drug release at 12 hour (84.32 ±8.30% w/w) during in-vitro dissolution studies indicating its unsuitableness. Formulation F-XII developed by reducing concentrations of Carbopol and HPMC and increasing amounts of Microcrystalline cellulose resulted in significant positive improvements like reduction in hardness (6.0 ±2.53), increase in swelling index (69.39 ±0.44) and higher drug release (95.74 ±3.36).However, the third formulation F–XIII prepared with increased Carbopol concentration and reduced Microcrystalline cellulose exhibited lower swelling index (67.89 ±0.51), increased muco-adhesive tension (0.687 ±0.03) and decreased drug release pattern (86.20 ±5.47). Since F-XII shows best drug release, its kinetic drug release pattern was investigated through in-situ models using goat vagina. After studying Zero order, First order, Higuchi's model, Korsmeyer and Peppas model & Korsmeyer and Peppas diffusion models, its release pattern was found closest to First-order Fickian diffusion controlled model. Comparison of its antimicrobial efficacy with standard Candid-V6 vaginal tablet using zone of inhibition method showed that F-XII demonstrates comparable but slightly lower activity against E. coli and Candida albicans microbes. HPTLC chromatography suggested presence of Quercetin, antioxidant flavonoidic compound which may be responsible for its significant exhibited anti-leucorrheal efficacy.

Keywords: Muco-adhesive, herbal, vaginal tablet, *Ficus glomerata, Symplocos racemosa.*

INTRODUCTION

Muco-adhesive drug delivery has been a topic of interest in the design of drug delivery systems to lengthen the residence time of the dosage form at the site of application or absorption and to facilitate intimate contact of the formulation with the underlying absorption surface, so as to improve and enhance the bioavailability of drug. Muco-adhesive controlled drug delivery systems are beneficial, since they give a controlled drug release over a period of time and can also be utilized for localizing the drug to a specific site in the body. Muco-adhesive delivery system is a complex process involving wetting, adsorption and interpenetration of polymer chains which offers several advantages over conventional drug delivery systems such as increased bioavailability due to prolonged residence time of the dosage form at the site of absorption, excellent accessibility, rapid absorption and onset of action, maximum utilization of drug enabling reduced dosing frequency and shorter treatment period. The common sites for muco-adhesive drug delivery systems include oral cavity, eye conjunctiva, vagina, nasal cavity and gastrointestinal tract.[1]

The vagina is a fibro-vascular tube connecting the uterus to the outer surface of the body. The vaginal epithelium consists of a stratified squamous epithelium and lamina propia. Bio-adhesives can control the rate of drug release and extend the residence time of vaginal formulations. Muco-adhesion is commonly defined as the adhesion between two materials, at least one of which is a mucosal surface. Over the past few decades, mucosal drug delivery has received a great deal of attention. It may be affected by a number of factors including hydrophilicity, molecular weight, cross-linking, swelling, pH, and the concentration of the active polymer. *Extended release* means the tablet is formulated so that the active drug is released slowly over time. These formulations may contain drug or act in conjunction with moisturizing agents as a control for vaginal dryness.[2-4]

Vaginitis is an inflammation of the vagina that can result in discharge, itching and pain. The cause is usually a change in the normal balance of vaginal bacteria or an infection. Leucorrhoea refers to the medical condition where excessive abnormal thick and sticky white or yellowish discharge occurs from the vagina accompanied with inflammation & associated with symptoms like itching, burning sensation and pain followed by body ache and tiredness. In the modern science, vaginal tablets were

prepared for patients suffering from Leucorrhoea known as *Swetapradar* in Ayurveda.

Now-a-days, muco-adhesive extended release vaginal tablets are very useful for the treatment of Leucorrhoea where the tablets adhere on mucus membrane of inner vaginal wall and the active drug is released slowly within around 8-10 hours in the vaginal fluid and inhibits the discharge by its anti-microbial activity. Hence, the tablets need not be taken repeatedly or orally, they can be just inserted into the vagina at bed-time.

Many herbal plants and their combinations in the nature of Ayurvedic drugs have been prescribed for oral administration and external application in the Ayurvedic text for the treatment of vaginitis or leucorrhoea. In the Ayurvedic system of Indian medicine, three types of medicines have been prescribed, either as single drug or as combination of drugs, for prevention and treatment of diseases – those of plant origin, mineral origin or animal origin. The research Ayurvedic vaginal herbal formulation has been newly prepared by adding equal amounts of dried parts of the stem bark of *Ficus glomerata* Roxb.and *Symplocos racemosa* Roxb because these two plants have been used since ancient times in the Ayurvedic system of medicine and elaborated in ancient texts such as Charak Samhita (Chikitsa Sthanam) as an astringent, anti-inflammatory & haemostatic and useful for arresting excessive abnormal vaginal discharge. This is a new herbal formulation which has not been evaluated till now although it is likely to exhibit sustained and significant antimicrobial action due to the synergetic effect of the phenolic and flavonoidic compounds present in this research drug and the pharmacological properties of its constituent herbs.[5-7]

This research formulation contains the plant *Ficus glomerata* Roxb. or Cluster Fig which belongs to the Moraceae family. It is a moderate sized spreading lactiferous tree without much prominent aerial roots found throughout India whose fruits are eaten by villagers. Its leaves are dark green, ovate or elliptical while the fruits contain 2-5 cm diameter sub-globose & smooth receptacles. The fruits are orange & dull reddish when ripe and having a pleasant smell. The stem bark is 0.5-1.8 cm. thick, grayish green in colour and having an uneven soft surface. On rubbing it, white papery flakes come out from the outer surface; the inner surface is light brown, fracture fibrous and mucilaginous taste. The stem bark, fruits, leaves and latex of this plant have been used since ancient times as mentioned in the Ayurvedic text book for treatment of dysentery, diarrhoea, toothache, stomach-ache, vaginal disorders, menorrhagia, haemoptysis, diabetes, piles and glandular swelling, etc. The Phytochemical compounds isolated from the stem bark are leucocyanidin-3-o-B-glucopyranoside, leucopela rogonidin 3-O-a- L-rhamnopyranoside, B- sitosterol, stigmasterol, tetracyclic triterpene- gluanol acetate and tiglic acid. The reported pharmacological properties of the different plant parts are hypoglycaemic, antiulcer, antioxidant, wound-healing, anti-inflammatory, anti-diarrhoeal, antibacterial, antifungal, antipyretic and antidiuretic.[6-8]

Symplocos racemosa Roxb. known as Lodhra belonging to the Symplocaceae family is found distributed throughout North Eastern India up to 2,500 ft. elevation. It is a small evergreen tree with stem up to 6 m in height and 15 cm in diameter. Its stem bark is useful in bowel complaints such as diarrhea & dysentery, in dropsy, eye disease, liver complaints, wound healing, excessive vaginal discharge, menstrual problems, fevers, ulcers, scorpion-string, etc. The bark is often employed in the preparation of plasters and is reported to promote maturation or resolution of boils, stagnant tumors and other malignant growths. A decoction of the bark or wood is used as gargle for giving firmness to spongy and bleeding gums and relaxed uvula. The phytochemical investigation of the n-butanol soluble fraction of the bark of stem of *Symplocos racemosa* Roxb. yielded two phenolic glycosides of salirepin series namely symplocuronic acid and sympocemoside while salirepin has also been isolated from this plant. The alcohol extract of stem bark indicated the presence of carbohydrates, glycosides, saponins, terpenoids & alkaloids while its ether extract indicated the presence of glycosides, phytosterol and steroids. The prominent pharmacological activities of its stem bark are antibacterial, anti-inflammatory, antiulcer, anti-tumor, antimicrobial and antioxidant.[9-10]

The aim of the present study was to design and standardize the novel vaginal herbal muco-adhesive extended released formulation in the tablet form by using standard parameters like hardness, friability, disintegration, surface pH, swelling index, muco-adhesion strength, in-vitro dissolution release and in-situ kinetic drug release pattern. These aqueous extract formulations have been prepared by mixing equal parts of stem barks of *Ficus glomerata* Roxb. and *Symplocos racemosa* Roxb. because both these plants are having antimicrobial, anti-inflammatory and astringent properties due to the presence of tannin & phenolic chemical compounds in them.

MATERIALS AND METHODS

Plant materials

The stem barks of *Symplocos racemosa* Roxb. *and Ficus glomerata* Roxb. Were purchased from crude drug supplier of Katwa Chowrasta, Burdwan district for the preparation of herbal vaginal tablet and the plant samples were authenticated by the Research Officer, Botanical Survey of India, Howrah, India (Ref. No. BSI/CNH/SF/Tech./2016) and herbarium specimen were stored in the Dravyaguna Museum of the Institute of Post Graduate Ayurvedic Education & Research, Kolkata.

Chemicals used for preparation of tablets

Di-calcium phosphate, Gum acacia, Lactose monohydrate, Sodium carboxy-methyl-cellulose, Sodium starch

glycolate, Starch (maize), Ferric Chloride (FeCl3), Magnesium stearate (IP grade), Microcrystalline cellulose (IP grade), Talc (IP grade), Folin-Ciocalteu's reagent, Sodium carbonate and Sulphuric acid were obtained from M/s Merck Specialties Pvt. Ltd., Mumbai. Carbopol 934P and Hydroxy-propyl-methyl-cellulose K4M were purchased from reputed company M/s HiMedia Laboratories Pvt. Ltd while Citric acid monohydrate was procured from M/s B.D. Pharmaceutical works Pvt. Ltd and Sodium bi-carbonate (IP grade) from M/s Indian Drug House. CANDID-V6 (Clotrimazole Vaginal Muco adhesive Extended Release Tablets 100 mg) Batchno: 18140320, Mfg.dt.: June 2014 & Exp.dt.: May 2018 was the marketed sample of M/s Glenmark Pharmaceuticals Limited.

Preparation of extracts

The stem barks of *Symplocos racemosa* Roxb. and *Ficus glomerata* Roxb. were taken in equal quantity by weight, washed, sun dried and crushed to particle size of 40 mesh. This coarse powder was sequentially extracted with petroleum ether (60 °C -80 °C), chloroform, acetone, ethanol and water using soxhlet apparatus. These extracts were filtered using a Buckner funnel and Whatman No. 1 filter paper at room temperature and concentrated at reduced temperature and pressure using rotary evaporator. All obtained extracts were stored in refrigerator below 10°C for subsequent experiments. The aqueous extract of the research formulation was used in the study.[11-12]

Formulation of drug-free tablets (i.e. Placebo)

The drug-free (placebo) tablets were prepared using a mixture of polymers and microcrystalline cellulose with or without effervescent. Carbopol & Hydroxy-Propyl-Methyl Cellulose (HPMC) were used as the polymers. Tablets loaded with effervescent agent consisted of sodium bicarbonate and citric acid in 3:1 molecular ratio and produced gas bubbles in liquid media. All used chemicals were of analytical reagent grade. For the various drug-free formulations, the effect of effervescent agent on polymers' bio-adhesive characteristics was investigated. The performances of these bio-adhesive polymers were evaluated, especially three main parameters - pH value, swelling index and the bio-adhesive strength. On the basis of these data, suitable polymers were selected to prepare the bio-adhesive effervescent vaginal tablets of active research drug.

Preparation of muco-adhesive vaginal tablets

The various types of bio-adhesive vaginal herbal tablets were prepared using the dry compression technique of tablet preparation. During this study, 15 types of muco-adhesive herbal vaginal formulations and 5 types of muco-adhesive effervescent vaginal formulations were prepared for testing. All these formulations were prepared by mixing the same amount of the active research drug extract powder (500 mg) with different amounts of excipients, binders and developers. The polymers Carbopol & Hydroxy-Propyl-Methyl Cellulose were used as excipients, while talc and magnesium stearate were added as glidant and lubricant respectively. Micro-Crystalline Cellulose and Lactose monohydrate were used as diluents. The binder hydroxyl-propyl-methyl-cellulose was used to form sustained-release matrix with the polymer carbopol, which swells to form hydrogel-like matrices through which drug molecules could be released at a controlled rate. All the ingredients were thereafter passed through # 44 mesh sieve and finally the mixture was compressed into tablet-form using single punch tablet compression machine.[13-14]

The effervescent muco-adhesive vaginal tablets were also prepared by direct mixing of required quantity of 500 mg active research drug, effervescent (consisting of sodium bicarbonate and citric acid in the ratio of 3:1), polymers (i.e. carbopol, hydroxy-propyl-methyl cellulose & sodium carboxy-methyl cellulose), microcrystalline cellulose, magnesium stearate and talc. Talc and magnesium stearate were added as glidant and lubricant respectively in the ratio of 2:1. Finally the mixture was compressed into tablets using single punch tablet compression machine. However, the bio-adhesive effervescent vaginal tablets could not produce very desirable results for vaginal drug delivery system.

Finally, three formulations F-XI, F-XII & F-XIII of active research drug have been selected for further standardization by using the in-vitro muco adhesive and in-vitro dissolution studies. Each of these tablets contained 500 mg active research drug, 100 mg Lactose monohydrate, 10 mg talc and 10 mg of Magnesium stearate. The amount of Carbopol 934P in F-XI, F-XII & F-XIII formulation was 35 mg, 15 mg and 45 mg respectively, while the quantities of HPMC K4M were 150 mg, 90 mg and 90 mg and the amounts of Microcrystalline cellulose were fixed at 95 mg, 175 mg and 145 mg respectively in these three drug formulations.

At last only Formulation XII was considered as a suitable vaginal herbal tablet on the basis of high muco-adhesive strength, swelling index and maximum sustained releasing pattern required for an effective muco-adhesive vaginal drug delivery system. Hence, further studies of in-situ kinetic drug release pattern and Chromatography (HPTLC) of F-XII were undertaken for its standardization.

Methods for standardization of vaginal tablets

Standard parameters like hardness, friability, disintegration, surface pH, swelling index, in-vitro muco adhesive strength & in-vitro dissolution profile of F-XI, F-XII & F-XIII vaginal tablet formulations were evaluated to standardize and find out the one most suitable formulation for further in-vitro dissolution and drug release pattern study using the vaginal route of administration. The following parameters were used for the standardization process:

Weight variation

Ten tablets from each formulation were weighed using an electronic balance and the average weight was calculated.

Thickness

The thickness of three randomly selected tablets from each formulation was determined in mm using a vernier caliper (Pico India). The average values were calculated.

Hardness of the tablet

The tablet hardness, the force required to break a tablet in a diametric compression force, was estimated using a hardness tester (Pfizer type) to determine the need for pressure adjustments on the tablet machine. Hardness can affect the disintegration properties. So if the tablet is too hard, it may not disintegrate in the required period of time. The hardness of the tablets was determined using Monsanto hardness tester and expressed in kg/sq.cm. Three tablets were randomly picked from each formulation and the mean and standard deviation values were calculated.

Friability

Friability is a measure of the resistance of the tablets to abrasion. It is an important parameter which tests the breaking point and structural integrity of a tablet under conditions of storage, transportation and handling before usage. The breaking point of a tablet is primarily based on its shape. The friability of the tablets was measured using Friability Test Apparatus (Imcorp, India) by tumbling them in a rotating drum. After tumbling, the integrity of the tablets and the weight loss are evaluated. Ten tablets are weighed initially, rotated at 25 rpm for 4 minutes (total 100 rotations) and re-weighed after removal of fines. Friability below 1.0 % w/w was considered acceptable. [15]

$$\% \text{ Weight loss} = \frac{\text{Initial wt.} - \text{Final wt.}}{\text{Initial wt.}} \times 100$$

Determination of the surface pH value

Surface pH of the tablets was determined in order to investigate the possibility of any in vivo side effects. Since an alkaline pH may cause irritation to the vaginal mucosa, it was determined to keep the surface pH as close to the system pH (4.0 – 5.0) as possible. A combined glass electrode was used for this purpose. The tablet was allowed to swell by keeping it in contact with 5 ml of distilled water (pH 6.5 ± 0.05) for 2 hours at room temperature. The pH was measured by bringing the electrode in contact with the surface of the tablets and allowing it to equilibrate for 1 minute.

Swelling study

The swelling behavior of tablets can be described in terms of their water absorbing capacity with time. Tablets were initially weighed individually (W_0), placed separately in 2% agar gel plates and incubated at (37±1) °C. At regular 0.5 hour time intervals until 4 hours, each tablet was removed from the Petri-dish and excess surface water was removed carefully using filter paper. The swollen tablet was then reweighed (W_t) and the percentage of swelling were calculated using the following formula:

$$\% \text{ Swelling} = \{(W_t - W_0)/ W_0\} \times 100$$

where W_t is the weight of the tablet at time t and W_0 is the initial weight of the tablet. The swelling was calculated and plotted as a function of time. The slope of the linear plots was taken as the swelling rate. [16]

In-vitro muco-adhesion study

Several types of mucosa, including rat intestine, pig oral, bovine sublingual and goat vaginal mucosa have been used as model biological tissues for evaluation of bio-adhesion. A simple apparatus was devised to measure the minimum detachment force. In evaluation of adhesion, it is important to use uniform surfaces that allow the formation of reproducible adhesive bonds. In the present study, goat vaginal mucosa was used as a model mucosal surface for bio-adhesion testing. For this purpose, the mucosa was removed immediately after slaughter and kept in phosphate buffer.

At the time of testing, a section of mucosa was secured keeping the mucosal side out on the upper glass vial using rubber band and aluminum cap. The diameter of each exposed mucosal membrane was 1 cm. Then one vial with section of mucosa and another vial were fixed on height adjustable pan. To a lower vial, a tablet was placed with the help of bi-layered adhesive tape, adhesive side facing downward. The height of the lower vial was adjusted so that a tablet could adhere to mucosa on the upper vial. A constant force was applied on the upper vial for 2 min, after which it was removed and the upper vial was then connected to the balance. Then the weight on right side pan was slowly added in increments of 0.5 gm till the two vials just separated from each other. The total weight required to detach the two vials was taken as a measure of the muco-adhesive strength. From this muco-adhesive strength, the force of adhesion was calculated using the following formula: [17, 18]

$$\text{Force of adhesion (Newton)} = \frac{\text{Muco-adhesive Strength (gm)}}{1000} \times 9.81$$

In-vitro dissolution profile

A standard curve was first generated relating the concentration of Active Research Drug (ARD) with its absorbance. The extract of ARD in different concentrations of 100 to 1100 mcg/ml was prepared in the desired medium (0.05 M phosphate buffer, pH 4.5) and the absorbance of prepared standard solutions was measured at 276 nm using UV-Visible Spectrophotometer. The standard curve prepared based

on concentration of the extract versus absorbance can be used to calculate the amount of ARD in an unknown sample solution.

The release profile of vaginal tablets was determined by using USP type-I dissolution test apparatus in 500 ml of phosphate buffer (pH 4.5) as the dissolution medium at 30 rpm & 37 ± 0.5ºC temperature. The tablet was placed in a settling basket to prevent the tablet from floating. Five ml of aliquot was withdrawn at predetermined intervals; filtered and equal volume of the dissolution medium was replaced in the vessel after each withdrawal to maintain sink condition. The filtrate was analyzed for the drug content by Shimadzu UV-2450 UV/Vis double-beam spectrophotometer at 276 nm. The percentage of drug released was calculated in the three finalized Formulations, F-XI, F-XII & F-XIII, from the standard curve of Active Research Drug and plotted as a function of time in order to study the pattern of drug release.[19]

In-situ drug release kinetic study

The in-situ release study of F-XII vaginal tablets was carried out using USP type-I dissolution test apparatus in 60 ml of 0.05M phosphate buffer (pH 4.5) as the dissolution medium at 30 rpm and 37 ± 0.5°C temperature. The tablet was placed into goat vagina mucosal membrane and kept inside the properly covered basket to prevent direct diffusion to medium. Five ml of aliquot was withdrawn at predetermined intervals, filtered and equal volume of the dissolution medium was replaced in the vessel after each withdrawal to maintain sink condition. The filtrate was evaluated for the drug content by Shimadzu UV-2450 UV/Visible double-beam spectrophotometer at 276 nm. In order to investigate the mode of release from tablets, the release data was analyzed with different mathematical models namely:

Zero order equation: $Q_t = Q_0 + K_0 (t)$ (1)

Plot: [Amount of drug remaining] *versus* [time].

First order equation: $Ln(Q_t) = Ln(Q_0) – K_1$ (2)

Plot: [Ln(cumulative percentage of drug remaining)] *versus* [time].

Higuchi equation: $Q_t = K_H (t)^{1/2}$ (3)

Plot: [cumulative percentage drug release] *versus* [square root of time].

Korsmeyer and Peppas equation: $Q_t/Q_\alpha = K_P (t)^n$ (4)

Plot: [Ln(cumulative percentage drug release)] *versus* [Ln(time)].

where, Q_0 is the drug remaining to be released at 0 hours and Q_t is the drug amount remaining to be released at time t and K_0 and K_1 are the coefficients of zero order and first order equations respectively, K_P is constant incorporating structural and genomic characteristics of the release device, $K_H$is a constant incorporating the surface-volume relation and n is the release exponent indicate the release mechanism.[20-21]

Higuchi model [18] is used to study the release of water soluble and poorly soluble drugs incorporated in semi-solid and/or solid matrices. Korsmeyer et al [22] derived a simple relationship which described drug release from a polymeric system equation and to find out the mechanism of drug release, first 60% drug release data is to be fitted in Korsmeyer-Peppas model where, $[Q_t/Q_\infty]$ is a fraction of drug released at time t, K_P is the release rate constant and n is the release exponent. The n value is used to characterize different release for cylindrical shaped matrices. In this model, the value of n characterizes the release mechanism of drug. For the case of cylindrical tablets, $n \leq 0.5$ corresponds to a Fickian diffusion mechanism, $n < 1.0$ to non-Fickian transport, $n = 1.0$ to Case II (relaxation) transport, and $n > 1.0$ to super case II transport.

Chromatography analysis

The High Performance Thin Layer Chromatography analysis of the placebo tablet, F-XII muco-adhesive vaginal tablet, aqueous extract and alcoholic extract was undertaken to understand the interaction of excipients with active research drug. Any adverse or synergistic effect of the formulation due to presence of similar chemical compounds in the aqueous and alcoholic extract were estimated on the basis of separated spots by trying different solvent systems and the following parameters were finalized for this analysis. The visualization of the spot was done under UV-Visible chamber at 254 nm and 366 nm wavelength but scanning of these compounds were done at 280 nm and 360 nm due to presence of maximum phenolic compounds in the extract of the formulation.

Chromatographic conditions:

Plate : Pre-coated silica gel $60F_{254}$ plate (10cm x 10cm)

Mobile phase : Toluene: Chloroform: Methanol: Formic acid = (7: 5: 1.5:0.5)

Wavelength : 280 nm & 360 nm

Applicator : CAMAG Linomat 5 automated TLC applicator

Scanner : CAMAG TLC scanner 3 equipped with WINCATS software

Sample concentration : 50 mg/ml

Standard concentration: 1 mg/ml

Stability studies

The stability of the formulated F-XII herbal vaginal tablets was assessed by tightly sealing 20 tablets in aluminum foil which were stored in two different environments at 30 ± 2 °C (65 ± 5 % RH) and at 40 ± 2 °C (75 ± 5 % RH) for 90 days. Initial and periodic analysis of the tablets for all the physical parameters, muco-adhesive strength, in-vitro dissolution and in-situ kinetic drug release in vagina was done on days 15, 45 and 90.[23]

Statistical data analysis

Statistical data analysis was performed using Student's t-test at 95 % level of confidence with the aid of Microsoft Office Excel 2007.

RESULTS

The obtained results after performing the various tests on formulations F-XI to F-XIII have been summarized in table 1 below.

Table 1: Comparison of important parameters of three selected tablet formulations

Sl. No.	Parameter	F-XI	F-XII	F-XIII
1	Weight variation (mg)	928.0 ± 42.2	927.8 ± 25.3	932.2 ± 35.6
2	Thickness (mm)	5.82 ± 0.03	5.80 ± 0.04	5.92 ± 0.05
3	Hardness (kg/sq.cm)	8.0 ± 4.52	6.0 ± 2.53	5.6 ± 4.04
4	Friability (% w/w)	0.42	0.66	0.68
5	Surface pH	4.68 ± 0.09	4.80 ± 0.04	4.86 ± 0.05
6	Swelling Index at 24 hour (% w/w)	65.26 ± 0.62	69.39 ± 0.44	67.89 ± 0.51
7	In vitro Mucoadhesive force (Newton)	0.706 ± 0.02	0.667 ± 0.02	0.687 ± 0.03
8	In vitro Dissolution at 12 hour (% w/w)	84.32 ± 8.30	95.74 ± 3.36	86.20 ± 5.47

In order to assess the dissolution from various tablets, first the spectroscopic analysis of placebo tablets was done which did not show any significant peaks or noticeable characteristics. Similarly, the spectroscopic profiles of the active research drug at various standard concentrations from 100 to 1100 mcg/ml were analyzed and the same are shown in figure 1.

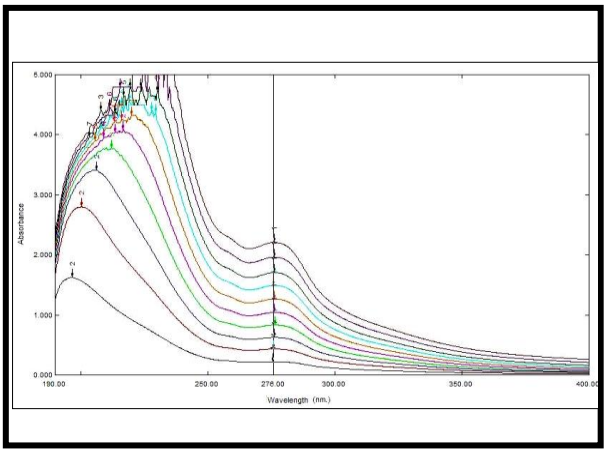

Figure 1: Overlay spectral representation of different concentrations of Active Research Drug

Analysis of the spectral patterns at different concentrations depicted in figure 1 showed a clear peak emerging at 276 nm in all cases. Therefore, the absorbance at 276 nm was noted at all concentrations and the standard curve for active research drug was prepared which isshown as figure 2.

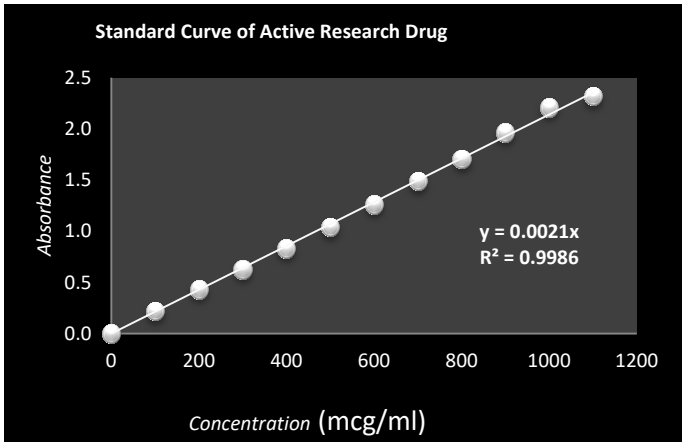

Figure 2: Standard curve of Active Research Drug

Using the standard curve mentioned above and the absorbance of the various drug formulations observed during the experiment, the dissolution profiles based on the drug release percentages were calculated which are shown in figure 3.

The above results indicated that all the three tablet formulations showed significant dissolution, good erodibility and maximum drug release within 10–12 hours. However, on comparing among the three formulations, F-XII shows better result parameters. In fact, its drug release profile exactly fits in with the ideal criteria for drug release which stipulates that a good muco-adhesive tablet should release more than 90% of drug within 8-10 hours to avoid vaginal irritation.[15]Hence, F-XII drug formulation was taken up for further in-situ drug release kinetic study and HPTLC analysis.

The data obtained during the drug release kinetic study are laid down in table 2 and the plots obtained in different models are shown in figure 4.

The obtained data indicates that while the cumulative drug release at 24 hour was 34.09 ± 2.22% w/w, the R^2 values were 0.836, 0.930, 0.914 and 0.975 for zero order, first order, Higuchi's kinetics and the Korsmeyer & Peppas kinetics models respectively. The results obtained from the four models suggest that the best fit during regression analysis is obtained in case of the Korsmeyer and Peppas kinetics model whose R^2 value (0.975) is closest to 1. The slope of this model which represents the diffusion coefficient (n) comes to 0.3616 which is less than 0.5, suggesting a Fickian diffusion drug transport mechanism. The findings indicate a combined mechanism of drug release (diffusion through the matrix and partially through water filled pores). Hence, this release pattern is the First-order Fickian diffusion controlled model, following the sphere geometry.

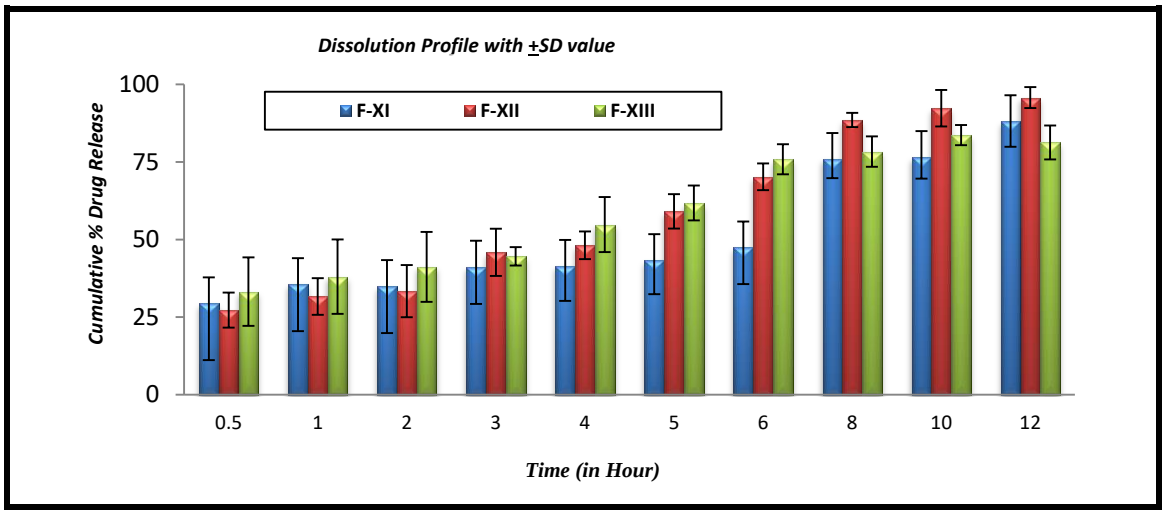

Figure 3: Dissolution profile of selected 3 tablet formulations showing variations in mean &SD values with time

Table 2: Data sheet relating to release kinetics of F-XII formulation

Time (Hour)	Square Root of Time (√t)	Ln (Time)	Drug amount remaining to be released = (Qt) in mg	Ln (drug amount to be released) = Ln(Qt)	Cumulative %w/w of drug release	Ln (cumulative %w/w of drug release)
0.00	0.00	-	500.00 ± 0.00	-	0.00	-
0.25	0.50	-1.39	472.82 ± 6.69	6.16 ± 0.01	5.44 ± 1.87	1.69 ± 0.23
0.5	0.71	-0.69	450.62 ± 6.57	6.11 ± 0.01	9.88 ± 2.00	2.29 ± 0.16
1.0	1.00	0.00	447.97 ± 8.93	6.10 ± 0.02	10.41 ± 2.38	2.34 ± 0.18
1.5	1.22	0.41	440.82 ± 10.36	6.09 ± 0.02	11.84 ± 2.51	2.47 ± 0.17
2.0	1.41	0.69	428.27 ± 7.34	6.06 ± 0.02	14.35 ± 2.26	2.66 ± 0.13
3.0	1.73	1.10	417.07 ± 8.54	6.03 ± 0.02	16.59 ± 2.40	2.81 ± 0.12
4.0	2.00	1.39	414.72 ± 7.90	6.03 ± 0.02	17.06 ± 2.23	2.84 ± 0.11
6.0	2.45	1.79	407.07 ± 8.78	6.01 ± 0.02	18.59 ± 2.66	2.92 ± 0.12
8.0	2.83	2.08	392.27 ± 9.07	5.97 ± 0.02	21.55 ± 2.37	3.07 ± 0.10
12.0	3.46	2.48	365.27 ± 3.65	5.90 ± 0.01	26.95 ± 1.35	3.29 ± 0.05
24.0	4.90	3.18	339.97 ± 10.73	5.83 ± 0.03	32.01 ± 2.22	3.47 ± 0.07
30.0	5.48	3.40	316.47 ±2.91	5.76 ± 0.01	36.71 ± 1.85	3.60 ± 0.05

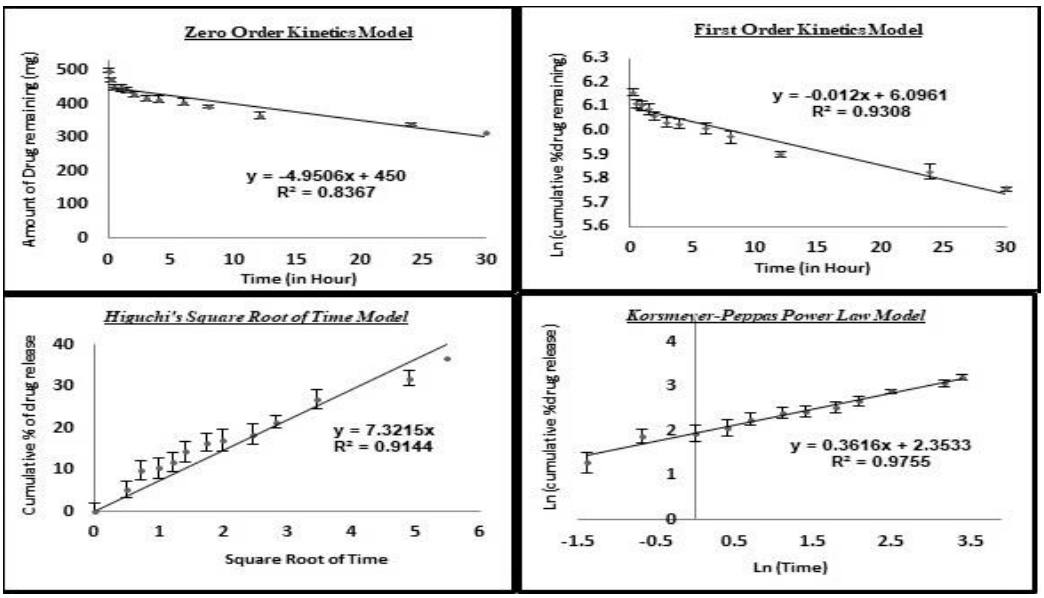

Figure 4: Plots obtained during drug release kinetic studies in different models

The parameters obtained in respect of F-XII drug research formulation were also compared with the standard drug preparation CANDID–V6 (Clotrimazole Vaginal Mucoadhesive Extended Release Tablets 100 mg) manufactured by M/s Glenmark Pharmaceuticals Limited. For this purpose a marketed sample of Candid –V6 having Batch no. 18140320, manufacturing date June 2014 & expiry date May 2018 was selected. The comparative position is shown in table 3 below. Comparison in the drug release and dissolution parameters could not be done due to wide differences in the composition of the two formulations in this regard.

Table 3: Comparison of the standard drug formulation with F-XII tablet formulation

Sl. No.	Parameter	CANDID-V6	F-XII tablets
1	Weight variation (mg)	1046.1 ± 15.2	927.8 ± 25.3
2	Thickness (mm)	6.00 ± 0.02	5.80 ± 0.30
3	Hardness (kg/sq.cm)	8.2 ± 1.5	6.0 ± 2.5
4	Friability (%w/w)	0.06	0.66
5	Surface pH	6.56 ± 0.04	4.80 ± 0.04
6	Swelling Index at 24 hour (%w/w)	10.41 ± 0.52	69.39 ± 0.44
7	Mucoadhesive force (Newton)	0.701 ± 0.018	0.667 ± 0.021
8	Dissolution study at 12 hour (%w/w)	-	95.74 ± 3.36
9	Release study at 12 hour (%w/w)	-	29.11 ± 1.35
10	Release study at 24 hour (%w/w)	-	34.09 ± 2.22
11	Zone of Inhibition for *E. coli* (mm)	20.2 ± 0.1	17.4 ± 0.2
12	Zone of Inhibition for *Candida albicans* (mm)	16.6 ± 0.1	12.0 ± 0.2

Chromatography analysis was performed under the following conditions:

Plate : Pre-coated silica gel $60F_{254}$ plate (10cm X 10cm)

Mobile phase : Toluene: Chloroform: Methanol: Formic acid = (7: 5: 1.5:0.5)

Wavelength : 280 nm & 360 nm

Applicator : CAMAG Linomat 5 automated TLC applicator

Scanner : CAMAG TLC scanner 3 equipped with WINCATS software

Sample concentration : 50 mg/ml

Standard concentration: 1 mg/ml

The Rf values obtained during HPTLC Chromatographic analysis are laid down and compared in table 4.

The comparison of *Rf* values obtained at scan wavelengths of 270 nm and 370 nm showed that at sl. no. 7, 9, 12 & 13, the Rf values of F-XII and aqueous extract are quite similar while the *Rf* value observed at sl. no. 13 is quiet similar to standard drug Quercetin.

DISCUSSION

The vagina is a most suitable site for bio-adhesive formulations as the product absorbs moisture, becomes a gel and releases medication in a time-controlled manner. Bio-adhesive polymers that have been used for vaginal formulation include hydroxyl-propylcellulose, polycarbophil and polyacrylic acid which is used to retain moisture and lubricate the vagina. The vaginal bio-adhesive preparations have been developed in recent years as a new type of controlled-release form for the treatment of both topical and systemic diseases. For drugs which are susceptible to gut or hepatic metabolism or which cause GI side effects, vaginal bio-adhesive delivery may offer a number of advantages over the other routes of administration. The greatest advantage of such dosage forms is the possibility of maintaining them in the vagina for extended periods of time including daytime and nighttime, thereby enabling lower dosing frequencies. Vaginal candidiasis is a common condition and up to 75% of all women suffer at least one episode of this infection during their lifetime.[24]

The purpose of the present study was to design and standardize a novel herbal vaginal tablet carrier system based on a muco-adhesive polymer exhibiting improved properties of drug delivery to the vaginal mucosa. Application of dosage forms to mucosal surfaces may be of benefit to drug molecules not amenable to the oral route, such as those that undergo acid degradation or extensive first-pass metabolism. The muco-adhesive drug delivery system is a very promising approach for delivering the drugs which have narrow absorption window at the target site to maximize their usefulness.

Table 4: Comparative Rf values obtained during HPTLC Scanned Chromatogram

Scanned at 270 nm				Serial no.	Scanned at 370 nm			
Placebo of F-XII Formulation	F-XII tablet formulation	Aqueous Extract	Alcoholic Extract		Placebo of F-XII Formulation	F-XII tablet formulation	Aqueous Extract	Alcoholic Extract
				1	0.03	0.16		
		0.27		2	0.06		0.27	
	0.34			3	0.08	0.34		0.33
				4	0.12	0.38		
0.10		0.42		5	0.15			
0.12	0.48		0.47	6	1.20	0.48		
0.15	0.57	0.59		7		0.57	0.54	0.58
0.17	0.65			8				
0.20	0.73	0.71		9		0.70	0.72	0.73
0.22				10		0.74		
0.27	0.85			11				0.85
0.40	0.89	0.88	0.89	12		0.90	0.88	0.91
Quercetin	0.96	0.95	0.95	13				

Muco-adhesive dosage forms may be designed to enable prolonged retention at the site of application, providing a controlled rate of drug release for improved therapeutic outcome. However, the muco-adhesive ability of a dosage form is dependent upon a variety of factors, including the nature of the mucosal tissue and the physicochemical properties of the polymeric formulation. In-vitro muco-adhesion retention studies have justified the prolonged retention of tablet inside the vaginal tract because the muco-adhesive form of drug would increase the time of contact with the vaginal mucosa and thus its therapeutic effect. In addition, the soft and rubbery nature of muco-adhesive polymers will minimize mechanical and frictional irritation to the surrounding tissue.[13-14]

The two basic ingredients which constitute the research drug were selected on the basis of evidence based properties of the stem barks of *Ficus glomerata* Roxb. and *Symplocos racemosa* Roxb. especially as elaborated in the Ayurvedic literature for their antimicrobial, anti-inflammatory, analgesic, antioxidant, wound-healing, anti-diarrhoeal, antibacterial, antifungal, antipyretic and therapeutic efficacy in arresting excessive abnormal vaginal discharge, excessive bleeding disorders and other gynaecological diseases since ancient times and possibly due to the presence of tannin, B-stiosterol and flavonoidic compounds in these medicinal plants.

The muco-adhesive vaginal herbal tablet formulations F-XI, F-XII & F-XIIIwere selected for this study and parameters such as average weight, hardness, thickness, surface pH, swelling index, muco-adhesive strength and in-vitro dissolution drug release in vagina were standardized. These vaginal herbal tablets were prepared by dried compressed method, each having 500 mg active research drug, fixed amounts of Lactose monohydrate, Talc and Magnesium stearate, and varying concentrations of polymers like Carbopol 934P, Hydroxy-Propyl-Methyl Cellulose K4M and Micro-Crystalline Cellulose.

After analysis of obtained results in respect of the important physical parameters, the formulation F-XI was not considered very suitable for further study due to high hardness (8.0 $\pm$ 4.52),low swelling index at 24 hour (65.26 $\pm$ 0.62 % w/w) and lower drug release at 12 hour during in-vitro dissolution study (84.32 $\pm$ 8.30% w/w). These properties of F-XI may be due to high concentrations of Carbopol and HPMC polymers and low concentration of Micro crystalline cellulose.

While developing the Formulation F-XII, the constituent composition used in formulation F-XI was modified by reducing concentrations of the two main polymers, Carbopol and HPMC, and increasing the concentration of Micro crystalline cellulose. Carbopol is a well-known pH dependent polymer which stays in solution form at acidic pH but forms a low viscosity gel at alkaline pH. HPMC is used in combination with Carbopol to impart the viscosity to Carbopol solution, while

reducing the acidity of the solution.[25]These modifications resulted in significant positive changes in the physical parameters observed in case of F-XII especially reduction in hardness, increase in swelling index and higher concentration of drug releasing percentage.

After observing the data from formulation F–XII, a new formulation F-XIII was developed by increasing the concentration of Carbopol from the previous two formulations (F-XI and F-XII) and reducing the concentration of Microcrystalline cellulose as compared to formulation F-XII in order to attempt for even better parameters. However, the results of this formulation F-XIII indicated lower swelling index, increased muco-adhesive tension and decreased drug release pattern when compared to Formulation F-XII possibly due to higher concentration of Carbopol and lesser concentration of Microcrystalline cellulose.

The results of the study suggest that all three tablet formulations (F-XI, F-XII and F-XIII) show significant dissolution, reasonable pH, adequate swelling index and high drug release within 10 – 12 hours, indicating good efficacy. However, among these three formulations, F-XII shows the best drug release profile and fits the desired criteria for drug release stipulating that the tablet should release more than 90% of drug within 8-10 hours to avoid vaginal irritation. [15] Therefore, after trying many combinations of constituents, the best results are observed in the combination of Carbopol 934P (15 mg), HPMC K4M (90 mg), Microcrystalline cellulose (175 mg), Lactose monohydrate (100 mg), Talc (10 mg), Magnesium Stearate (10 mg) and 500 mg active research drug found in the muco-adhesive vaginal formulation F-XII whose physical parameters are observed to be 927.8 ±25.30 mg average weight, 5.80 ±0.04 mm thickness, 6.0 ±2.53 kg/sq.cm hardness, 0.66 %w/w friability,4.80 ±0.04 surface pH, 69.39 ±0.44 % w/w swelling index at 24hour and 0.667 ±0.02 Newton muco-adhesive force.

Drug release mechanism and kinetics are the two important characteristics of a delivery system in describing the drug dissolution profile. Hence, formulation F-XII was further investigated for its kinetic release pattern of drug delivery following the standard in-situ release models in goat vagina. After studying the Zero order kinetics model (R^2), First order kinetics model (R^2), Higuchi's kinetics model (R^2), Korsmeyer and Peppas kinetics model (R^2) & Korsmeyer and Peppas diffusion exponent (n) models, it was found that its release pattern is closest to the First-order Fickian diffusion controlled model, following the sphere geometry.

The results of the kinetic release study using in-situ methods revealed that the drug is released into the mucous membrane of the vagina consistently in a very slow and steady pattern which shows a decreasing trend with time. Thus, while 14.35 % was released in first 2 hours, 4.24 % was released in the next 4 hours, 8.36 % in subsequent 6 hours and 5.06 % in the next 12 hours leading to a total cumulative release of 36.71 % after 30 hours. Therefore, it is evident that maximum concentration of the drug remained in the mucus membrane of the vagina which proves the local action of the vaginal tablets as already indicated by its primary physical parameters like significant swelling index, pH value below 5 and high muco-adhesive strength.

E. coli and Candida albicans are the two microbes which are the primary causative factors for vaginitis and the efficacy of vaginal tablet F-XII in respect of these microbes was compared with the standard vaginal tablet Candid-V6 whose shape, size, weight, thickness and muco-adhesive force parameters are quite similar. The data obtained using the zone of inhibition method suggests that F-XII demonstrates comparable but slightly lower antibacterial and antifungal activity against E.coli and Candida albicans microbes as compared to Candid-V6.

Scanned chromatograms obtained at 270nm & 370 nm wavelengths during HPTLC analysis of placebo, F-XII muco-adhesive formulation, aqueous extract and alcoholic extract showed four clear common peaks (spots) except in case of placebo suggesting the presence of four common chemical compounds in the muco-adhesive form, aqueous and alcoholic extracts of the research formulation. Spot number 13 also showed the presence of standard chemical flavonoidic compound Quercetin in these three samples (except placebo) as demonstrated by almost same Rf value (0.96), which could be directly related to the antimicrobial, antioxidant and anti-inflammatory activities of the herbal vaginal tablet. Quercetin is a plant pigment (flavonoid) found in many plants and foods such as red wine, onions, green tea, apples, berries and Ginkgo biloba. On account of its antioxidant and anti-inflammatory properties, it is used for treating high cholesterol, heart diseases, diabetes, cataract, peptic ulcer, asthma, gout, viral infections, chronic fatigue syndrome, etc.

The analysis of data relating to the stability test of F-XII vaginal tablets revealed that no statistically significant changes ($p > 0.05$) in drug content and release pattern of the formulations occurred over the period of the stability study. No significant changes were observed in the mean values of the other physical parameters also over different time intervals. Thus, the use of the chosen polymers did not adversely affect the stability of the drug; furthermore, the tablet formulations were physically stable over this duration.

CONCLUSION

The study aims to design and standardize a muco-adhesive extended release vaginal herbal tablet prepared from aqueous extract of stem barks of *Ficus glomerata* Roxb. and *Symplocos racemosa* Roxb. using standard parameters like hardness, friability, disintegration, surface pH, swelling index, muco-adhesion strength, in-vitro dissolution release and in-situ kinetic drug release studies. The results suggest that while all

three tablet formulations (F-XI, F-XII and F-XIII) show significant positive physical parameters indicating good efficacy, F-XII formulation shows the best attributes and fits the desired criteria for drug release vaginal tablets. After trying several combinations of polymers, the best results are exhibited in F-XII containing low levels of Carbopol, moderate levels of HPMC and high amounts of Micro crystalline cellulose.

Further validation was done by the in-situ kinetic release study which indicated a very slow but steady decreasing first-order Fickian diffusion drug release pattern in the vaginal mucosa suggesting effective local action. Comparison with the standard vaginal tablet CANDID-V6 indicated that F-XII demonstrates comparable but slightly lower antibacterial and antifungal activity against E. coli and Candida albicans microbes. HPTLC chromatography suggested the presence of standard chemical flavonoidic compound Quercetin, well known as an antioxidant and anti-inflammatory plant compound which may be responsible for the exhibited anti-leucorrheal efficacy of F-XII formulation.

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