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In vitro and *in vivo* investigation of drug-eluting films for the treatment of periodontal disease using herbal drugs.

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ABSTRACT

A novel periodontal film for the treatment of periodontitis was developed by using herbal drugs Quercetin and Rutin, it is a plant flavanol from the flavonoid group of polyphenols. It is found in many fruits, vegetables, leaves, seeds and grains; capers and red onions. It has a bitter flavour and is used as an antimicrobial agent and effective against infecting microorganisms in the periodontal pocket. Periodontal films were prepared by solvent casting technique using Eutragit and HPMC as polymers Dibutyl phthalate as plasticizers and PEG as surface active agent. The patches were cut in 1 cm^2 and placed in desiccator for further study . *In vivo* animal study was performed by using Male Albino rabbit (New Zealand rabbit) 8–12 weeks. The operative area was cleaned and periodontal patch of 1cm^2 was placed in the gingival sulculus of rabbit's lower incisors. The gingival crevicular fluid of the lower incisors was then extracted using #30 standardized sterile paper point on 1, 2, 4, 7, 10, and 14hrs.

Keywords:; Quercetin; periodontal film; Periodontitis Controlled release and Gingivitis.

1.INTRODUCTION

Periodontal diseases is recognized as the major public health problem throughout the world. Daily oral hygiene plays a vital role in maintaining healthy teeth and gums. Periodontal disease can do occur in all age groups, ethnicities, races, genders and socioeconomic levels. Periodontal diseases, including gingivitis and periodontitis, are serious infections that left untreated can lead to tooth loss. The word periodontal literally means "around the tooth." Periodontal disease is a chronic bacterial infection that affects the gums and bone supporting the teeth. Periodontal disease can affect one tooth or many teeth. It begins when the bacteria in plaque (the sticky, colourless film that constantly forms on teeth) causes the gums to become inflamed. Periodontal diseases range from simple gum inflammation to serious disease hence results in major damage to the soft tissue and bone that support the teeth. In the worst cases, teeth are lost [1]. Periodontitis is caused by microorganisms that adhere to and grow on the tooth's surfaces, along with an overly aggressive immune response against these microorganisms[2]. A diagnosis of periodontitis is established by inspecting the soft gum tissues around the teeth with a probe and x-ray films by visual analysis, to determine the amount of bone loss around the teeth. Specialists in the treatment of periodontitis are periodontists their field is known as "periodontology" or "periodontics". The main cause of periodontal disease is bacteria plaque, a sticky, colourless film that constantly forms on teeth. However, factors like smoking/ tobacco use, genetics, pregnancy and puberty, stress, medication, clenching or grinding teeth, diabetes and poor nutrition also lead to periodontal diseases. Periodontal pathogens grow only

where atmosphere and nutrient composition are strictly conductive to their requirements and the disease once established, causes major changes in the periodontal microenvironment. The gingival crevicular fluid (GCF) flow occurs at extremely low levels in healthy gingival sulci but increases enormously to 3.5 ml/day or more. The most commonly grown anaerobic pathogenic bacteria are Actinobacillus actinomycetencomitans, Bacteroides gingivitis; Bacteroides melanogenic sub species intermedius, Porphyromonas gingivitis and Prevotella intermedia. Clinical signs such as bluish red thickened marginal gingiva, bluish red vertical zone from the gingival margin to the oral mucosa, gingival bleeding and localized pain are suggestive of the presence of periodontal pockets microorganisms [3,4,5]

FIGURE 1 Representing the healthy gums, gingivitis, periodontal disease and periodontitis.

2.MATERIALS AND METHOD

Quercetin and Rutin was purchased from Vasa lab Bangalore, HPMC, Eudrgit RL100, Dibutyl phthalate and alcohol was obtained from laboratory. Ethical clearance was obtained for this work ,IAEC approval no **IAEC SSCP NO :175/2021-21**

2.1 Method of preparation of Quercetin and Rutin periodontal films

1. Quercetin and Rutin was dissolved in polyethylene glycol and polymer HPMC and eurtagit was dissolved in alcohol at room temperature using magnetic stirrer in separate beaker and beaker containing drug solution was mixed with polymers solution.
2. Later dibutyl phthalate was added as plasticizer.
3. The solution was poured on a petri dish and dried at room temperature.
4. Films were removed from petri dish and were cut into specific size packed in aluminum foil and stored in desiccators for further evaluation[6].

2.2 Formulation containing Quercetin periodontal films

Table 1 composition of Quercetin periodontal films.

Ingredients	F1	F2	F3	F4
Quercetin (mg)	100	100	100	100
HPMC K 15M(mg)	100	100	125	150
Eudrgit RL100 (mg)	-	75	100	125
Dibutyl phthalate(ml)	1	1	1	1
PEG 800 (ml)	5	5	5	5
Alcohol(ml)	10	10	10	10

2.3 Formulation containing Rutin periodontal films

Table 2 composition of Rutin periodontal films

Ingredients	F1	F2	F3	F4
Rutin (mg)	100	100	100	100
HPMC K 15M(mg)	100	100	125	150
Eudrgit RL100 (mg)	-	75	100	125
Dibutyl phthalate(ml)	1	1	1	1
PEG 800 (ml)	5	5	5	5
Alcohol(ml)	10	10	10	10

2.4. *In-vitro* drug release study:

As the P^H of the gingival fluid lies between 6.5-6.8 phosphate buffer of P^H 6.6 was used as the simulated gingival fluid and the films remains immobile in periodontal pocket. A static dissolution method was adopted for *in-vitro* drug release studies. Patches of known weight and dimension (1×1 cm^2) were placed separately into small test tubes containing 1ml phosphate buffer P^H 6.6 the tubes were sealed and kept at 37º C for 24hrs. the buffer was drained off and replaced with 1ml of phosphate buffer P^H 6.6. Then the concentration of the drug in buffer were measured at 369nm for Quercetin and 256nm for Rutin in UV. *In-vitro* drug release studies were carried out for 10 days[7].

2.5.*In vivo* Animal studies:

To study the drug release *in vivo* animal study was performed on male Albino rabbit (New Zealand rabbit) 8–12 weeks/ 1.5 kg. Each rabbit was sedated with 2% xylazine-HCl (5 mg/kg body weight) and ketamine-HCl (Ketasol, 30 mg/kg body weight, administered intramuscularly). The operative area was cleaned and sterilized the periodontal patch of 1cm^2 was placed in the gingival sulculus of each rabbit's lower incisors. The gingival crevicular fluid of the lower incisors was then extracted using #30 standardized sterile paper point on 1, 2, 4, 7, 10, and 14days. The paper point was inserted 1 mm into the gingival crevice and was left *in situ* for 30 seconds during each procedure. Immediately after collection, the paper point was eluted with 0.1 ml phosphate buffer solution and then stored at −20°C until it was analysed [8].

2.6.HPLC Analysis:

Quercetin was analysed by HPLC using a C_{18} column with UV detection at 369 nm. The mobile phase was acetonitrile HPLC grade (50:50 v/v) at a flow rate of 0.9 mL min^{-1}. The curve range was linear for the concentration range 0.1–25 μg mL^{-1}

Standard and working solutions of Quercetin:

Quercetin was dissolved in methanol in a 1.0 mL volumetric flask to a final concentration of 1.0 mg μmL^{-1} . The solution was sonicated for 5 min in an ultrasonic bath and completed to the final volume. Working solutions (0.1–25.0 μmL^{-1}) were prepared by dilution of aliquots of the standard solution with methanol (1.0 mg mL^{-1}) in a 5.0 mL volumetric flask [9].

Rutin was analysed by HPLC using a C_{18} column with UV detection at 256 nm. The mobile phase was acetonitrile HPLC grade (50:50 v/v) at a flow rate of 1.0 mL min^{-1}. The curve range was linear for the concentration range 0.1–25 μg mL^{-1}

Standard and working solutions of Rutin:

Rutin was dissolved in methanol in a 1.0 mL volumetric flask to a final concentration of 1.0 mg μmL^{-1} . The solution was sonicated for 5 min in an ultrasonic bath and completed to the final volume. Working solutions (0.1–25.0 μmL^{-1}) were prepared by dilution of aliquots of the standard solution with methanol (1.0 mg mL^{-1}) in a 5.0 mL volumetric flask[10].

3.RESULTS AND DISCUSSION:

Time(days)	% of drug released of F 1	% of drug released of F 2	% of drug released of F 3	% of drug released of F 4
0	0	0	0	0
1	41.66	31.281	26.204	26.067
2	57.328	41.732	41.949	36.520
3	67.865	57.320	47.132	46.462
4	78.363	62.691	57.760	49.638
5	88.871	69.009	65.679	57.508
6	94.174	73.227	68.888	65.383
7	99.472	83.624	74.721	73.267
8	--------	94.758	83.180	81.682
9	---------	95.273	89.029	84.891
10	-------	-------	99.928	95.051

Table number 3 Comparative *In-vitro* drug release study of Quercetin patch from F1-F4

In vitro drug release study was carried out for 10 days in phosphate buffer P^H 6.6 .The release was in the range of 26.067 to 99.928 and F3 released the drug for 10 days.

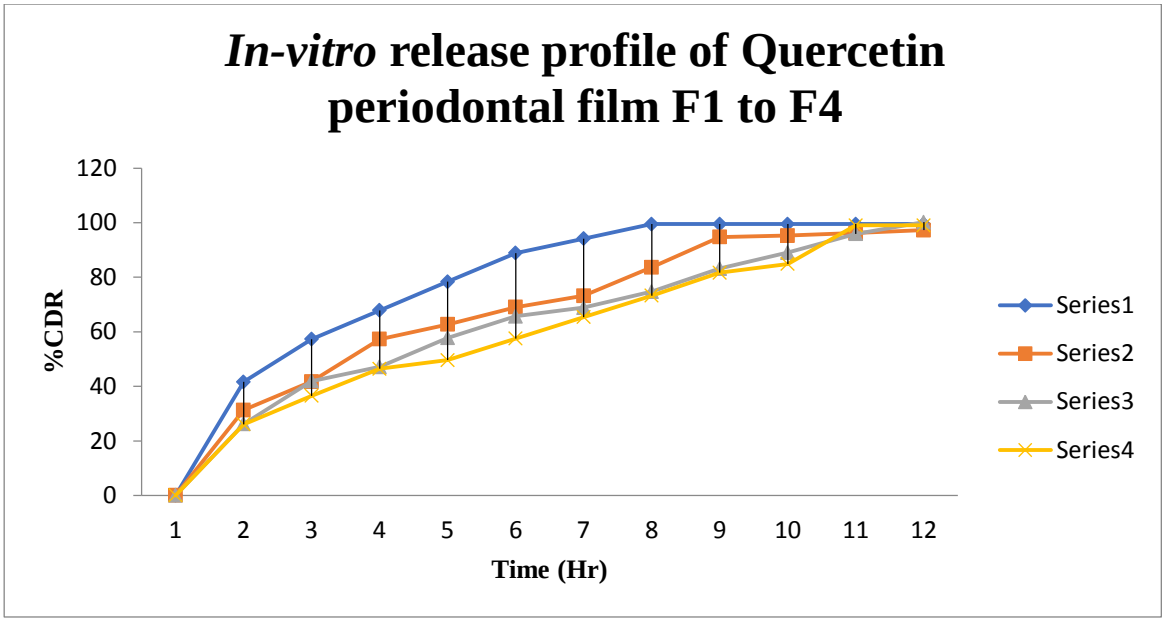

Figure number 3 Comparative *In-vitro* drug release study of Quercetin patch from F1-F4

Time(days)	% of drug released of F1	% of drug release of F2	% of drug released of F3	% of drug released of F4
0	0	0	0	0
1	50.37	44.02	12.57	19.048
2	64.823	61.67	25.162	31.480
3	76.649	79.981	31.477	56.698
4	79.087	87.607	62.958	75.643
5	87.716	88.323	75.490	82.634
6	91.587	94.690	83.790	84.224
7	98.605	--------	87.608	88.469
8	100.76	--------	89.577	94.227
9	--------	---------	94.080	96.737
10	---------	----------	95.44	-------
11	---------	----------	99.93	--------

Table number 4 Comparative *In-vitro* drug release study of Rutin patch from F1-F4

In vitro drug release study was carried out for 11 days in phosphate buffer P^H 6.6 .The release was in the range of 12.57 to 100.76 and F3 released the drug for 11days

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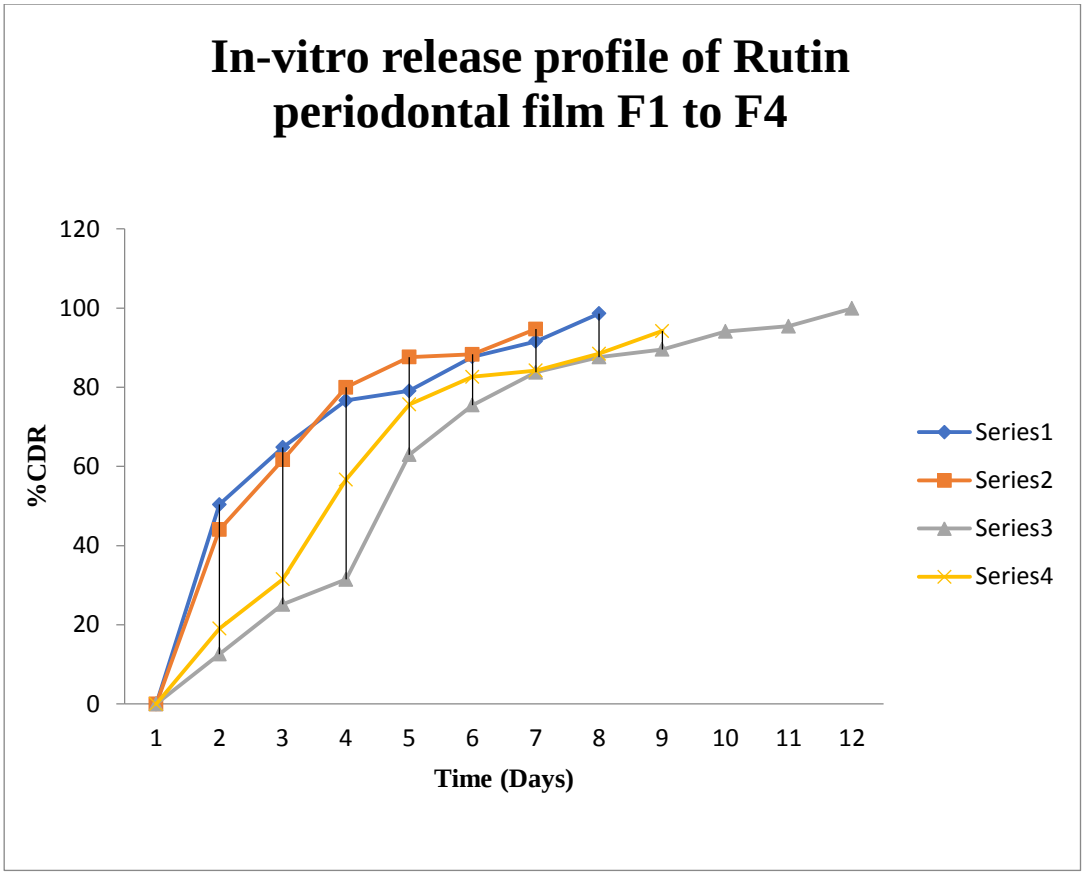
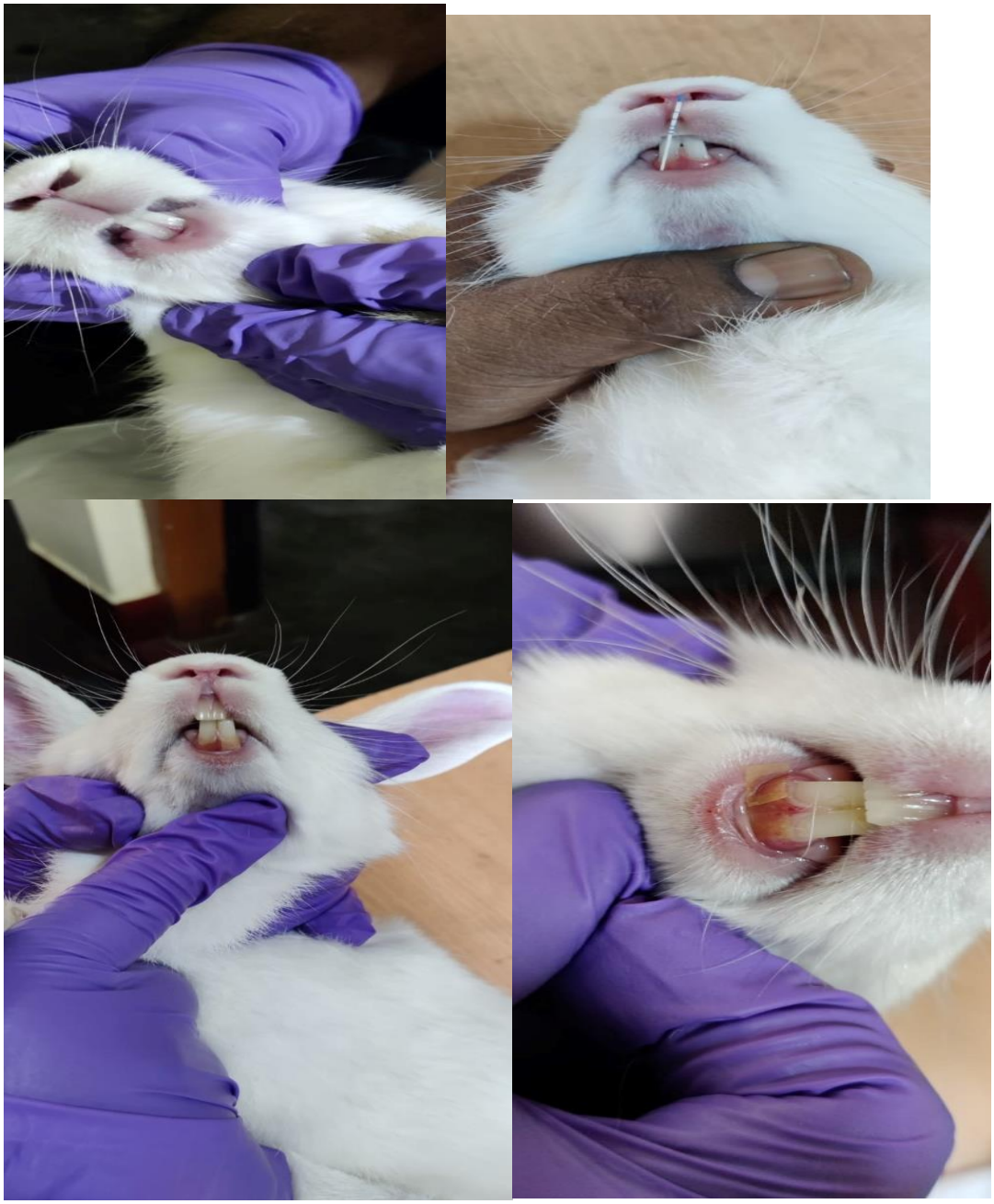


Figure 4 Comparative *In-vitro* release profile of Rutin periodontal film F1 to F4 in phosphate buffer P^H 6.6



Figures number 5: Insertion of the patch in gingival sulculus of rabbit's lower incisors.

3.1.HPLC Analysis

In vivo animal study was performed by placing patch of 1 cms ^{2} in the gingival sulculus of rabbit's lower incisors. The gingival crevicular fluid of the lower incisors was then extracted using #30 standardized sterile paper point on 1, 2, 4, 7, 10, and 14days. The paper point was inserted 1 mm into the gingival crevice and was left *in situ* for 30 seconds during each procedure. Immediately after collection, the paper point was eluted with 0.1 ml phosphate buffer solution and then stored at −20°C until it was analysed.

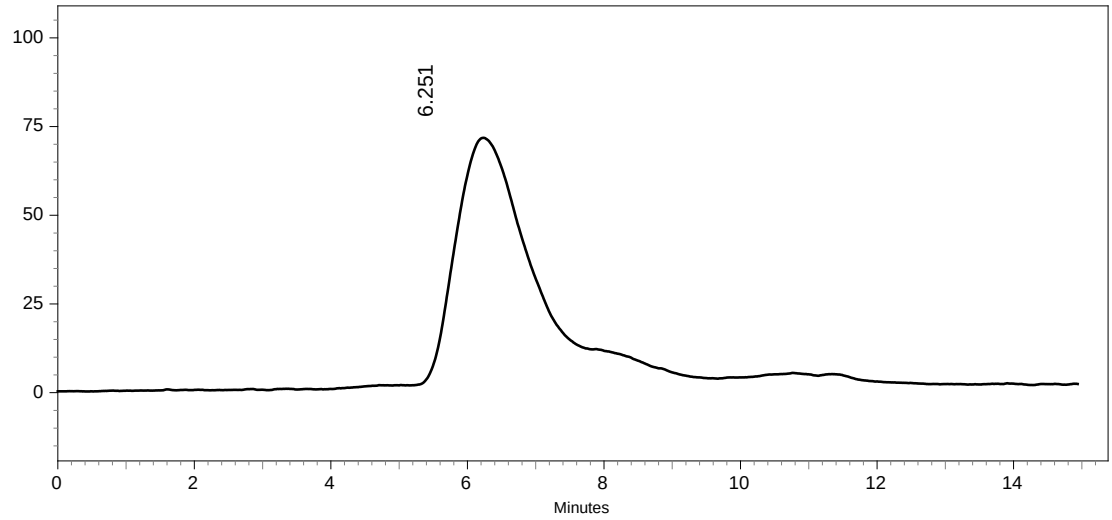


Figure number 6 HPLC peak of Quercetin

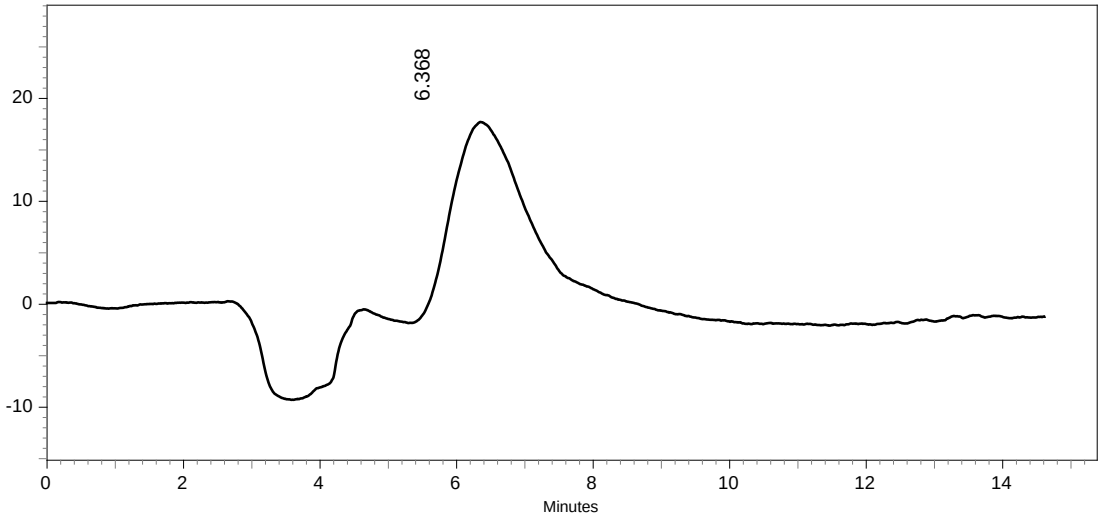


Figure no 7 HPLC peak of Quercetin containing periodontal films

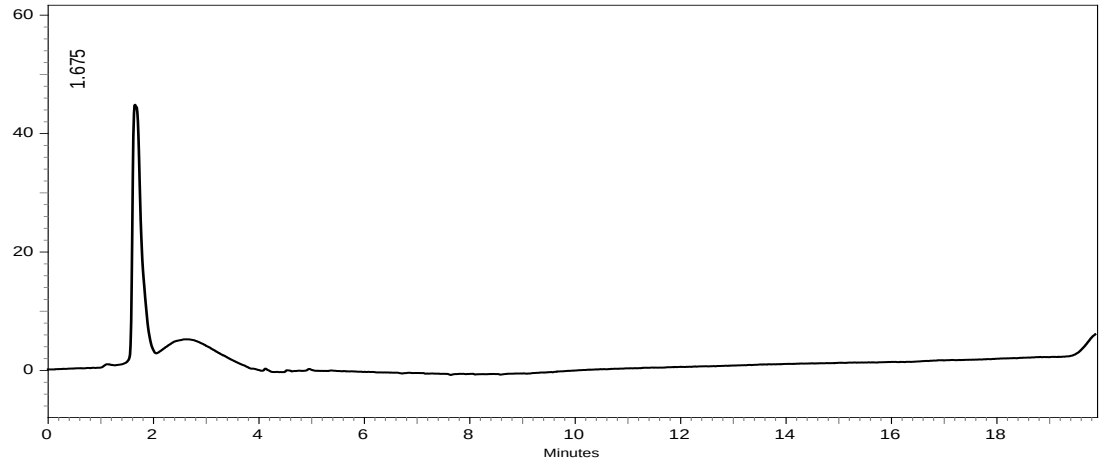


Figure number 8 HPLC peak of Rutin

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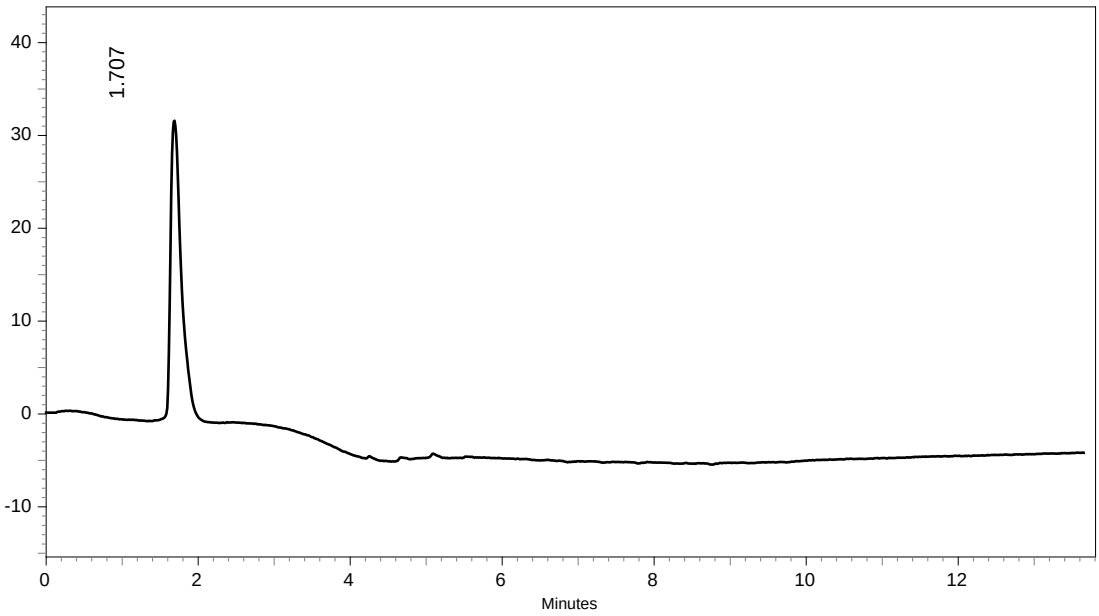

Figure number 9 HPLC peak of Rutin containing periodontal patch.

Table number 5 Showing time in days and AUC of Rutin periodontal film and Quercetin periodontal film.

Time in days	AUC Rutin periodontal film	AUC Quercetin periodontal film
1	18976	4440
2	24699	51464
4	50307	57589
7	168367	70247
10	499538	81111
14	3912552	197440

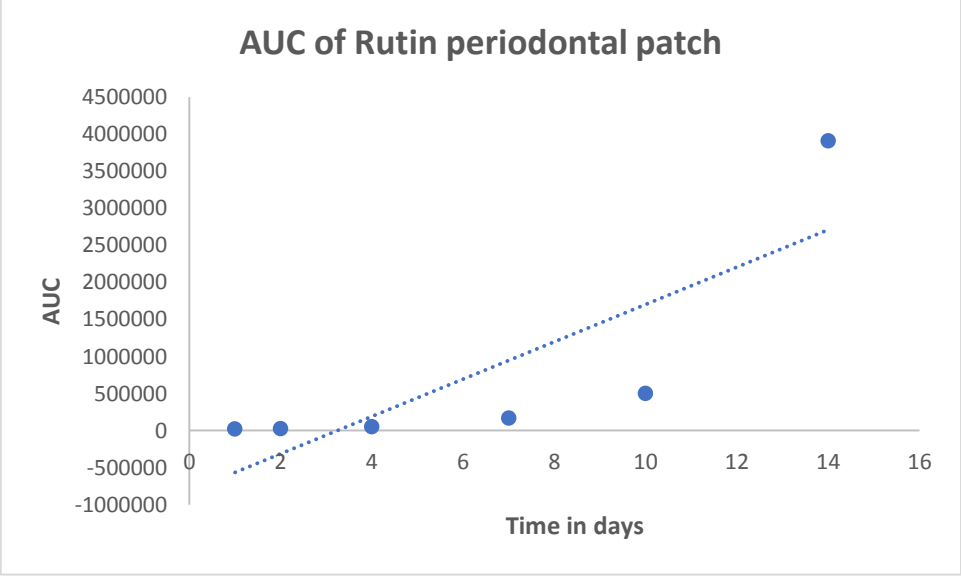

Figure number 10 AUC and Time in days of Rutin containing periodontal films

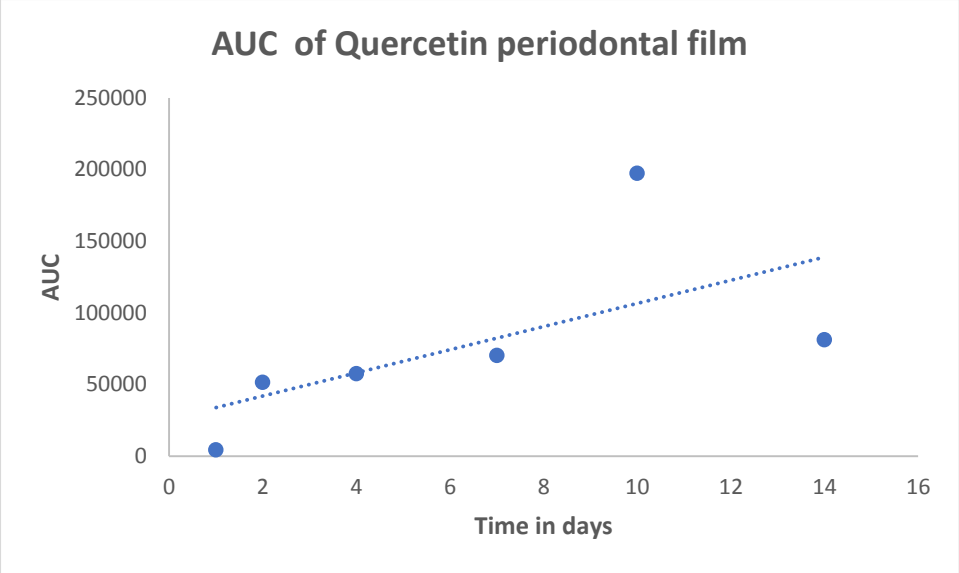

Figure number 11 AUC and Time in days of Quercetin containing periodontal films

4.CONCULSION

In the present research work periodontal patch of Quercetin and Rutin was prepared by solvent casting method using HPMC and Eudragit as polymers. The formulations were studied for *In vitro* release in phosphate buffer P^H 6.6 The study was carried out for 11 days F3 of both the formulations released for 11 days .*In vivo* animal study was performed by placing the patch of 1 cm^2 placed in rabbits gingival sulcus and studied for drug release by using HPLC ,and this method was found suitable for analytical determination of Rutin and Quercetin concentrations in gingival crevicular fluid of rabbit .Retention time for standard Rutin was 1.675minutes and for the formulation of (F3) was 1.707minutes . Retention time for standard Quercetin was 6.251min and for the formulation was Quercetin(F3) 6.368min. This method was capable of detecting low concentrations of Rutin and Quercetin in the gingival crevicular fluid of rabbit and the calibration curve was found to be linear over the concentration range of Rutin and for Quercetin (0.1 to 25 μgmL^{-1}) *In vivo* animal study showed the drug release in gingival sulcus were able to inhibit the bacteria .

COMPLIANCE WITH ETHICAL STANDARDS

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DISCLOSURE OF CONFLICT OF INTEREST

The authors have declared that, there is no conflict of interest exist in this research article.

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