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TRANSDERMAL PATCH FORMULATION WITH *NIGELLA SATIVA* EXTRACT FOR SYNERGISTIC ANTI-BACTERIAL AND ANTI-INFLAMMATORY THERAPY

Silpa G.*, Aysumma C. U. and Gowrishankar N. L.

Department of Pharmaceutics, Prime College of Pharmacy, Palakkad.

***Corresponding Author: Silpa G.**

Department of Pharmaceutics, Prime College of Pharmacy, Palakkad.

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ABSTRACT

The objective of the present project was to extract Nigella sativa L. seed and incorporate it into a transdermal drug delivery system. Extraction was done with 100 ml of ethanol in a Soxhlet apparatus. The ethanol extract of Nigella sativa has antibacterial and anti-inflammatory activity. Hence, this extract was used for phytochemical screening and as an anti-bacterial and anti-inflammatory Phytoconstituent. An albumin degradation and protein denaturation method demonstrates anti-inflammatory activity. Six patches are formulated by using two solvent systems: water and EtOH, and HPMC 50CPS, along with PEG-400 10% w/v polymer weight by solvent evaporation technique, were suitable for good flexibility and elasticity. It was found that the formulations F3 and F4 (HPMC4%) have achieved acceptable mechanical properties and extended-release, prolonged zero-order release, and first-order kinetics, as well as regression values.

KEYWORDS: *Nigella sativa L*, EtOH, HPMC4%, PEG-400, HPMC 50CPS.

INTRODUCTION

The main goal of the pharmaceutical sector is to treat and care for patients to relieve the symptoms of a disease drugs. Drugs are available as natural drugs, semi synthetic and synthetic drugs. Natural drugs are still untouched in the pharmaceutical industry because of poor pharmacokinetic studies. All the existing synthetic drugs are taken in oral form due to a lack of certain parameters, like poor absorption and a lower percentage of bioavailability. Due to this entire phenomenon, pure natural drugs were taken via the transdermal route of drug delivery.

The transdermal patches with ant-bacterial and anti-inflammatory skin infection such as caused by *Eschericia coli*, also known as *E. coli*, *E. coli* strains are harmless, but some serotypes can cause serious bacteria associated with skin and soft tissue infections in hospitalized patients, which have been recorded over the years in several databases, And also skin infection caused by *Staphylococcus aureus* bacteria are classified as gram-positive cocci based on their appearance under a microscope They are only able to invade via broken skin or mucosa membranes; hence, intact skin is an excellent human defense. Skin infection is the most common form of *S. aureus* infection. The inflammatory skin infections such as Psoriasis is a chronic, life-long inflammatory skin condition in which red, itchy plaques commonly occur on the knees and elbows. It is estimated to affect between 1 and 3% of the population. It is characterized by epidermal hyper proliferation, and dermal inflammation is the result of a delayed skin production process.

Transdermal drug delivery systems

The TDDS allows for pharmaceuticals to be delivered across the skin barrier. In theory, transdermal patches work very simply. The drug is added in a relatively high dosage to the patch, which acts on the skin for an extended period of time. And in the diffusion process, the drug enters the bloodstream through the skin.

Advantages of TDDS

- Transdermal delivery can increase the therapeutic value of many drugs by avoiding specification due to hepatic "*first pass.*"
- It achieves consistent plasma levels.
- It is of great advantage to patients who are nauseated or unconscious.
- When toxicity develops from a drug administered transdermally, it can be corrected by removing the patch.

Disadvantages of TDDS

- May not be economical.
- The barrier function of the skin changes from one site to another on the same person, person to person, and with age.

MATERIALS AND METHOD

Plant Profile

Nigella sativa (**black caraway**, also known as *nigella* or *kalonji*), often called black cumin, is an annual flowering plant in the family *Ranunculaceae*, native to south and southwest Asia. The flowers are delicate and usually pale blue and white, with five to ten petals. The black caraway fruit is a large and inflated capsule composed of three to seven united follicles, each containing numerous seeds that are used as spice.

Fig 1: ***Nigella Sativa Seed.***

Collection of seed material

The *Nigella sativa* L. seeds were purchased from Kottakal Aaryavydhyashala, Palakkad district, Kerala, India. The black seeds were thoroughly rinsed with plenty of water to get rid of the adherent impurities. The clean seeds were used for the preparation of the extract.

Preparation of the extract

Sativa seeds were first dried at room temperature and then grinded into fine powder by using an electric grinder. 10 g of dried powdered seeds were subjected to extraction with 100 ml of ethanol in a Soxhlet apparatus. The extracts obtained were filtered and stored at low temperatures for further studies.

Phytochemical analysis

Chemical tests were carried out to evaluate the presence of the phytochemicals present in the extract.

METHODOLOGY

Table 2: Phytochemical Screening of seeds of *N. sativa.*

CONSTITUENTS	TEST
Phenols	Ferric Chloride test
Alkaloids	Dragendorff's reagent test
Tannins	Ferric test
Cardiac Glycosides	Keller killani test
Steroids	Liebermann-Burchard's test
Flavonoids	Alkaline test
Saponins	Salkowski reaction
Carbohydrates	Fehling's test
Proteins and amino acids	Biuret test

Investigation of anti-bacterial activity

Micro titre plate assay

The anti-bacterial assay was carried out using a micro titer plate assay, as mentioned below. The assay was done using an ethanolic extract of *Nigella sativa* against E. coli *and Staphylococcus aureus*. Plates were prepared under aseptic conditions. A sterile 96-well plate was labeled, and further procedures are given below. After incubation for 24 hours, the micro titre plate was fixed to the ELISA titre plate reader, and the values were noted.

Table 3: Experimental setup for anti-bacterial assay.

	T1	C1	T2	C2	T3	C3	T4	C4	T5	C5	Control
Test sample	10µl	10µl	20µl	20µl	40µl	40µl	60µl	60µl	100µl	100µl	-
Culture	100µl	-	100µl	-	100µl	-	100µl	-	100µl	-	100µl
Nutrient broth	100µl	100µl	100µl	100µl	100µl	100µl	100µl	100µl	100µl	100µl	100µl

% OF inhibition = [Test – Control] / Test × 100.

Investigation of anti-inflammatory activity

Anti-proteinase activity

The reaction mixture (2 ml) contained 0.06 mg of trypsin, 1 ml HCl buffer (7.4), and 1 ml of a of a test sample of different concentrations (100–500 µg/ml). The mixture was incubated at 37 oC for 5 minutes, and 1 ml of per chloric acid was added. The cloudy suspension was centrifuged, and the absorbance was read out at 210 nm against the buffer as blank. The percentage of inhibition was calculated.

% of inhibition = (Abs control – Abs sample) × 100 / Abs control.

Formulation and Evaluation of Transdermal Films

Preparation of Transdermal films

The transdermal patch containing *Nigella sativa* seed extract was prepared by using polyvinyl alcohol as a backing membrane and different ratios of HPMC 50CPS as dispersion polymer by solvent evaporation technique. The backing membrane of polyvinyl alcohol 4% w/v was

prepared and allowed to dry for 24 hours at room temperature. Weighed 10 ml of seed extract and different ratios of HPMC with solvent were continuously stirred for 1 hour, then polyethylene glycol was added as a plasticizer and stirred for another 1 hour. Then, a 10 ml solution containing seed extract was slowly poured over the previously prepared backing of PVA by pipette. The solvent was allowed to evaporate. After 24 hours of drying at room temperature, the film was collected by peeling and evaluated.

Table 4: Formulation chart of *Nigella sativa* seed extract transdermal patches.

Formulation code	PVA % w/v	HPMC-50 CPS %w/v	PEG-400 %w/v of total polymer	Ethanolic extract of *Nigella sativa* seed(ml)	Solvent Used
F1	4	6	10	25	water
F2	4	5	10	25	Water
F3	4	4	10	25	Water
F4	4	4	10	25	EtOH
F5	4	5	10	25	EtOH
F6	4	6	10	25	EtOH

EVALUATION OF TRANSDERMAL PATCH

A. Surface pH

The surface pH of the patch was determined by allowing it to swell in a closed Petri dish at room temperature for 30 minutes in 0.1 ml of double-distilled water. The swollen device was removed and placed under a digital pH meter to determine.

B. Uniformity of weight

Weight variation is studied by individually weighting randomly selected patches and calculating the average weight. The individual weight should not deviate significantly from the average weight.

C. Thickness

The thickness of transdermal patches was measured at three different places using a screw gauge, and then the mean value was calculated.

D. Moisture content

The prepared film was weighed individually and kept in vacuum desiccators containing phosphorus pentoxide at room temperature for 24 hours. The patches were weighted again and again individually until they showed a constant weight. The percentages of moisture content were calculated as a difference between initial and final weight with respect to final weight.

E. Uptake moisture

The drug polymer films were weighed and then kept for drying up to a constant weight in a vacuum desiccator at normal room temperature for 24 hours exposed to 80% relative humidity (a saturated solution of potassium chloride).

F. Flatness

One strip is cut from the center and two from each side of the patch. The length of each stripe is measured, and variation in length is measured by determining percent constriction. Zero percent constriction is equivalent to 100 percent flatness.

G. *In vitro* drug permeation studies using the egg membrane

Procedure for Drug Permeation through the Egg Membrane.

The in vitro permeation studies were conducted using a vertical test tube cell with a receptor compartment capacity of 50 ml. The excised egg membrane was mounted with the prepared patch on the top of the test tube, with the membrane in contact with receptor fluid (phosphate buffer pH 7.4), and was equilibrated for 24 hours. The receiving chamber had a volume of 50 ml, and the area available for diffusion was about 1.13 cm2. The fluid in the receptor compartment was maintained at 37±.5°C. Initially, the egg membrane was slightly dipped in the receiving chamber. The entire assembly was kept on a magnetic stirrer, and the solution in the receiver compartment was stirred continuously using a magnetic bead. The sample solution was withdrawn from the receiving chamber at regular time intervals, replaced by equal volumes of fresh receptor medium, and assayed using a spectrophotometer. The concentration of seed extract permeated was determined spectrophotometrically at 350 nm after suitable dilution against a blank of phosphate buffered saline at 7.4 pH by a UV spectrophotometer.

H. Optimization of *Nigella sativa* TDDS

Optimization of the best patch was done based on the drug release profile, i.e., the patch with the highest cumulative drug release in 24 hours, in a controlled manner and also based on other evaluated parameters.

a. Zero order kinetics

$$At = A_0 - K_0 t$$

Where, *At* = drug release at time t.A_0 = initial drug concentration, K_0 = Zero order rate constant (hr^{-1}), when data is plotted as cumulative percent drug release versus time, if the plot is linear then the data obeys zero-order equal to K.

b. First order kinetics

Log C = log C_0 – Kt / 2.303.

Where, C= amount of drug retained at time t, C_0 = initial amount of drug, K = First order rate constant (hr^{-1}) when data is plotted as log cumulative percent drug remaining versus time yield a straight line indicated that the release follow first order kinetics. The constant 'K' can be obtained by multiplying 2.303 with the slope values.

c. Higuchi model

$Q = Kt^{1/2}$

When data is plotted cumulative drug release versus square root of time yield a straight line, indicating that the drug was released by diffusion mechanism, the slope is equal to K.

RESULTS

Phytochemical Evaluation

Table 5: The result of the various active constituents is given below.

CONSTITUENS	INFERENCE
Phenols	+
Alkaloids	+
Tannins	+
Glycosides	-
Steroids	-
Flavonoids	+
Saponnins	-
Carbohydrates	+
Proteins and amino acids	+

Anti-bacterial activity of *Nigella sativa seed* extract by **Micro-titreplate method**

Table 6: Anti-bacterial activity of *Nigella sativa* seed extract on the tested micro-organism.

Against *E.coli*		
Sample	**OD(test-extract)**	**% of inhibition**
T1(10)	0.643	31.31
T2(20)	0.357	61.87
T3(40)	0.132	85.89
T4(60)	0.124	86.7
T5(100)	0.071	92.4
Control	0.936	
Against *Staphylococcus aureus*		
T1(10)	0.183	22.45
T2(20)	0.164	30.50
T3(40)	0.111	52.96
T4(60)	0.03	97.28
T5(100)	0.023	90.25
Control	0.236	-

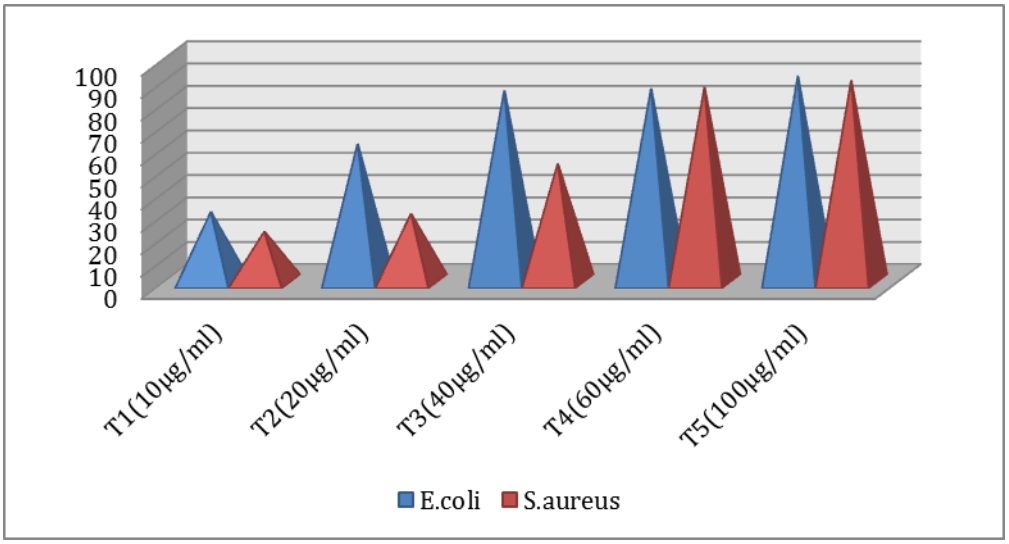

Figure 2: Anti-bacterial activity of different concentration *Nigella sativa* seed extract with these pathogenic microorganisms.

Anti-inflammatory activity of *Nigella sativa seed* extract by Anti-proteinase method.

Table 7: anti-inflammatory activity of *Nigella sativa* seed extract.

Treatment	Concentration (µg/ml)	Absorbance at660nm	% of inhibition of proteinase action.
Control	-	0.38	-
Extract of *N.*s seed	100	0.22	42
Extract of *N.s* seed	500	0.18	53
Aspirin	100	0.17	56

Formulation of Transdermal Patches

Figure 3: Formulated transdermal patches.

Evaluation of transdermal films

A. Surface pH

Table 8: The surface pH was measured by using digital pH meter.

Code	Surface pH
F1	5.20
F2	5.33
F3	5.49
F4	5.5
F5	5.39
F6	4.9

B. Uniformity of weight

Table 9: Uniformity of weight of *Nigella sativa* seed extract patch.

Code	Weight of the patch in mg			Mean ±S.D* (n=3)
	Trail 1	Trail 2	Trail 3	
F1	0.3413	0.3416	0.3415	0.3414±0.02
F2	0.2824	0.2810	0.2822	0.2818±0.06
F3	0.3048	0.3047	0.3047	0.3047±0.03
F4	0.3331	0.3332	0.3334	0.3333±0.03
F5	0.3380	0.3387	0.3394	0.3394±0.02
F6	0.3199	0.3198	0.3203	0.3300±.03

C. Uniformity of Thickness

Table 10: Uniformity of thickness of *Nigella sativa* seed extract.

Code	Weight of the patch in mg			Mean ±S.D* n=3
	Trail 1	Trail 2	Trail 3	
F1	0.30	0.32	0.32	0.31±0.01
F2	0.34	0.37	0.36	0.36±0.05
F3	0.33	0.33	0.33	0.33±0.03
F4	0.34	0.33	0.35	0.34±0.01
F5	0.29	0.29	0.30	0.29±0.05
F6	0.31	0.29	0.30	0.30±0.06

D. Percentage of moisture uptake

Table 11: Percentage of moisture uptake.

Code	Day 1 weight (mg)				Day 2 weight(mg)				% of moisture uptake
	Trail 1	Trail 2	Trail3	Avg.	Trail 1	Trail 2	Trail 3	Avg.	
F1	91	89	89	89.66	96	94	93	94.33	4.95
F2	148	144	144	145.3	160	159	158	159	8.69
F3	78	77	77	77.33	99	99	98	98.67	21.63
F4	58	57	58	57.67	71	74	73	72.67	20.65
F5	98	98	97	97.67	104	100	103	102.3	4.55
F6	133	134	134	133.6	144	147	146	145.6	8.24

E. percentage of moisture content

Table 12: Percentage of moisture content.

Code	Day 1 weight (mg)				Day 2 weight(mg)				% of moisture content
	Trail1	Trail2	Trail3	Avg. Wgt	Trail1	Trail2	Trail3	Avg. wgt	
F1	56	57	56	56.3	55	55	55	55	2.41
F2	98	96	97	97	92	92	90	91.3	6.2
F3	79	79	78	78.6	70	69	72	70.3	11.84
F4	63	65	66	64.6	59	58	58	58.3	10.86
F5	146	146	144	145	141	142	141	141.3	2.83
F6	122	126	128	1253	120	120	119	119.6	4.74

F. Flatness

Table 13: Flatness.

Code	Initial length (cm)				Final length (cm)				% constriction	% flatness
	Trail 1	Trail 2	Trail 3	Avg.	Trail 1	Trail 2	Trail 3	Avg.		
F1	12.0	3.0	6.5	7.1	12.0	3.0	6.5	7.1	0	100
F2	10.5	2.5	7.1	6.7	10.5	2.5	7.1	6.7	0	100
F3	11.0	4.0	5.8	6.93	11.0	4.0	5.8	6.9	0	100
F4	10.9	3.0	6.2	6.7	10.9	3.0	6.2	6.7	0	100
F5	12.2	2.9	7.3	7.4	12.2	2.9	7.3	7.4	0	100
F6	9.9	3.3	7.8	7	9.9	3.3	7.8	7	0	100

G: Drug permeation study

Table 14: % CDR of formulated patches after 24 hours.

TIME (HRS)	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
2	8.57	7.84	7.4	8.89	6.66	5.55
4	15.44	13.22	14.77	12.99	9.79	14.34
6	22.30	18.54	20.56	16.78	18.14	18.87
8	28.79	24.57	29.55	24.59	22.73	27.13
10	33.48	29.99	35.90	29.83	32.17	32.84
12	38.80	32.87	41.34	36.48	38.53	39.37
16	44.67	37.66	49.62	48.30	45.73	44.10
20	49.67	48.22	58.90	58.84	49.90	48.91
24	57.39	59.88	69.86	69.75	55.00	60.55

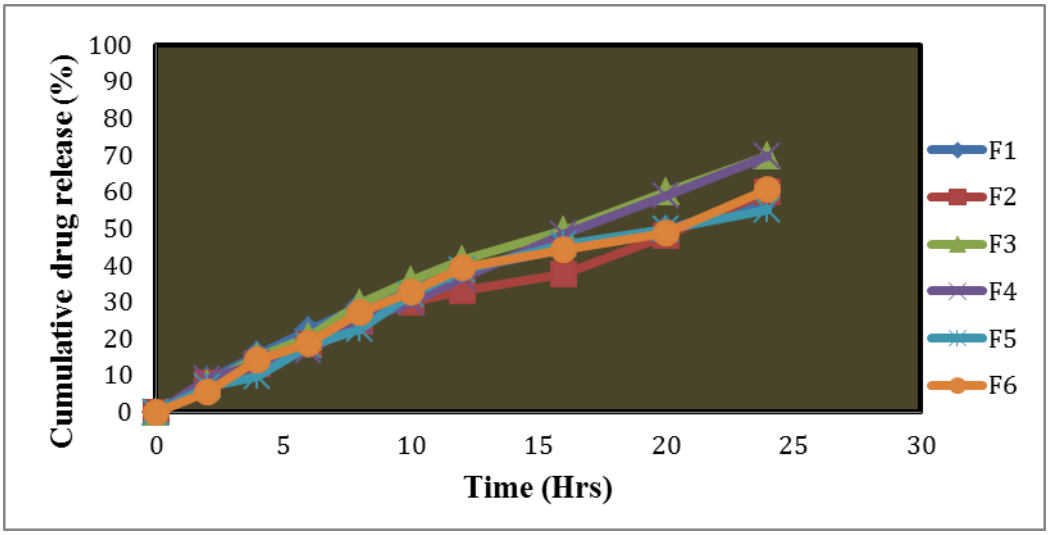

Figure 4: *In-vitro* drug release profile of *Nigella sativa* seed Transdermal patch According to zero order kinetics.

I. Optimization of best patches

Out of six formulation F3 and F4, exhibited good mechanical propertices, and release pattern. so F3 and F4 had been optimized as the best patches. To discribe the, cure fitting analysis done with F3 and F4.

1) Zero order kinetics of F3

Table 15: Zero order kinetics of F3.

SL.NO	TIME (HOURS)	%CDR
1	0	0
2	2	7.4
3	4	14.77
4	6	20.56
5	8	29.55
6	10	35.90
7	12	41.34
8	16	49.62
9	20	58.90
10	24	69.86

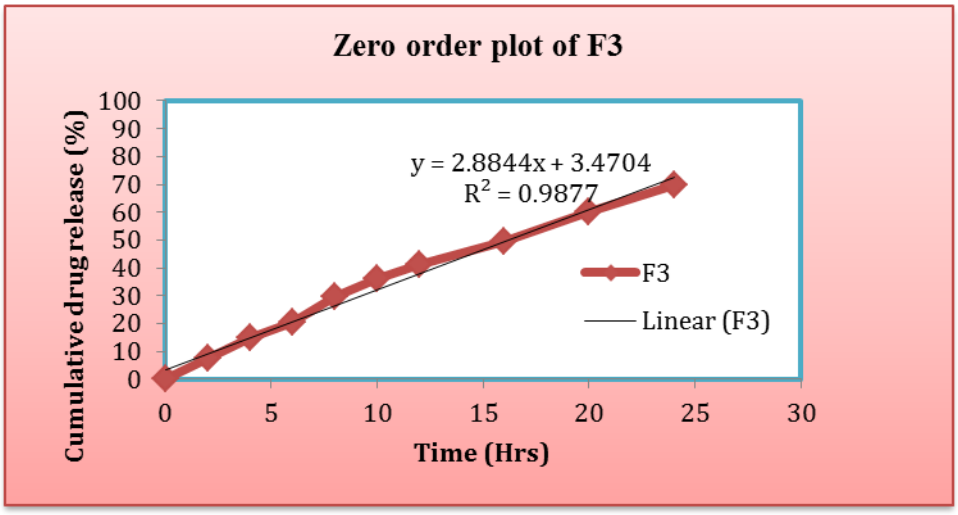

Figure 5: Graph indicating Zero order kinetics of F3.

2) Frist order Kinetics of F3

Table 16: Frist order kinetics of F3.

SL.NO	TIME (HRS)	LOG% CDR
1	0	0
2	2	0.869
3	4	1.1693
4	6	1.3130
5	8	1.4705
6	10	1.5550
7	12	1.6163
8	16	1.6955
9	20	1.7701
10	24	1.8442

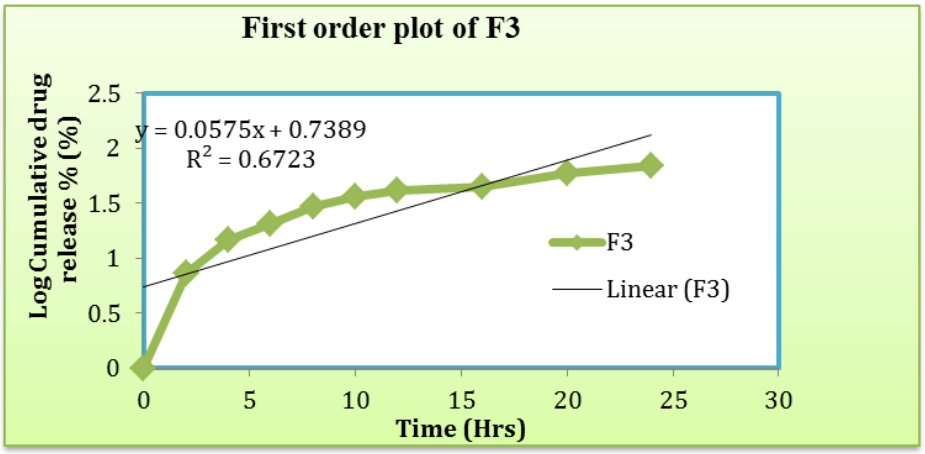

Figure 6: Graph indicating First order kinetics of F3.

3) Higuchi model of F3

Table 17: Higuchi model of F3.

SL. NO	SQUARE ROOT TIME	% CDR
1	0	0
2	1.414	7.4
3	2	14.77
4	2.449	20.56
5	2.828	29.55
6	3.162	35.90
7	3.464	41.34
8	4	49.62
9	4.472	58.90
10	4.898	69.86

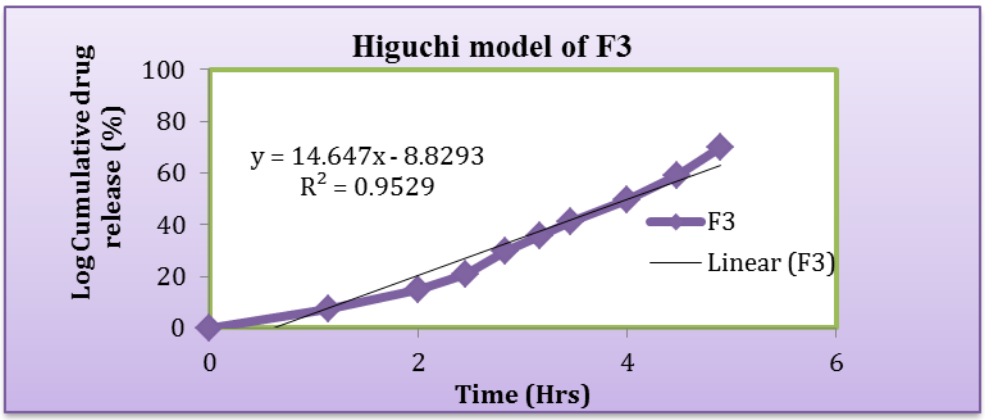

Figure 7: Graph indicating Higuchi model of F3.

4) Zero order plot of F4

Table 18: Zero order kinetics of F4.

SL. NO	TIME (HRS)	% CDR
1	0	0
2	2	8.89
3	4	12.99
4	6	16.78
5	8	24.59
6	10	29.83
7	12	36.48
8	16	48.30
9	20	58.84
10	24	69.75

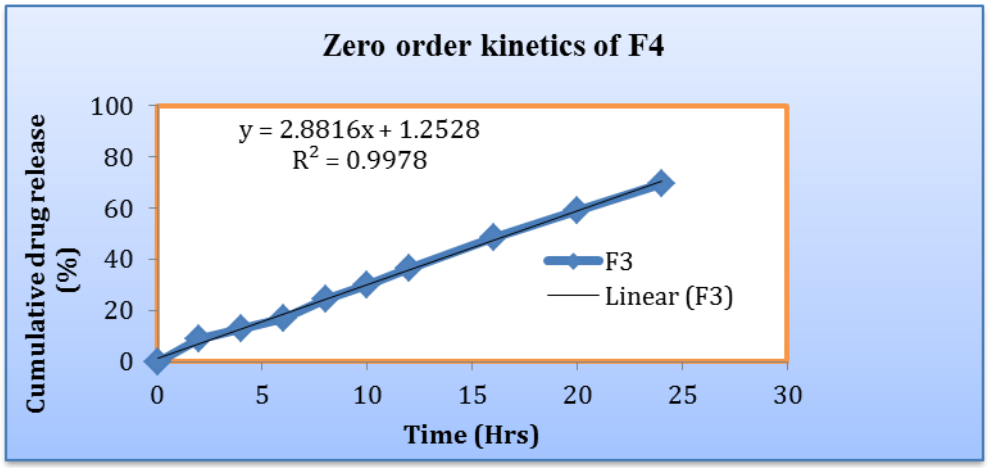

Figure 8: Graph indicating Zero order of F4.

5) First order kinetics of F4

Table 19: First order kinetics of F4.

SL. NO	TIME (HRS)	LOG % CDR
1	0	0
2	2	0.811
3	4	1.1136
4	6	1.2247
5	8	1.3907
6	10	1.4746
7	12	1.5620
8	16	1.6839
9	20	1.7696
10	24	1.8435

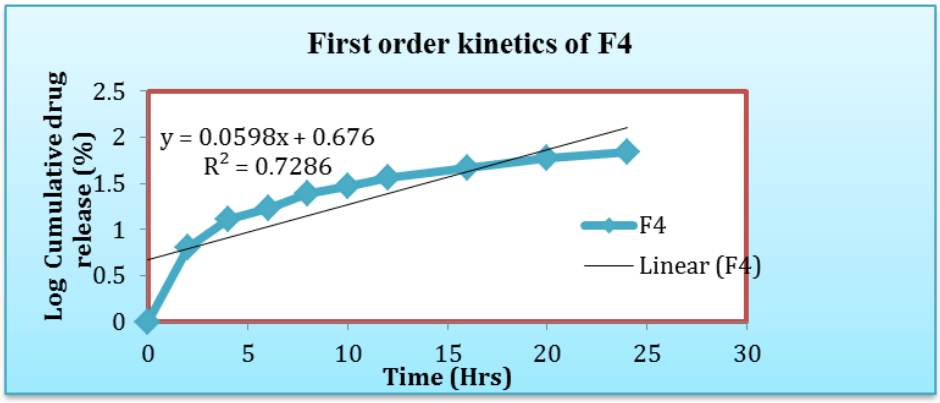

Figure 9: Graph indicating First order kinetics of F4.

6) Higuchi model of F4

Table 20: Higuchi model of F4.

SL. NO	SQUARE ROOT TIME	% CDR
1	0	0
2	1.414	6.5
3	2	12.99
4	2.449	16.78
5	2.828	24.59
6	3.162	29.83
7	3.464	36.48
8	4	48.3
9	4.472	58.84
10	4.898	69.75

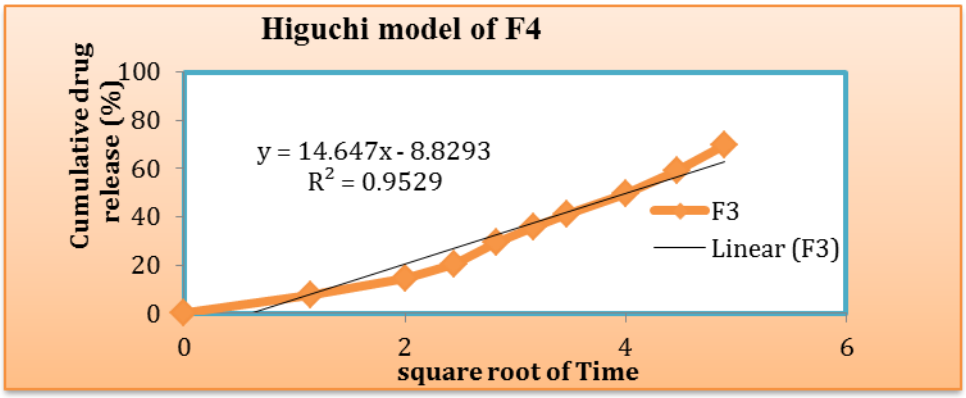

Figure 10: Graph indicating Higuchi model of F4.

CONCLUSION

The present investigation shows the ethanolic extract of *Nigella sativa* (Ranunculaceae) has anti-bacterial and anti-inflammatory activity. Hence, this extract was used for phytochemical screening, which showed the presence of anti-bacterial and anti-inflammatory phytoconstituents. Ethanolic extracts of *N. sativa* have high anti-bacterial activities against gram-positive and gram-negative bacteria. An albumin degradation and protein denaturation method shows the anti-inflammatory activity of psoriasis skin rashes.

Hence attempt was done to formulate transdermal patch for anti-bacterial and anti-inflammatory therapy. Six patches are formulated by using two solvent system water and EtOH. The polymers are polyvinyl alcohol and HPMC 50CPS, along with PEG-400 10% w/v polymer weight by solvent evaporation technique, were suitable for good flexibility and elasticity. And it was found that the formulation F3 and F4 (HPMC4%) has achieved acceptable mechanical property and extended release, prolonged zero order release and first order kinetics, regression values. Developed film formulation F3 and F4 has the best effective combination of polymer concentration to achieve therapeutic plasma concentration.

Based on the observations, it was concluded that as the as the concentration of polymers increases, there will be a decrease in the release of drugs. The attempt at formulation and evaluation of the ethanolic extract of *Nigella sativa* seed patches for anti-bacterial and anti-inflammatory therapy was found to be successful in the release of the drug for an extended period of 24 hours.

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