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Research Article

FORMULATION AND EVALUATION OF HYPTIS SUAVEOLENS BASED BIOADHESIVE VAGINAL GELS

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ABSTRACT

The aim of the present undertaken work is to formulate and evaluate vaginal gel containing Hyptis suaveolens leaf petroleum leaf extract. The prepared extract was subjected to phytochemical screening to determine the presence of various phytoconstituents. The extract was subjected to formulation of gels incorporating gelling agents (Carbopol 934P, HPMC K100, Pemulen TR-1, Pemulen TR2, Xanthan gum, Lutrol) in various concentrations by standard gelling techniques. All the HS Gel formulations were subjected to characterization of various physical parameters like Appearance, Spreadability, Viscosity, pH and Extrudability as per the standard methods. pH values of various HS Gel formulations were found to be unsuitable, as the desired vaginal pH is between 4.5 and 5.5. Owing to the inferior results of physical parameters, which were not considered for further characterization. CF3, HF3, P1F3, P2F3, LF3 XF3 Gel formulations of Hyptis suaveolens consisting high concentrations of respective gelling agents have shown encouraging results with respect to physical parameters. These Gel formulations of Hyptis suaveolens consisting high concentrations of respective gelling agents were subjected to in vitro diffusion studies. By comparing the results of in vitro diffusion studies, Hyptis suaveolens Gel formulations consisting Carbopol 934P (CF3) was optimized due to its retarding ability and consistency. In vivo studies of the optimized vaginal gel formulations were carried out using rats as the animal model to evaluate the antifertilty activity. Results of Antifertility studies indicated that the petroleum ether extract from the leaves of Hyptis suaveolens was found to be efficient to arrest sperm motility and conceiving.

KEYWORDS: *Hyptis suaveolens, Carbopol 934P, vaginal gel, Xanthan gum, Antifertility.*

INTRODUCTION

In today's context, the problem of over population and the resulting need for birth control are becoming very important. Contraceptives have proven their worth to some extent to control this menace. However, the synthetic oral contraceptives available today for fertility control produce severe side effects such as hormonal imbalance, hypertension, increased risk of cancer, weight gain, etc. Therefore, many more efforts are still needed in this field [1].

Spermicides represent an accepted method of vaginal contraception. Many compounds with diverse pharmacological activity have been evaluated *in vitro* and *in vivo* for their spermicidal activity. However there is a paucity of data regarding the safety profile of most of these agents. Spermicidal agents are defined as drugs that have the ability to immobilize or kill the sperm upon contact. An ideal spermicide should immediately and irreversibly produce immobilization of the sperm, nonirritating to the vaginal and penile mucosa, not have adverse effects on the developing fetus, free from long term topical and systemic toxicity and should not be systemically absorbed. Hence, the spermicidal agents should be critically evaluated for these aspects. Understanding the morphology of spermatozoa is essential to appreciate the mechanism of action of spermicide [1].

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Today, only nonoxynol-9 is marketed as a spermicide, and that too suffers from side effects of vaginal irritation on prolonged use. The major disadvantage of nonoxynol-9 is that it lacks entry into cervical mucus at the concentrations that would be present vaginally after the use of spermicides. The rate of penetration of a spermicide into the genital tract is an important consideration in the prevention of infection [2].

MATERIALS AND METHODS

Collection and Authentication of the plant material:

The *Hyptis suaveolens* plant material was collected and authenticated from Dr. K. Madhava chetty, Assistant Professor, S.V University, Tirupati. Leaves of Hyptis *suaveolens* were used for the study.

Preparation of Leaves Powder and Extraction:

250gms of plant material (leaves) was ground to course powder. The procedure recommended in Indian Pharmacopoeia (Anonymous, 1966; 1985; 1996). All chemicals and solvents used for different studies were of analytical reagent / spectroscopy grade.

The powdered leaf was extracted with petroleum ether by using soxhlet extraction method [3].

Qualitative phytochemical screening:

The petroleum ether extract of Hyptis *suaveolens was* subjected to qualitative phytochemical screening to detect presence or absence of various phytoconstituents using standard methods [4].

Formulation development and Characterization:

Procedure:

Carbopol 934 P was soaked in water over night. The gelling agent was then stirred on an overhead stirrer (Remi Motors) for

uniform mixing. The exact quantity of prepared extract was then added to the gelling agent with stirring to obtain gel. Propyl paraben & methyl paraben were added as preservatives. The final pH of gel was adjusted to 4.5-5.5 using 10% sodium hydroxide solution [5].

The same procedure is used for preparing HPMC K 100, Pemulen TR-1, Pemulen TR-2, Lutrol F127, Xanthan gum based *Hyptis suaveolens* gels.

Evaluation of *Hyptis suaveolens* Gel Formulations:

Prepared gel formulations were evaluated for physical appearance, pH, extrudability, spreadability, viscosity, and drug content.

pH measurement:

pH measurement of the gels was carried out of the formulation was measured by using a digital pH meter, dipping the glass electrode completely into the gel system.

Extrudability: [6, 7]

In conducting the test, a closed collapsible tube containing gel was pressed firmly at the crimped end and a clamp was applied to prevent any rollback. The cap was removed and the gel was extrudes until the pressure was dissipated.

Spreadability:

Two glass slides of standard dimensions were selected. The gel formulation whose spreadability had to be determined was placed over one of the slide. The other slide was placed on top of the gel in such a way that the gel was sandwiched between the two slides across a length of 6 cms along the slide.

Spreadability = M.L/T

M= wt tied to upper slide= 33.1gms
L =length of glass slide=6cms
T = time taken in sec.

Viscosity: [8]

A Brookfield digital viscometer with a suitable sample adaptor was used to measure the viscosities of the prepared vaginal gels in cps.

In vitro Drug release studies: [9]

The *in vitro* diffusion studies of the gels were performed by using dialysis membrane (Sigma Inc. MO, USA; dry, unwashed, pre-cut and open ended; fiat width: 35 mm; inflated diameter, 21mm; Length: 30mm). The membrane soaked in phosphate buffer pH 4.5 for 6-8 h was clamped carefully to one end of the hollow glass tube of dialysis cell (2.3 cm diameter, 4.16 cm2 area).

100ml of phosphate buffer was taken in a beaker, which was used as receptor compartment for the study. 1gm of respective gel formulations was spreaded uniformly on the membrane. The donor compartment was kept in contact with the receptor compartment and the temperature was maintained at 32.6±5^{0}C. The solutions on the receptor side were stirred by externally driven Teflon-coated magnetic bars. At pre-determined time intervals, 5ml sample of solution from the receptor compartment was withdrawn and immediately replaced with 5ml fresh buffer solution so as to maintain sink conditions.

The drug concentration of the receptor fluid was determined by using the developed HPLC method, (Cyberlab LC UV 100) at 220nm against appropriate blank. This experiment was carried out in triplicate and expressed as Mean ±Standard deviation.

Assessment of *in vivo* antifertilty activity:

***In-vivo* Studies of Vaginal Gels:**

In vivo studies of the optimized vaginal gel formulations were carried out using rats as the animal model to evaluate the antifertilty activity.

Experimental Animals:

Sexually mature, 2-month-old male and female Wistar rats (150–200 g) were used in the present study. The animals were maintained at laboratory conditions (12:12, dark: light cycle) and fed with standard pellet diet and water supplied *ad libitum*. The Institutional Animal Ethics Committee of the P. Rami reddy memorial College of Pharmacy, Kadapa, Andhra Pradesh, approved the study. Guidelines for the care and use of animals were approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) was followed.

Procedure: [10]

The estrous cycle of the female rats was observed by the following procedure:

Few drops of saline were taken into a dropper, inserted it into the vagina, taking care not to touch the cervix. Saline was expelled into the vagina and withdrawn two or three times. The contents of the dropper were placed on a microscope slide. The cells (epithelial, cornified, leukocyte) of the smear were observed using a microscope and the estrous cycle of the female rat was determined.

Vaginal irritation test:

Hyptis suaveolens gels (0.3 ml) were applied in to the vagina of the female Wistar rats. After 72 hours, the *Hyptis suaveolens* gel was removed and the following characteristics such as sensitization (allergic reaction), edema and excess redness were observed in test animals and in control by visual inspection.

Grouping of animals:

Six healthy female rats were used in each group (3 groups).

Group I : Placebo Gel
Group II : Marketed Gel
Group III : *Hyptis suaveolens* Gel

A total of 0.3 ml of the optimized *Hyptis suaveolens* gel was taken in 1-ml syringe and administered into the vagina during the proestrus-estrus transition phase. Group I animals received the placebo gel, group II animals received marketed gel and group 3 animals received *Hyptis suaveolens* gel. The female animals were allowed to mate with young proven fertile males (3:2). The animals were observed for any vaginal leakage post administration of gels. During cohabitation, mounting was seen, and mating was confirmed by the presence of spermatozoa in the vaginal lavage.

RESULTS AND DISCUSSION

Percentage yield:

Plant part used: Leaves
Weight of leaf powder: 250 g
Weight of petroleum ether extract obtained: 9 g
Percentage yield in average: 4%

Preliminary Qualitative Phytochemical Screening of Petroleum Ether Extract of *Hyptis suaveolens* Leaves:

By performing preliminary qualitative phytochemical tests to petroleum ether extract of *Hyptis suaveolens* leaves it was revealed that Alkaloids, Saponins, Glycosides, Aldehydes, Ketones were not present. Tannins, Carbohydrates, Flavanoids, Steroids, and Terpenoids were present. The results are tabulated in Table No. 1.

Evaluation of Physical Parameters of Hyptis suaveolens Formulations:

All the HS Gel formulations were subjected to characterization of various physical parameters like Appearance, Spreadability, Viscosity, pH and Extrudability as per the standard methods. All the formulations were appeared to be yellowish green in color exhibiting smooth and fine texture.PIF1 and P2F2 HS gels comprising Pemulen as gelling agent had shown more viscosity when compared to other HS formulations. pH values of various HS Gel formulations were found to be unsuitable, as the desired vaginal pH is between 4.5 and 5.5. All the HS formulations have shown very inferior extrudable characters. Owing to the inferior results of physical parameters, CF1, CF2, HF1, HF2, P1F1, P2F2, LF1, LF2, XF1 and XF2 Gel formulations of HS were not considered for further characterization. CF3, HF3, P1F3, P2F3, LF3 XF3 Gel formulations of HS consisting high concentrations of respective gelling agents have shown encouraging results with respect to physical parameters characterization when compared to that of HS Gel formulations containing moderate and low concentrations of gelling agents. The results were tabulated in Table No. 2.

***In vitro* Diffusion Studies of Optimized Formulations:**

CF3, HF3, P1F3, P2F3, LF3 XF3 Gel formulations of HS consisting high concentrations of respective gelling agents were subjected to in vitro diffusion studies up to 8 hours abiding standard procedures through dialysis membrane (Sigma Inc. MO, USA; dry, unwashed, pre-cut and open ended; fiat width: 35 mm; inflated diameter, 21mm; Length: 30mm). CF3 Gel formulation consisting Carbopol 934P as gelling agent has shown good retarding ability by releasing 95.90% of drug release at the end of 8 hours exhibiting its ability as firm gel strength in controlling the release of active principle of HS extract. HF3, P1F3 Gel formulation consisting HPMC K100 and Pemulen TR-1 as gelling agents failed to retard the release of the active principle, as they have shown maximum amount of release within 4 and 3 hours respectively. P2F, LF3 Gel formulation consisting Pemulen TR-2 and Lutrol as gelling agents have retarded the drug at effective manner by releasing 89.33% and 93.65% upto 8 hours. XF3 Gel formulation consisting Xanthan gum as gelling agent has shown its retardation ability by releasing 94.56%, but up to 6 hours only.

By comparing the results of in *vitro* diffusion studies, HS Gel formulations consisting Carbopol 934P (CF3) was optimized due to its retarding ability and consistency. CF3 Gel formulation was subjected to further characterization. The results were shown in Table No.3 and Figure No. 1.

Analysis of Release Data:

The Optimized formulations was subjected to release kinetic studies like zero order, first order, Higuchi, Erosion and Peppa's models. The order of drug release from matrix systems was described by using zero order or first order kinetics. The mechanism of drug release from matrix systems was studied by using higuchi equation and erosion equation and Peppas equation (Power Law) and the plots were given as Figure No.-2 to 6 and the regression values are shown in Table No. 4. By fitting the *in vitro* diffusion data into popular five regression and exponential models, all HS Gel formulations were found to be accepting zero order kinetics which was evident by higher R2 values of zero order plot compared to R2 values of first order plot that specifies independency of the concentration. HS Gel formulations were found to be following Diffusion mechanism, which were evident by higher R2 values of Higuchian plot compared to R2 values of Erosion plot. In order to assess the exact release mechanism, dissolution data of HS Gel formulations were fitted to Korsemeyer Pappas (Power Law) plot. All the exponent (n) values were found to be between 1.5 -1, which specifies that the Gel formulations were exhibiting Anomalous (Non-Fickian) transport mechanism for the drug release at rate controlled fashion.

***In vivo* antifertilty activity assessment:**

The estrous cycle was confirmed by means of observing vaginal smear which was shown in Figure No. 7. The *Hyptis suaveolens* gel formulations were found to be non irritant to vagina of female Wistar rats. The marketed product applied intravaginally before mating during proestrus- estrous phase resulted none of these animals became pregnant, indicating 100%. In the control group, all the six animals became pregnant and delivered 38 pups. In the *Hyptis suaveolens* gel treated group, two out of six animals delivered pups. The spermicidal effect and potency of extract was proved as contact spermicidal.

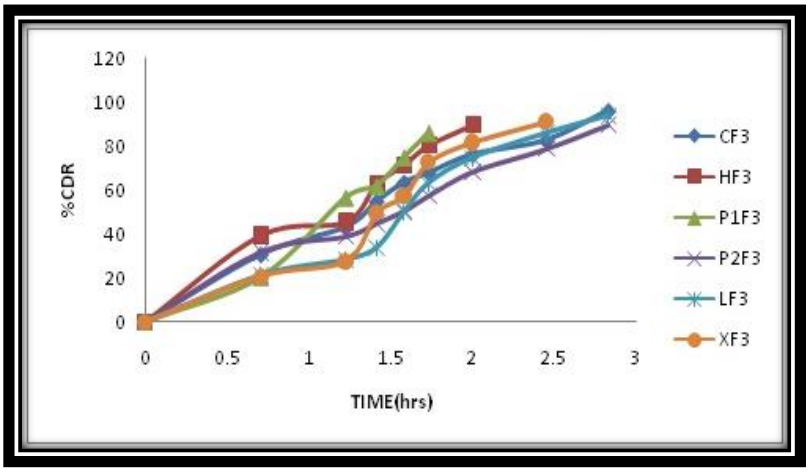

Fig. 1: Comparative release profiles of HS Gel Formulations

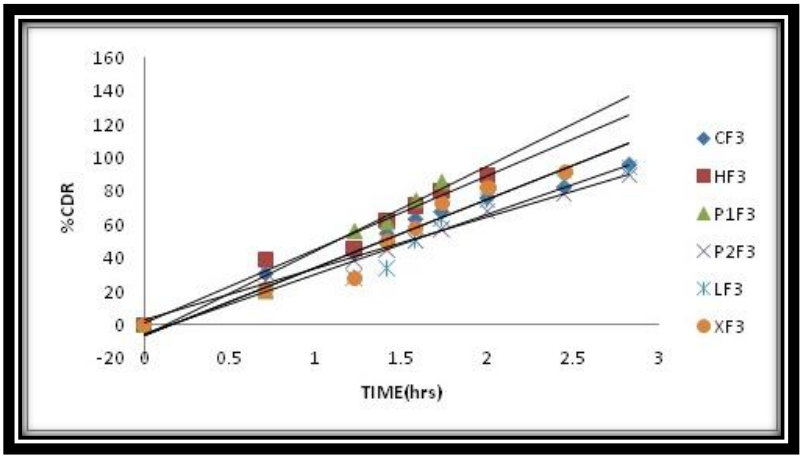

Fig. 2: Regression Plot-Zero Model

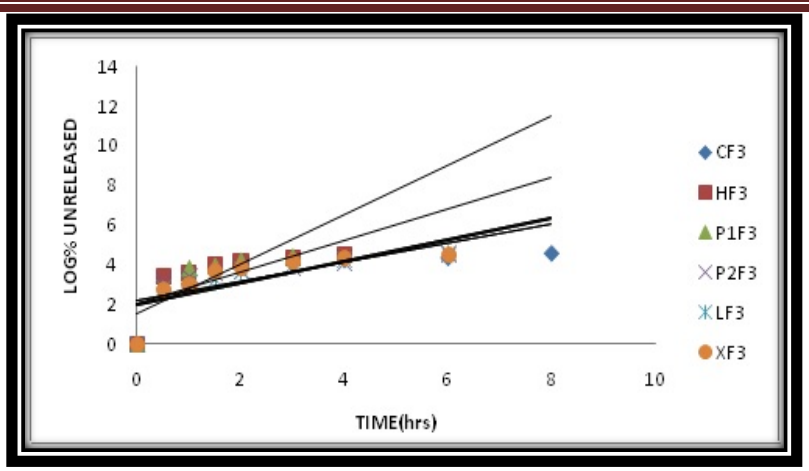

Fig. 3: Regression Plot-First Model

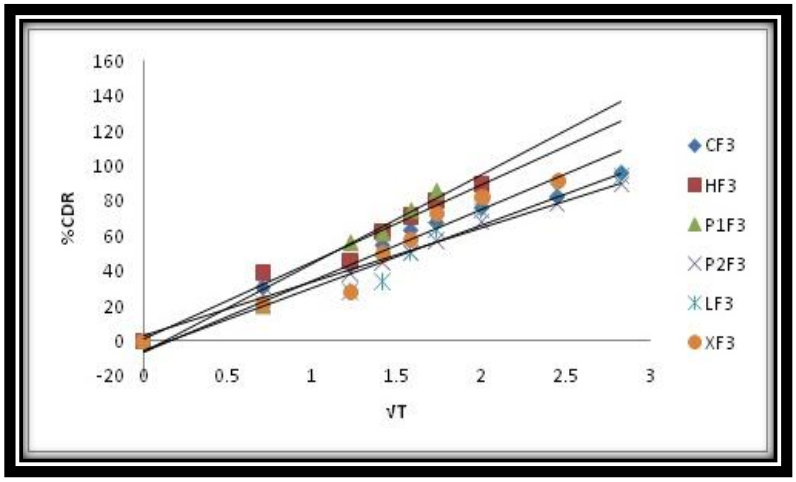

Fig. 4: Regression Plot-Higuchi Model

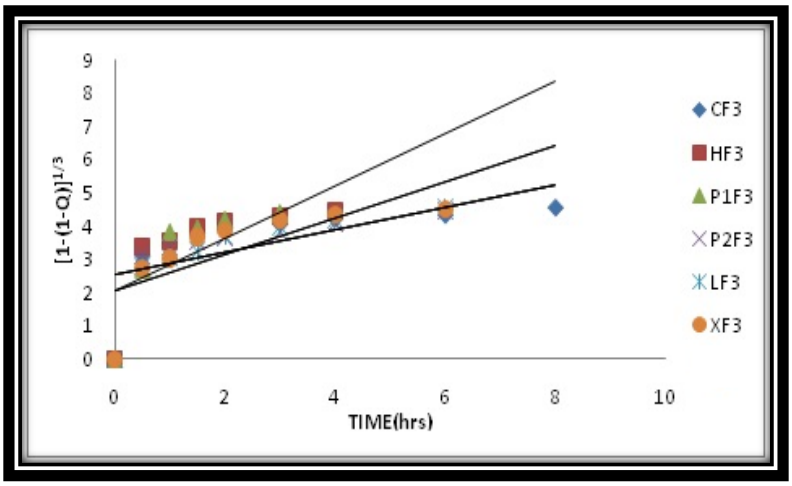

Fig. 5: Regression Plot-Erosion Model

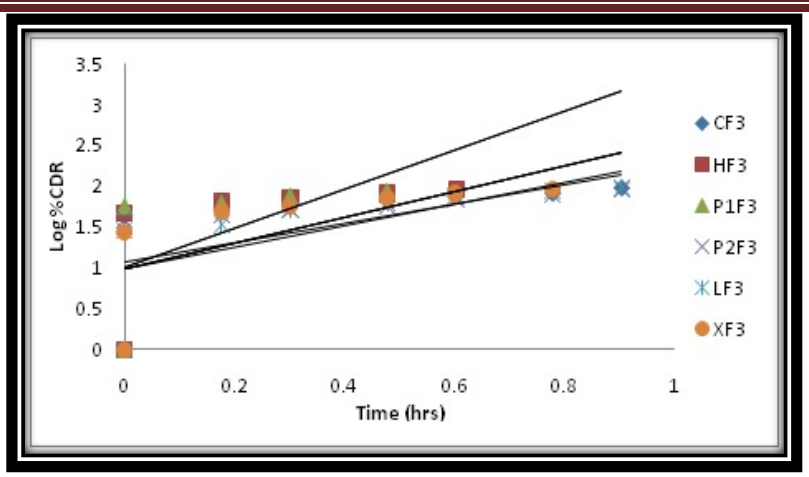

Fig. 6: Regression Plot-Preppas Model

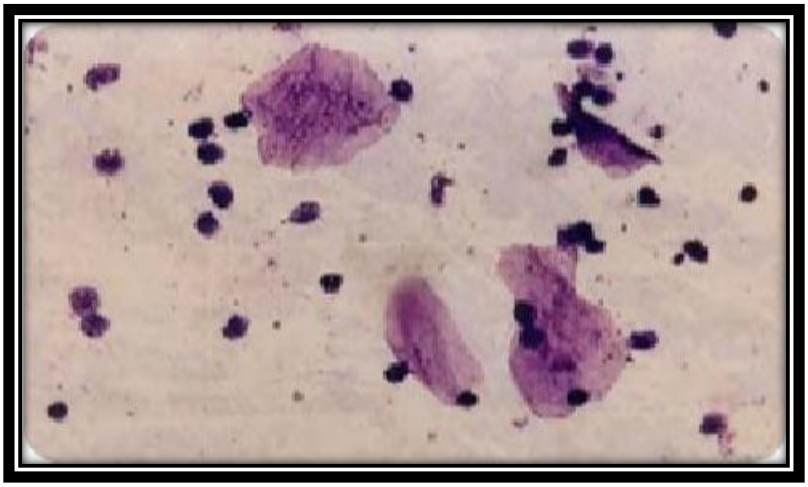

Fig. 7: Vaginal smear of Rat in Estrous phase

Table No.1: Preliminary Qualitative Phytochemical Screening of Petroleum Ether Extract of *Hyptis suaveolens* Leaves

S .NO	PHYTOCHEMICAL TESTS	RESULT
1	Test for Alkaloids	-
2	Test for Tannins	+
3	Test for Carbohydrates	+
4	Test for Flavanoids	+
5	Test for Saponins	-
6	Test for Steroids	+
7	Test for Glycosides	-
8	Test for Terpenoids	+
9	Test for Aldehydes	-
10	Test for Ketones	-

Table No. 2: Evaluation of Physical Parameters of *Hyptis suaveolens* Formulations

Formulation Code	Physical Appearance	Spreadability (gm/sec)	Viscosity	p^H	EXTRUDABILITY
CF1	Yellowish green	4.6	6.555	3.5±0.011	Very poor
CF2	Yellowish green	4.4	2.533	6.0±0.011	Poor
CF3	Yellowish green	1.57	1.555	5.5±0.011	Very good
HF1	Yellowish green	4.95	5.397	3.1±0.025	Poor
HF2	Yellowish green	4.55	5.397	6.1±0.025	Poor
HF3	Yellowish green	1.62	2.397	5.1±0.025	Good
P1F1	Yellowish green	4.25	2.6.015	3.8±0.027	Poor
P1F2	Yellowish green	3.26	2.6.111	6.8±0.027	Poor
P1F3	Yellowish green	1.58	2.015	4.8±0.027	Good

P2F1	Yellowish green	4.7	6.234	3.1±0.033	Poor
P2F2	Yellowish green	3.24	6.012	2.1±0.033	Poor
P2F3	Yellowish green	1.59	3.012	4.1±0.033	Good
LF1	Yellowish green	5.15	4.702	3.1±0.015	Very poor
LF2	Yellowish green	3.15	4.702	4.1±0.015	Poor
LF3	Yellowish green	1.58	2.702	5.1±0.015	Very good
XF1	Yellowish green	5.5	6.576	3.9±0.024	Poor
XF2	Yellowish green	4.5	5.787	5.9±0.024	Very poor
XF3	Yellowish green	1.6	4.175	4.9±0.024	Good

Table No. 3: Percentage Cumulative Drug Release Profile of Optimized Formulations

Time (hrs)	CF3	HF3	P1F3	P2F3	LF3	XF3
0.5	30.61±0.12	39.25±0.12	20.32±0.24	31.60±0.03	21.25±0.23	20.82±0.13
1	43.08±0.17	45.30±0.15	56.55±0.27	38.68±0.12	28.24±0.08	22.6.73±0.17
1.52	54.68±0.13	62.65±0.17	61.95±0.23	44.59±0.02	33.78±0.09	49.63±0.15
2.5	63.32±0.11	71.30±0.18	74.92±0.22	50.28±0.03	49.99±0.78	52.6.±0.16
3	62.72±0.16	80.18±0.10	86.09±0.21	52.6±0.05	63.18±0.06	72.76±0.05
4	76.00 ±0.18	89.33±0.19	-	68.08±0.07	74.56±0.07	81.77±0.14
6	82.49 ±0.19	-	-	78.52±0.08	85.73±0.09	91.13±0.17
8	95.90 ±0.12	-	-	89.33±0.06	93.65±0.08	-
% Assay	92.6.03	93.45	92.56	94.02	92.52	94.56

Table No. 4: Release Kinetics of optimized formulations

GEL FORMULATION CODE	Zero order	First order	Higuchi	Erosion	Peppas
	R^2 value	R^2 value	R^2 value	R^2 value	'n' value
C F3	0.996	0.778	0.978	0.413	0.712
HF3	0.985	0.835	0.972	0.515	0.549
P1F3	0.983	0.881	0.971	0.65	0.749
P2F3	0.98	0.847	0.986	0.482	0.718
LF3	0.996	0.889	0.95	0.599	0.647
XF3	0.995	0.871	0.941	0.556	0.538

CONCLUSION

Our data demonstrate that the petroleum ether leaf extracts of Hyptis suaveolens has potent antifertility activity. However, it needs further clinical evaluation before consideration for the treatment. Further studies with purified constituents may be done to clearly understand the complete mechanism of antifertility activity of Hyptis suaveolens.

REFERENCES:

1. Singh, Kamalinder Kaur. Herbal Contraceptive Formulations. International publication date 10.08. **2006**.
2. Ankita Singh, Pramod Kumar Sharma, Nitin Kumar, Rupesh Dudhe, Shushank Dixit. Novel spermicidal agent. Der Pharma Chemica **2010**;2(4):278-292.6.
3. Agarwal SS and Paridhavi M. Herbal Drug Technology, 2nd ed., Universities press, Hyderabad : **2012**;325.
4. Khandelwal KR. Practical Pharmacognosy. 16th ed., NiraliPrakashan, **2006**;93.
5. Gupta. A, Formulation and Evaluation of Topical Gel of Diclofenac Sodium Using Different Polymers, Drug Invention Today **2010**;2(5):250-253.
6. Varshosaz J, Tavakoli N, Roozbahani P. Formulation and In vitro Characterization of Ciprofloxacin floating and bioadhesive extended-release Tablets. Drug Deliv **2006**;13:277-285.
7. Carstensen JT. Drug Stability, Principles & Practices. New York: Marcel Dekker; **1989**;17-58.
8. Ritger PL, Peppas NA. Modelling of water transport solute release in physiologically sensitive gels. J Control Release **1987**;5:37-4.
9. Prabhudutta Panda and Arpita Ghosh. Formulation and Evaluation of Topical Dosage form Eupatorium Odoratum Linn. and their Wound Healing Activity. Int J Pharm Bio Sci **2010**;1(2).
10. Kamalinder K. Singh, Shruti Parmar, Pratima Arun Tatke. Contraceptive efficacy and safety of HerbOshieid TM vaginal gel in rats, Contraception **2012**;85(6):122-122.

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