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Advances in Bioresearch

ORIGINAL ARTICLE

Formulation and Evaluation of Herbal Gel for Management of Dysmenorrhea and Menorrhagia

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ABSTRACT
Dysmenorrhea and menorrhagia are common gynecological disorders affecting a significant number of women worldwide, leading to discomfort, pain, and excessive bleeding during menstruation. Conventional treatments often use non-steroidal anti-inflammatory drugs and hormonal therapies, which can have adverse effects. This study aimed to formulate and evaluate an herbal gel formulation as a potential alternative for the management of dysmenorrhea and menorrhagia. The gel was formulated using the dispersion method with carbopol 934, propylene glycol, and methylparaben. The formulated batches were evaluated using physicochemical properties, such as spreadability, viscosity, pH, drug content, compatibility, and in-vitro drug release where F4 formulation was found as an optimized batch. This study suggests that the formulated herbal gel is a promising alternative for managing dysmenorrhea and menorrhagia, offering potential benefits over conventional therapies. Further research is warranted to explore this herbal formulation's long-term effects and potential large-scale production.
Keywords: *Dysmenorrhea, Cynodon Dactylon, Hibiscus Rosa Sinensis, Menorrhagia, Gel.*

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INTRODUCTION

The female reproductive system is a remarkable biological system responsible for various functions, including menstruation, pregnancy, and childbirth. Menstruation, also known as a period, is a natural process that occurs in females of reproductive age when the uterus sheds its inner lining (endometrium) through vaginal bleeding. Menstruation is a cyclical process regulated by hormones, primarily estrogen and progesterone and it is a monthly physiologic shedding of the endometrium. During this time, the body prepares for a potential pregnancy by thickening the uterine lining. The lining is shed if pregnancy does not occur, and the menstrual cycle begins again. While menstruation is a natural process, it can be accompanied by various problems or complications. Unfortunately, menstrual problems are often overlooked as minor health issues and are not given due attention to the public health agenda, especially for women in developing countries who may be contending with life-threatening conditions. [1] It encompasses major issues associated with menstruation including:

1. Dysmenorrhea (painful menstruation)
2. Menorrhagia (excessive bleeding)

1. Dysmenorrhea (painful periods): Dysmenorrhea is classified into two types:

1) Primary dysmenorrhea
2) Secondary dysmenorrhea.

The release of prostaglandins in menstrual fluid causes dysmenorrhea, which is characterized by painful and painful uterine contractions. Vasopressin may potentially play a role by producing vasoconstriction, which increases uterine contractility and ischemia discomfort. Women with primary dysmenorrhea have been found to have elevated vasopressin levels. This pain usually first manifests in women aged 20 years or younger, following the establishment of their ovulatory cycles, and occurs in the absence of any visible

pathological pelvic disease. It is characterized by cramping, lower abdominal pain, and discomfort before or during menstruation. Secondary dysmenorrhea is prevalent in women beyond the age of 20, and is caused by underlying pelvic difficulties or pathology. It includes conditions like pelvic inflammatory disease, endometriosis, submucosal fibroid, and cervical stenosis. [2,3]

Common causes of Dysmenorrhea – Age, smoking, higher BMI, nulliparity, earlier age at menarche, menorrhagia, and family history of dysmenorrhea. Additionally, depression, stress, alcohol consumption, and physical activity increase the risk of dysmenorrhea. [3]

Symptoms of Dysmenorrhea –

Headaches, Nausea, Sleep deprivation, Breasts soreness, Abdominal bloating, Constipation or diarrhea, Frequent urination, Vomiting [3]

Menorrhagia (excessive bleeding)*:* Menorrhagia is characterized by massive menstrual blood loss over multiple consecutive cycles in an adult woman which may lead to anemia and other health issues. Idiopathic ovulatory menorrhagia refers to the condition of experiencing heavy and excessive bleeding during menstrual periods, despite the absence of any identifiable pelvic disease or heavy bleeding. This condition occurs in women who have regular ovulatory cycles. Objective menorrhagia is considered when the total amount of blood loss during each menstrual period is 80 ml or more. [4]

Causes of Menorrhagia –

Systemic conditions - von Willebrand's disease, hypothyroidism, hemophilia carriage, anovulation

Local conditions - pelvic inflammatory disease, benign or malignant tumors, endometrial hyperplasia, endometriosis

Iatrogenic disorders - Illness caused due to poor control of anticoagulation treatment and intrauterine contraceptive devices [5]

Symptoms of Menorrhagia –

Experiencing rapid saturation on tampons or pads within one to two hours

Passing blood clots that are the size of quarters or larger.

Severe and very painful menstrual cramps

A period lasting for seven days or more.

Herbal Remedies for Dysmenorrhea and Menorrhagia*:*

Medicinal plants are a rich source of natural compounds and extracts that possess immense chemical diversity. This diversity presents numerous prospects for discovering new lead compounds that could potentially be developed into drugs. Whether in the form of pure isolated compounds or standardized plant extracts containing multiple compounds, natural products derived from medicinal plants offer an unmatched array of chemically diverse molecules that can be explored and exploited for their therapeutic potential. Throughout history, humans have utilized plant extracts from various plants to treat numerous ailments and alleviate physical discomfort. Plants play a crucial role in human therapeutic remedies, with many plants and herbs having long-standing medicinal uses. Essential oils and pastes of certain herbs are used in various ways, including massage, inhalation, and bathing. Studies have shown the positive impact of aromatherapy abdominal massage is beneficial for easing the pain associated with menstruation, shortening its duration, and minimizing heavy bleeding throughout the menstrual cycle. To increase patient acceptability for such herbal medications, it is necessary to investigate or research them. Among the plants with analgesic properties are *Cynodon Dactylon, Emblica Officinalis, Eclipta Alba, Hibiscus Rosa Sinensis,* and *Asparagus Racemosus*. [6, 7]

Cynodon Dactylon – (Durva grass or Bermuda grass) It has a pungent smell and bitter nature but also cold potency and a characteristic fragrance. Various phytochemical analyses have revealed that it contains flavonoids, glycosides, terpenoids, alkaloids, triterpenoids, resins, steroids, saponins, tannins, phytosterols, proteins, carbohydrates, volatile oils, reducing sugars, and fixed oils. It has been used in traditional remedies for a wide range of conditions, including headaches, cramps, tumors, epilepsy, measles, dropsy, diarrhea, dysentery, hemorrhage, snakebite, stones, urogenital problems, and warts. Ayurvedic physicians recommend this grass for conditions such as menorrhagia (excessive bleeding during the menstrual cycle) and irregular menstrual cycles. Local application of a paste made from the plant extract on the lower abdomen has been found to reduce severe vaginal bleeding. [8]

Fig 1: ***Cynodon Dactylon***

***Hibiscus Rosa Sinensis* –**

It contains Beta-sitosterol, campesterol, cholesterol oxalic acid, palmitic acid, tartaric, glucose, citric acid, fructose, sucrose, flavonoids and flavonoid glycosides, chrysanthemin, quercetin, ergosterol, lipids. It has Biological Activities including antipyretic, anti-inflammatory, anticonvulsive, antispasmodic, analgesic, CNS depressant, antiestrogenic, uterine stimulant, and anti-fungal. Stomachaches are treated with a mixture of crushed leaf juice and salt water. Young flower shoot infusion is consumed for menstruation irregularities, fever, and mild body aches and pains. An aqueous preparation of these flowers helps to control menstruation. Research on animals has shown that *Hibiscus Rosa Sinensis* effectively reduces pain and inflammation. [9]

Fig 2: ***Hibiscus Rosa Sinensis***

Gel is a highly versatile semi-solid substance used extensively in personal care, and medical applications. When applied to the lower abdomen, gels provide significant benefits, including effective relief from lower abdominal pain, menstrual cramps, back pain, and muscle aches, along with a cooling and soothing effect. The unique advantages of gel lie in its inert properties, compatibility with various additives, and stable storage conditions, ensuring patient compliance. Research assuredly supports the effectiveness of *hibiscus* flower extract and *Cynodon Dactylon Linn*. paste in managing irregular menstruation troubles, cramps, and heavy blood flow. [7]

MATERIAL AND METHODS:

Materials:

Sr. No.	Materials	Suppliers
1	*Cynodon Dactylon extract*	Janani Organics
2	*Hibiscus Rosa Sinensis extract*	Janani Organics
3	Carbapol 934	Research lab Fine Chem. Mumbai
4	Propylene glycol	Research lab Fine Chem. Mumbai
5	Methylparaben	Research lab Fine Chem. Mumbai
6	Triethanolamine	Research lab Fine Chem. Mumbai

Formulation of herbal gel: To make 10 grams of herbal gel, carbopol 934, propylene glycol, methylparaben, triethanolamine, and required amount of distilled water were used in the preparation process.

Procedure –

The required amount of Carbopol 934 was dispersed in 5ml distilled water with continuous stirring using a magnetic stirrer. [Phase 1]

The calculated amount of Propylene glycol and methylparaben are dissolved in water. [Phase 2]

The drug extract is added to Phase 2.

Phase 2 is then added to Phase 1 with continuous stirring.

Triethanolamine is added at the end for pH adjustment.

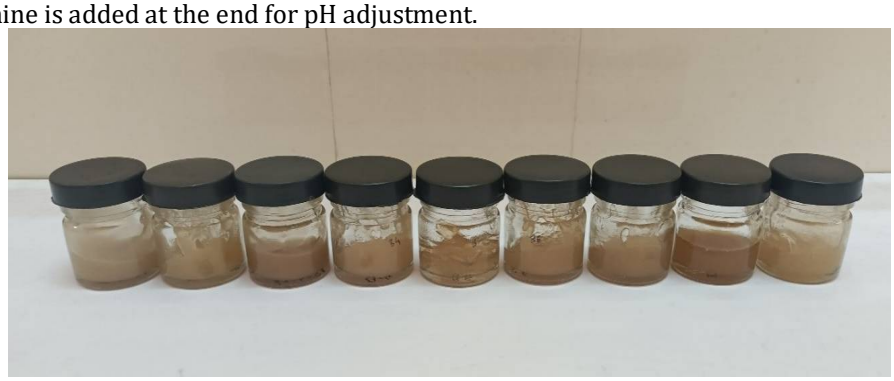

Fig 3: Formulation batches

Table 1- Composition of different formulation batches

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Cynodon Dactylon extract (gm)	1	1	1	**1**	1	1	1	1	1
Hibiscus Rosa Sinensis extract (gm)	0.5	0.5	0.5	**0.5**	0.5	0.5	0.5	0.5	0.5
Carbapol 934 (gm)	0.05	0.23	0.05	**0.2**	0.12	0.12	0.12	0.01	0.2
Propylene glycol (ml)	1	1.1	1.2	**1.2**	1.2	0.9	1.1	1.1	1
Methyl paraben (gm)	0.1	0.1	0.1	**0.1**	0.1	0.1	0.1	0.1	0.1
Triethanolamine	2 Drops	2 Drops	2 Drops	**2 Drops**	2 Drops	2 Drops	2 Drops	2 Drops	2 Drops
Distilled water	Q. S	Q. S	Q. S	**Q. S**	Q. S	Q. S	Q. S	Q. S	Q. S

Evaluation of Gel:

Organoleptic properties: Organoleptic properties included the study of colour, homogeneity, uniformity, texture, appearance, etc. The colour was observed visually and recorded. The homogeneity of the gel was confirmed by rubbing it between fingertips. To assess its texture and consistency, gel was applied to the skin. [11]

pH: The pH of the batches of prepared gel was determined using a digital pH meter. An electrode was dipped into 0.5 grams of dissolved material in 10 ml of distilled water to determine the pH. [10]

Viscosity: A Brookfield viscometer with spindle number L4 was used to measure the viscosity of the prepared preparations. A thermostatic jacketed beaker was filled with the formulation whose viscosity needed to be measured. The device was kept at 25 °C while the spindle was permitted to pass through the gel and a reading at 60 rpm was recorded. [12]

Spreadbility: Glass slides in two sets of the same size were taken. The herbal gel was put on one of the slides. When the other slide was placed on top of the gel, it occupied a space of 7.5 cm along the slide, sandwiching the gel between the two slides. To evenly press the gel between the upper slides into a thin layer, a 20-gram weight was applied to them. When the weight was applied, the time it took for the upper slide to travel 5 cm and detach from the bottom slide was noted. The findings of the experiment were calculated using the average time for three runs. [13]

Drug content: In solvent (distilled water), 1gm of gel was dissolved and filtered to obtain a clear solution and absorbance was determined by UV spectrophotometer at 246nm and 345nm for the presence of the drug *Cynodon Dactylon* and *Hibiscus Rosa Sinensis* respectively. A standard plot of the respective drug was prepared in a similar solvent. The same standard plot using the absorbance values was used to determine concentration and drug content. [13]

In vitro drug release study: Franz diffusion cell was used for the in vitro drug release from gel at 37 ^{0}C ± 0.5 ^{0}C and 100 RPM. The dissolution medium used was phosphate buffer (pH 6.8). 1 ml of the

dissolution medium was removed at pre-arranged intervals and replaced with a fresh dissolution medium. The samples were taken out at regular intervals and subjected to UV spectrophotometer analysis to determine the presence of *Cynodon Dactylon* and *Hibiscus Rosa Sinensis* at 246 and 345 nm, respectively. [14, 15]

Apparatus: Franz diffusion cell

Media: Phosphate Buffer pH 6.8

Time interval: 0, 15**,** 30, …, 90 min**.**

Temperature: $37^0C \pm 0.5^0C$

Centrifugation test: For the centrifugation testing all 9 batches of gel formulation were placed in centrifugation testing apparatus and the separation of 2 phases was observed. [16]

Pre-formulation studies:

UV- Spectroscopy:

Estimation of *Cynodon Dactylon* extract using UV spectroscopic method:

A 10 mg of *Cynodon Dacytlon* extract was weighed and diluted in 70 ml of distilled water and then volume was adjusted to obtain a solution with 100 ug/ml. *Cynodon Dactylon* λmax determined was 246 nm which was in the range of 200-400nm. Transferred 0.2-1 ml of stock solution into various 5 volumetric flasks (10 ml) yielded standard extract solutions ranging from 2 to 10 ug/ml and volume made up with the distilled water. Beer's Lambert law was used to determine absorptivity and test the absorbance of various solutions. [18]

Estimation of *Hibiscus Rosa Sinensis* extract using UV spectroscopic method:

A 10 mg of *Hibiscus Rosa Sinensis* extract was weighed and diluted in 70 ml of distilled water and then volume was adjusted to obtain a solution with 100 ug/ml. *Hibiscus Rosa Sinensis* λmax determined was 345 nm which was in the range of 200-400nm. Transferred 0.2-1 ml of stock solution into various 5 volumetric flasks (10 ml) yielded standard extract solutions ranging from 2 to 10 ug/ml and volume made up with the distilled water. Beer's Lambert law was used to determine absorptivity and test the absorbance of various solutions. [19]

Drug excipient compatibility studies:

I) Physical compatibility test: A physical compatibility test was performed to examine the interaction/compatibility of drug excipients. The drug and excipient were properly combined in a 1:1 ratio. The mixtures were placed in individual glass vials that were stored at room temperature. After 15 days, samples were examined for colour/appearance in preparation for drug-excipient compatibility testing.

II) FT-IR Spectroscopy: The FTIR study was carried out by using Shimadzu affinity 1S with DRS Attachment in that IR solution software was used. It was measured with an FT-IR spectrophotometer against a background of potassium bromide (KBr) salt plates. The spectra were scanned in the 4000-400 cm-1 frequency band. [17]

HPTLC: The HPTLC study was conducted using a Merck HPTLC silica gel 60 F254 stationary phase with a 20-minute saturation time.

Mobile Phase for *Cynodon Dactylon*: Formic Acid: Water : Methanol : Ethyl Acetate : Toluene (2.0 : 1.0 : 1.0 : 1.0 : 6.0 V/V/V/V/V).

Mobile Phase for *Hibiscus Rosa Sinensis*: Formic Acid: Toluene: Ethyl Acetate: Methanol (2.0: 6.0 : 12.0: 1.0 V/V/V/V) [19]

RESULT AND DISCUSSION

Evaluation of organoleptic properties: Organoleptic evaluation parameters of all batches were performed. The Colour, homogeneity, and texture were assessed, and the formulation F4 exhibited excellent homogeneity and a smooth texture.

Formulations	Physical appearance		
	Colour	Texture	Homogeneity
F1	Pale Brown	Smooth	Homogenous
F2	Light brown	Smooth	Homogenous
F3	Dark brown	Smooth	Homogenous
F4	**Light brown**	**Smooth**	**Homogenous**
F5	Light brown	Smooth	Homogenous
F6	Light brown	Smooth	Homogenous
F7	Light brown	Smooth	Homogenous
F8	Dark brown	Smooth	Homogenous
F9	Pale Brown	Smooth	Homogenous

Table 2- Evaluation of organoleptic properties

pH, viscosity, and spreadability of gel: The F4 batch exhibited a pH of 6.54, a viscosity of 3648 cps, and a spreadability of 7.4 gm*Cm/sec.

Formulations	pH	Viscosity(cps)	Spreadability (gm*Cm/sec)
F1	6.27	2143	5.3
F2	6.50	3136	4.6
F3	6.38	2486	6.7
F4	**6.54**	**3648**	**7.4**
F5	6.60	3047	7
F6	7.2	3233	7.1
F7	6.43	3587	6.3
F8	6.22	3486	6.8
F9	6.32	3600	5.7

Table 3- pH, viscosity, and spreadability, of gel

Drug content:

For the F4 batch, *Cynodon dactylon* had a drug content of 94.19% ± 0.14%, indicating high average content and consistency. *Hibiscus rosa-sinensis* in the F4 batch showed a drug content of 95.75% ± 0.24%, also reflecting excellent average concentration and precision.

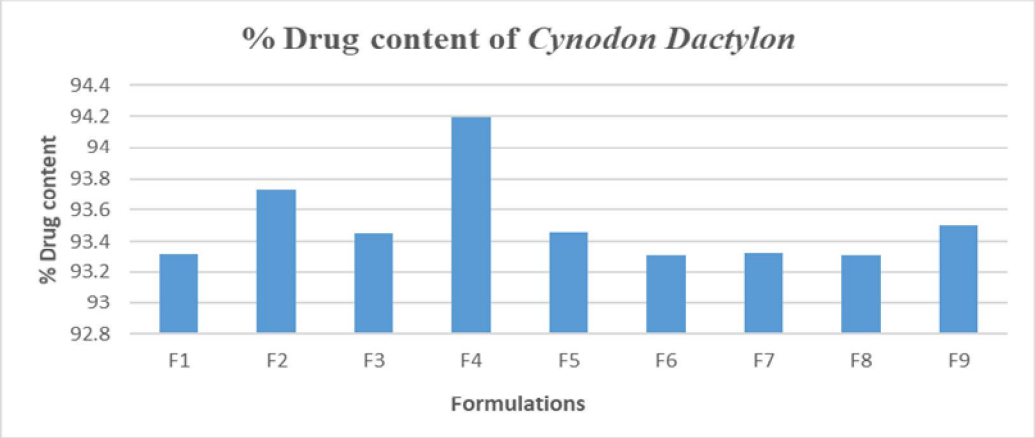

Fig 4: % Drug content of *Cynodon Dactylon*

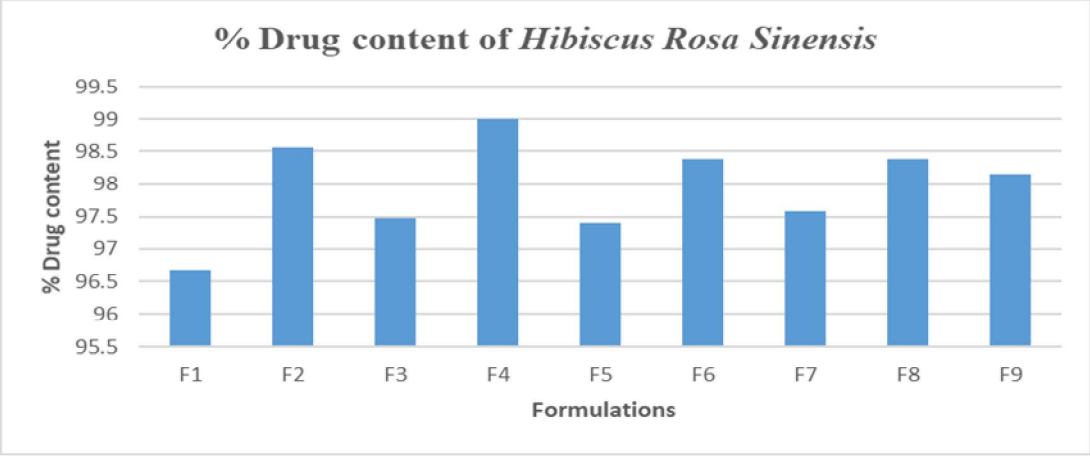

Fig 5: % Drug content of *Hibiscus Rosa Sinensis*

***In vitro* drug release:** The drug release study of all 9 batches was carried out by using a Franz diffusion cell with phosphate buffer (pH 6.8) solution. The F4 batch of *Cynodon dactylon* showed maximum in-vitro

drug release, with cumulative percentages reaching 21.02% at 15 minutes, 36.27% at 30 minutes, 52.20% at 45 minutes, 68.81% at 60 minutes, and 86.78% at 90 minutes. For *Hibiscus rosa sinensis*, the F4 batch demonstrated cumulative drug release percentages of 18.93% at 15 minutes, 39.15% at 30 minutes, 56.80% at 45 minutes, 71.01% at 60 minutes, and 89.94% at 90 minutes.

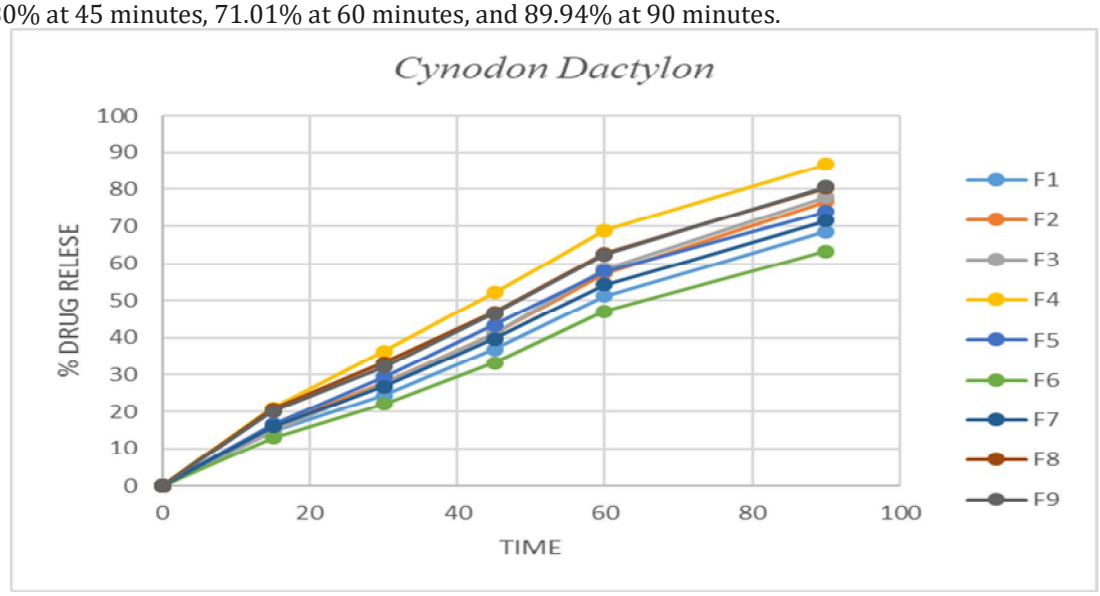

Fig 6: % Drug release of *Cynodon Dactylon* at 246 nm

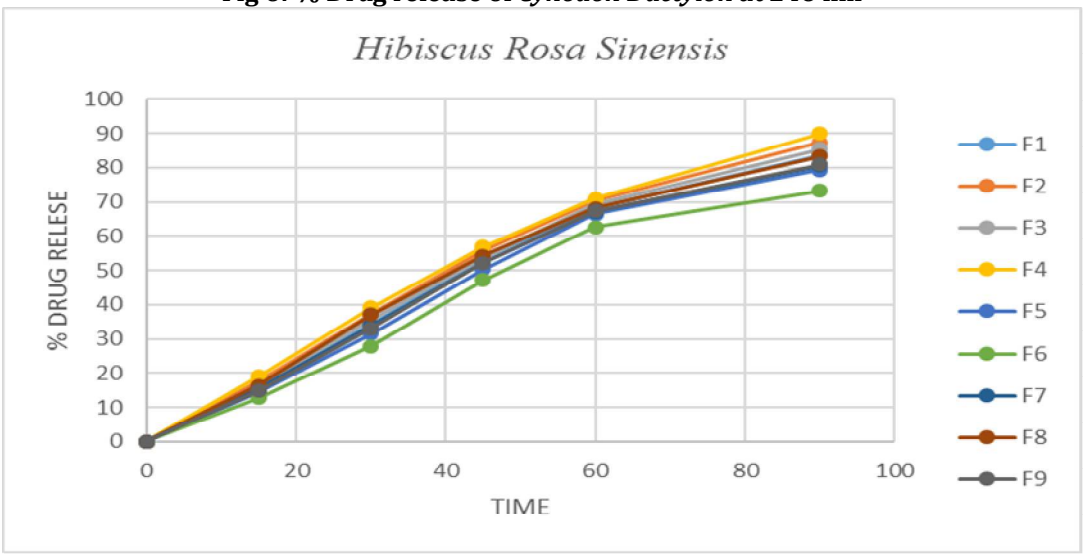

Fig 7: % Drug release of *Hibiscus Rosa Sinensis at 345 nm*

Centrifugation test: All batches were tested for phase separation on centrifuge. The F4 batch showed no phase separation during the centrifugation test.

Table 4- Centrifugation test

Batch	F1	F2	F3	F4	F5	F6	F7	F8	F9
Result	No	No	No	No	No	No	No	Yes	No

UV analysis of *Cynodon Dactylon* extract:

For *Cynodon dactylon*, the calibration curve showed a maximum absorbance at λmax 246 nm, following Beer-Lambert's law in the range of 2 to 10 µg/ml, with a regression equation of y = 0.059x + 0.0488 and an R^2 value of 0.9979.

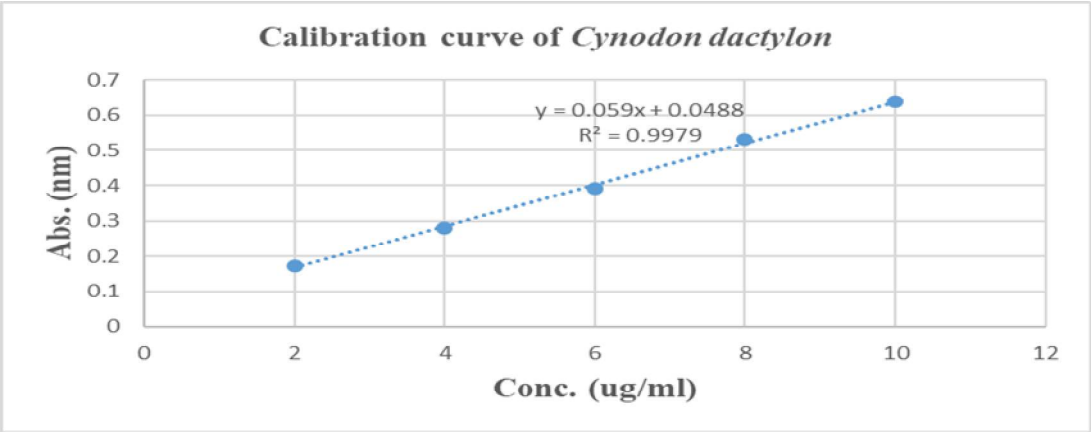

Fig 8: Calibration curve of *Cynodon Dactylon* at 246nm

UV analysis of *Hibiscus Rosa Sinensis* extract:

For Hibiscus rosa sinensis, the calibration curve showed a maximum absorbance at λmax 345 nm, following Beer-Lambert's law in the range of 2 to 10 µg/ml, with a regression equation of y = 0.0169x + 0.0332 and an R^2 value of 0.9944.

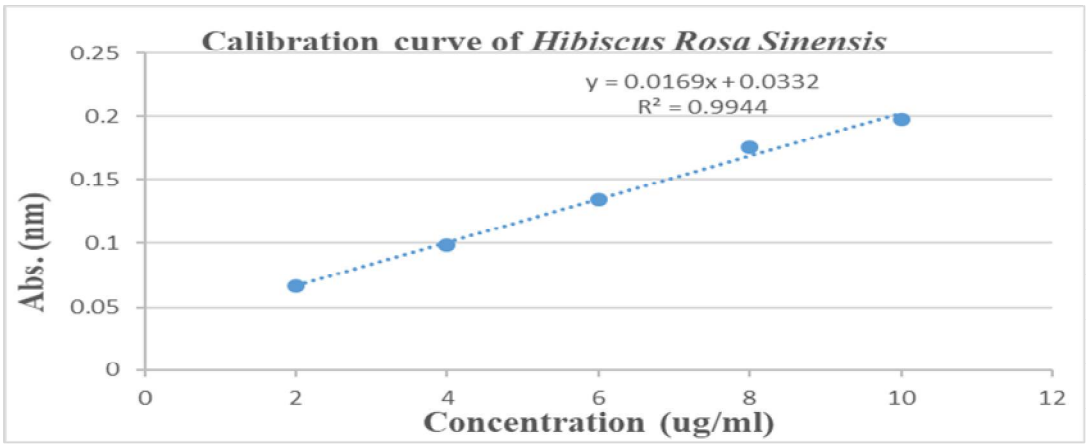

Fig 9: Calibration curve of *Hibiscus Rosa Sinensis at 345nm*

FT-IR Spectroscopy: The possibilities of drug-excipient interactions were investigated by recording the FT- IR spectrum. In the FTIR spectrum, the frequency range of formulation lies is nearly similar to the frequency region of the both extract.

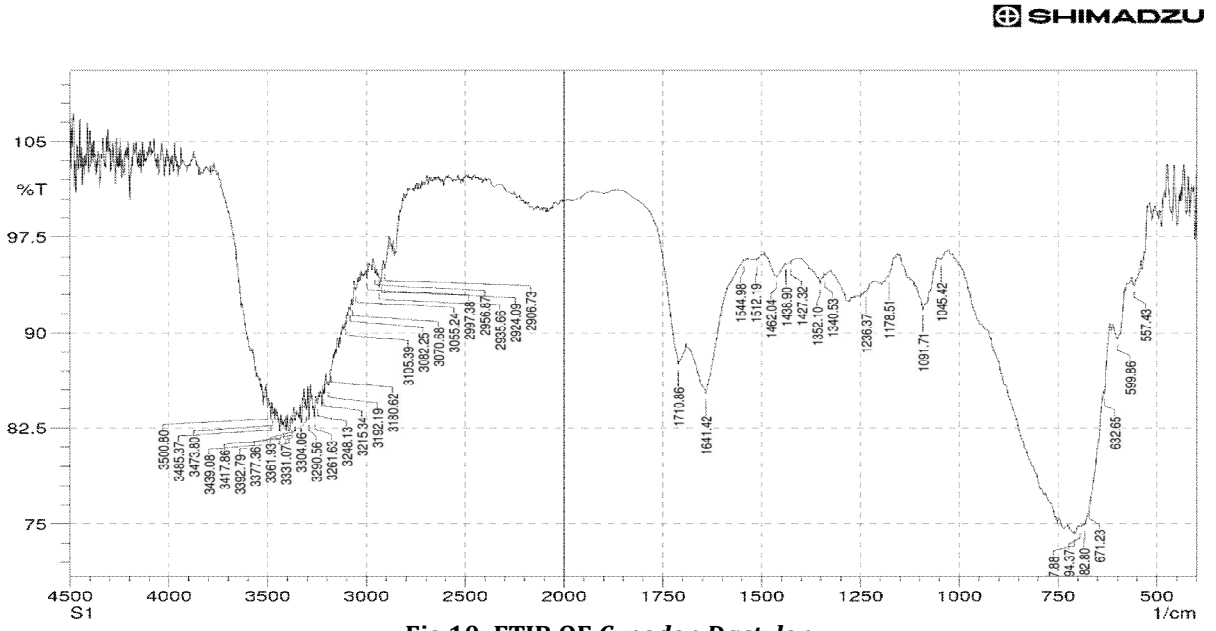

Fig 10: FTIR OF *Cynodon Dactylon*

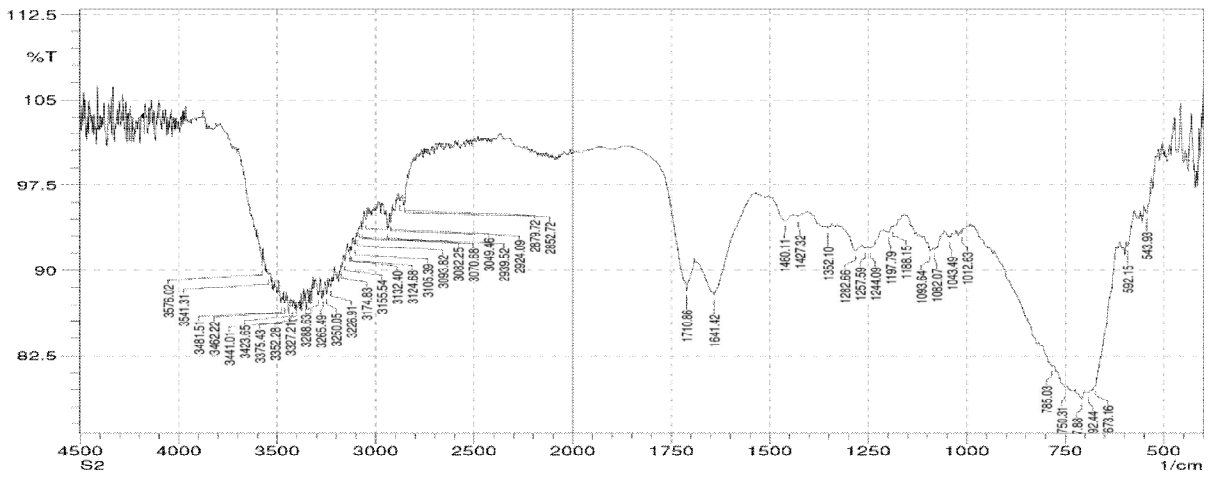

Fig 11: FTIR OF *Hibiscus Rosa Sinensis*

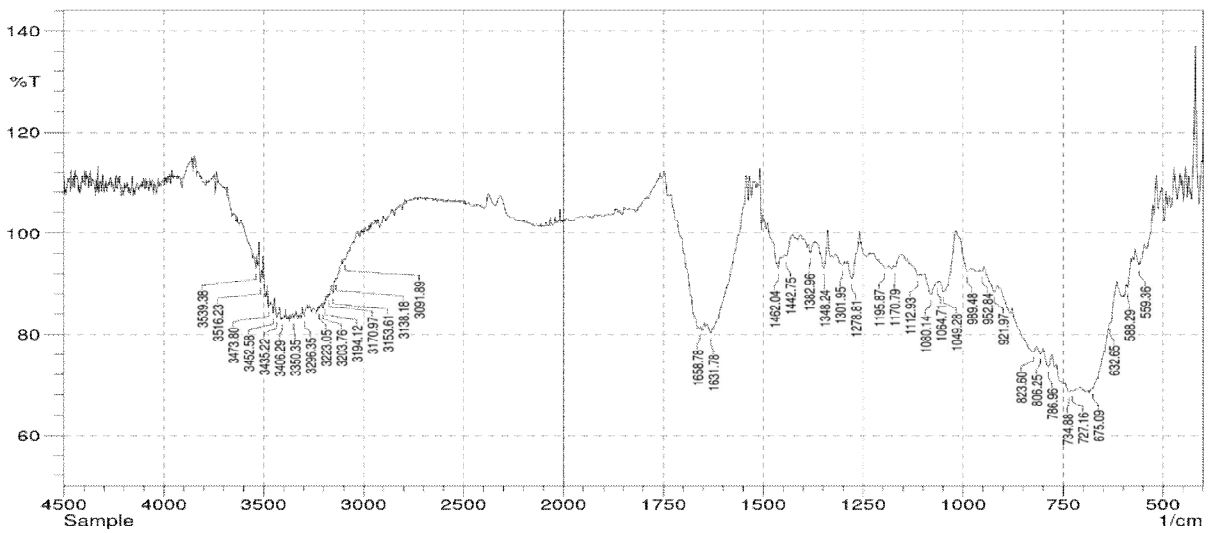

Fig 12: FTIR OF GEL FORMULATION

Estimation of herbal drugs by HPTLC:

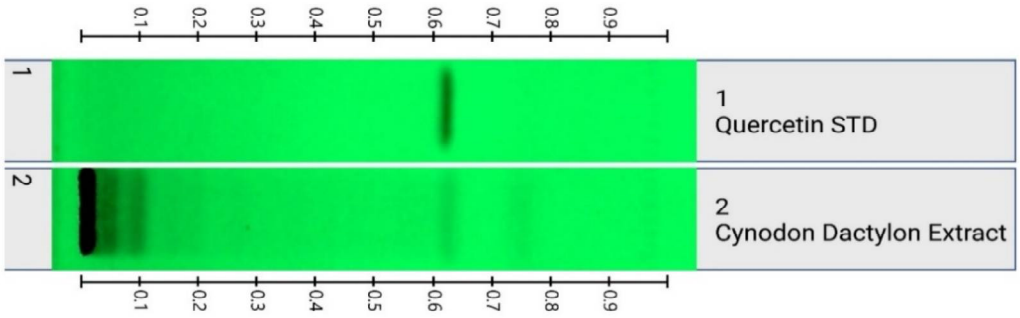

Fig 13: HPTLC image of *Cynodon Dactylon* Extract after developed at 347 nm

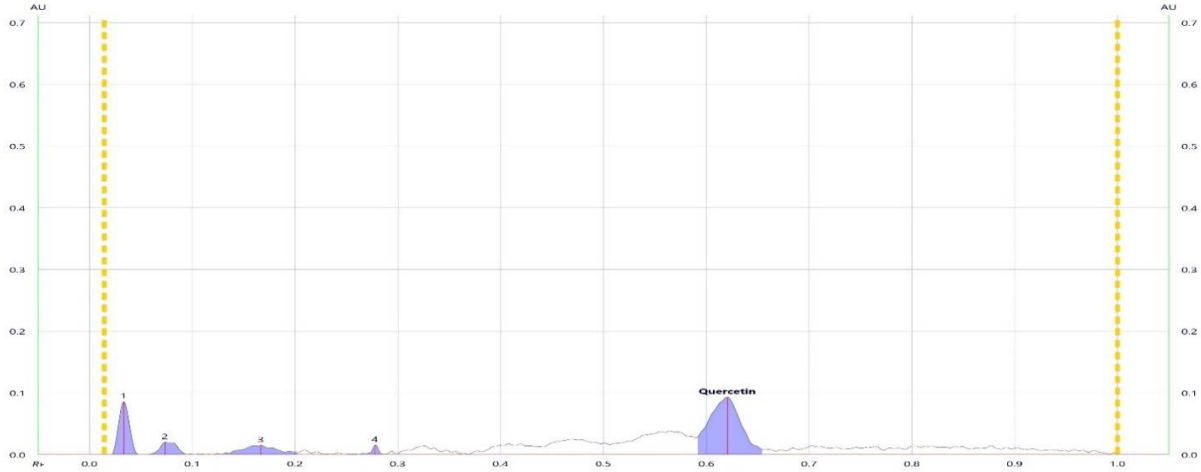

Fig 14: HPTLC Densitogram of Quercetin in *Cynodon Dactylon* Extract

The analysis identified five distinct peaks, with Peak 5 being the most prominent, accounting for 61.55% of the total area. Other significant peaks include Peak 1, contributing 18.73% of the total area, while Peaks 2, 3, and 4 represented 6.98%, 10.67%, and 2.07% of the total area, respectively.

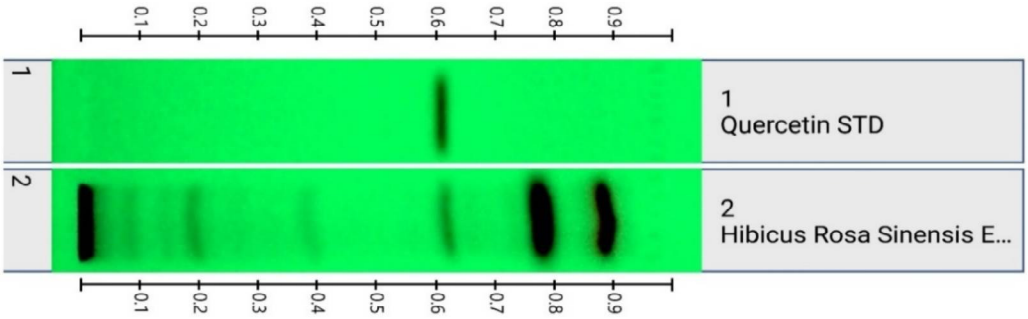

Fig 15: HPTLC image of *Hibiscus Rosa Sinensis* Extract after developed at 347 nm

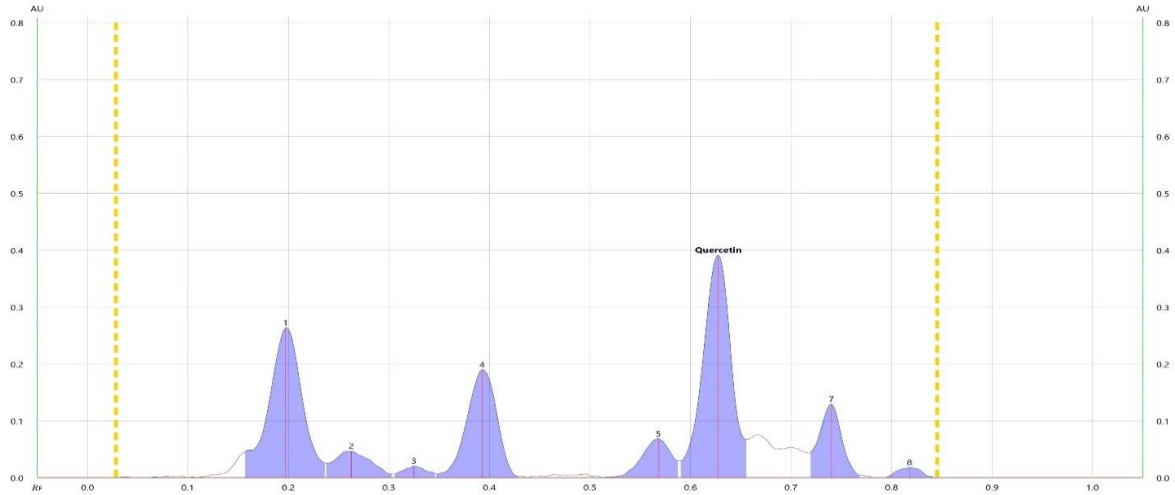

Fig 16: HPTLC Densitogram of Quercetin in *Hibiscus Rosa Sinensis* Extract

The analysis revealed eight distinct peaks. The most prominent peaks by area percentage were Peak 1 at 26.51% and Peak 6 at 33.37%. Other notable peaks include Peak 4 at 17.18% and Peak 7 at 8.98%, with the remaining peaks (2, 3, 5, and 8) collectively contributing the smaller percentages of 5.23%, 1.50%, 5.95%, and 1.29% respectively.

Experimental Design:

Viscosity - **P-values** less than 0.0500 indicate model terms are significant. In this case A is a significant model term.

The **Predicted R^2** of 0.9645 is in reasonable agreement with the **Adjusted R^2** of 0.9799; i.e. the difference is less than 0.2.

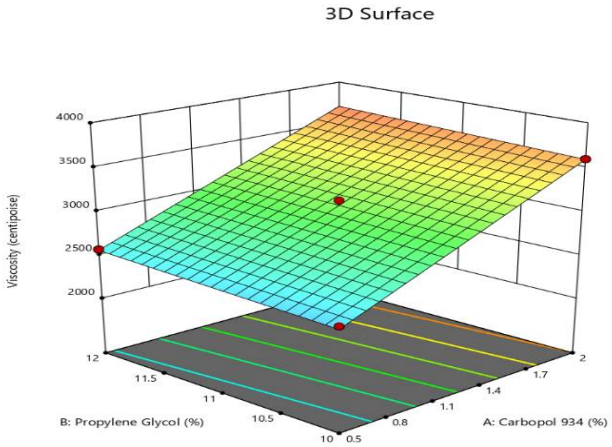

Fig 17: Responses surface curve representing a 3D effect of carbopol 934 and propylene glycol on viscosity.

Spreadability - P-values less than 0.0500 indicate model terms are significant. In this case A is a significant model term. The Predicted R^2 of 0.7914 is in reasonable agreement with the Adjusted R^2 of 0.8828; i.e. the difference is less than 0.2. [20]

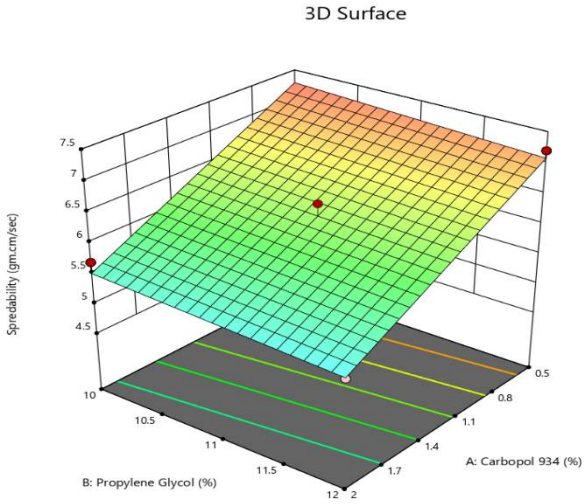

Fig 18: Responses surface curve representing a 3D effect of carbopol 934 and propylene glycol on spread ability.

CONCLUSION

The present study successfully formulated and evaluated an herbal gel formulation for managing dysmenorrhea and menorrhagia using *Cynodon Dactylon* and *Hibiscus Rosa-Sinensis* extracts. The gel formulation was prepared using a simple and cost-effective method, and all batches were evaluated for their physicochemical properties, including viscosity, pH, spreadability, and drug content. Among the batches, F4 exhibited the most desirable characteristics, indicating its potential as an effective and stable topical formulation. The optimized batch, F4, demonstrated suitable pH, viscosity, and spreadability, ensuring ease of application and patient compliance. Additionally, the drug content analysis revealed a uniform distribution of the active ingredients, ensuring consistent therapeutic efficacy. Furthermore, the herbal extracts used in the formulation, *Cynodon Dactylon* and *Hibiscus Rosa-Sinensis*, are known for their anti-inflammatory, analgesic, and antispasmodic properties, making them potential for the management of menstrual disorders such as dysmenorrhea and menorrhagia. Overall, the formulated herbal gel, F4, demonstrates promising potential as a safe, effective, and convenient topical formulation for the management of dysmenorrhea and menorrhagia.

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