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Research Paper

To Develop and Characterize a Novel Herbal Extract Formulation for enhanced Anticancer efficacy

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ABSTRACT

The present study was carried out to prepare and evaluate a herbal formulation containing parthenium hysterophorus extract and turmeric for potential anticancer activity. The ethanolic extract of the plant material was prepared using suitable extraction method and further incorporated into the formulation. Various evaluation parameters such as description study, solubility study, pH determination, assay, percentage yield, foreign particle test, microbiological test, environmental condition test, stability study, and diffusion study were performed. UV spectrophotometric analysis was carried out for quantitative estimation of the formulation and the calibration curve showed good linearity with satisfactory correlation coefficient value. The formulation exhibited acceptable physical appearance, good stability, suitable pH, and satisfactory drug content. No foreign particle and microbial contamination were observed during the study. The formulation also shows satisfactory stability under different environmental and storage condition. The overall results indicated that the prepared herbal formulation passed good pharmaceutical characteristics and may be useful for further studies related to anticancer activity and drug delivery application.

INTRODUCTION

Traditional phytomedicines are widely used as a primary health care option for nearly 75% to 80% of the world's population because they are affordable and usually have fewer side effects. Herbal medicines have been used for many years to treat different diseases and also help in strengthening the immune system against various

health problems. The beneficial effects of medicinal plants mainly come from the presence of different bioactive compounds produced through their natural metabolic processes. Cancer is a leading cause of mortality worldwide. Modern chemotherapeutics often cause severe toxicity. The parthenium hysterophorus and turmeric are

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show Anti-cancer activity and anti-Inflammatory activity.^[1]

Parthenium hysterophorus is herbaceous flowering species belonging to family Asteraceae. It contains so many chemical constituents such as Sesquiterpene lactones, Flavonoids and Phenolic acids and etc. *Parthenium hysterophorus* also known as Congress weed, Carrot grass, wild fever, bitter weed, star weed, white top and some peoples as called “Scourge of India”. It is agricultural weed

rapidly spreading through out Asia and other areas beyond its native range in the central region. In *parthenium hysterophorus* Parthenin (Sesquiterpene lactones) are the main chemical compound that show Anti-cancer activity. This compound is quire some type of cancer treatment such as Breast cancer (MCF-7), Liver cancer (HepG2), Colon cancer (HT-29), acute leukemia cancer (HL-60) and Promyelocytic leukemia cancer (THP-1).^[1,2,3]

Table No.1: *Parthenium hysterophorus* (parthenin) Discription

Property	Description
Plant Name	<i>Parthenium Hysterophorus</i>
Drug Name	Parthenin
Chemical Structure	Sesquiterpene lactone
Molecular Formula	C ₁₅ H ₁₈ O ₄
Molecular Weight	262.3 g/mol
Solubility	Soluble in Water And ethanol
Melting point	163 -166 °C
Appearance	Dark Green Color
Identification	UV Spectroscopy IR Spectroscopy
Category	Anticancer Activity

Turmeric, a rhizomatous herbaceous eternal plant (*Curcuma longa*) it's belonging to family Zingiberaceae it has been received attention due to its immense anti-oxidant, anti-inflammatory, anti-mutagenic, anti-microbial, anti-cancer and analgesic activity. These medicinal values of turmeric are mainly ascribed to curcumin. Curcumin known chemically as 1,7-bis-(4-hydroxy-3-methoxyphenyl)-hepta-1,6-diene-3,5-dione, is a lipophilic polyphenol believed to

exhibit anticancer, antibiotic, anti-inflammatory, and anti-aging properties, as indicated by various in vitro, in vivo studies, and clinical trials. Despite its potential, the therapeutic application of curcumin is hindered by challenges such as poor aqueous solubility, limited bioavailability, and unfavourable pharmacokinetic profiles. To overcome these issues, numerous formulations of curcumin have been developed.^[4,5,23]

Table No. 2: Turmeric (curcumin) Description

Property	Discription
Plant Name	Turmeric
Drug Name	Curcumin
Chemical Structure	Curcuminoids
Molecular Formula	C ₂₁ H ₂₀ O ₆
Molecular Weight	368.39 g/mol
Solubility	Poorly Soluble in water and sparingly soluble in Lipophilic Solutions



Melting Point	183 ⁰ C
Appearance	Yellow And Orange Colour
Identification	UV Spectroscopy IR Spectroscopy
Category	Anti-inflammatory Anti-cancer Anti-oxidant

Parenteral dosage forms are sterile pharmaceutical preparations that are administered directly into the body through injections, bypassing the gastrointestinal tract. The term “parenteral” refers to the administration of drugs outside the intestine. These dosage forms are particularly useful when a rapid therapeutic effect is required, when the drug is unstable in the digestive system, or when the patient is unable to take medicines orally. Common routes of parenteral administration include intravenous, intramuscular, subcutaneous, and intradermal injections. Parenteral preparations must be carefully manufactured to ensure sterility, absence of pyrogens, and freedom from particulate matter in order to maintain patient safety. Since the drug is delivered directly into the bloodstream or body tissues, parenteral dosage forms provide faster action and better bioavailability compared to oral medications. Although they offer several advantages such as accurate dosing and rapid drug action, these formulations require strict sterile conditions during preparation and administration to prevent infection and ensure drug stability.

Breast cancer is heterogeneous and one of the most common cancers affecting women worldwide. Breast cancer is not a single disease but includes different subtypes, and each behaves differently in the body. These subtypes are mainly identified based on hormone receptors (ER, PR) and HER2 status, which help doctors decide the most suitable treatment and predict the outcome. For example, Luminal A type usually has a good prognosis because it is ER and PR positive with low growth rate (low Ki-67), and it responds well to hormonal therapy. Luminal B type is slightly more

aggressive, showing higher cell growth, so patients often need both chemotherapy and hormonal treatment. In the case of HER2-enriched subtype, the cancer cells produce excess HER2 protein, but targeted drugs specifically designed for HER2 have significantly improved patient outcomes. On the other hand, triple-negative breast cancer does not have ER, PR, or HER2 receptors, making it more aggressive and difficult to treat, although newer treatments like immunotherapy are now being explored. Overall, understanding these subtypes is very important, as it helps in selecting the right treatment and improving patient care. [25,26,27,28]

➤ MATERIALS & METHODS:

❖ Materials:

The younger parthenium hysterophorus are collected from well maintain agriculture and Turmeric rhizome are collected from college medicinal garden. The Parthenium & Turmeric are wash with water and remove the impurity form plant and then dry at the Room 25⁰C. and also for extraction process Soxhlet apparatus are used. Identification and authentication of theses leaves were done at Analytical lab. Also, Ethanol as a solvent and Tween 80 as a Surfactant are used provided form College chemical storeroom. We use some instrument for analysis purposed we used UV spectrophotometer, pH meter, Weighing Balance, and some other instrument form college instrumental room & machine room. And IV formulation conformation test are performed in Analytical Lab.



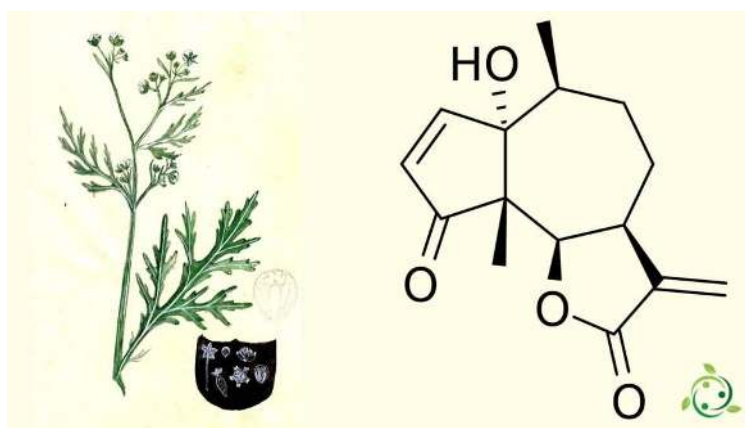


Figure 1. *Parthenium hysterophorus* (Parthenin)

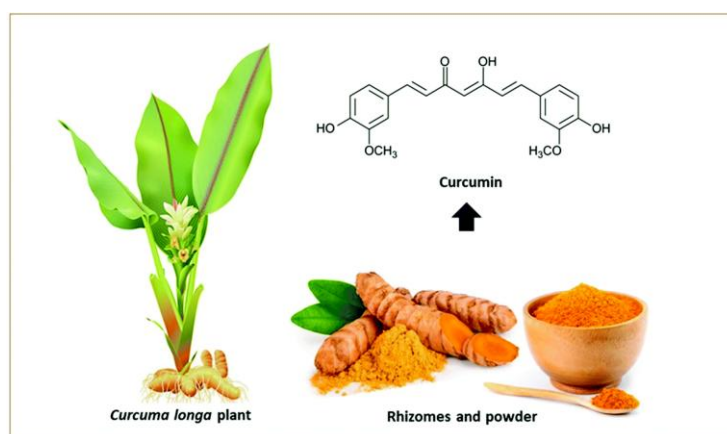


Figure 2. *Turmeric* (curcumin)

❖ Methods:

Preparation of Ethanolic Extract of *Parthenium hysterophorus* (PH):

The shed dried plant of *parthenium hysterophorus* were crumbled by a mechanical grinder and after grinding dry the powder in shed and then the grind powder passed to sieve to remove unwanted particles. We have used Soxhlet apparatus for the extraction of parthenin with the help of ethanol as a solvent, for that we have maintained the temperature at 75 to 80⁰ C, this process is kept for 6 – 8 hours till the extract gets, after completing the extraction process evaporate the ethanol by using the simple distillation process or rotary evaporator method.^[6,8,10,14]

Preparation of Ethanolic Extract of Turmeric:

The shed dried turmeric rhizomes were pulverized into fine powder in a mechanical grinder and then passed the powder into sieve for proper fine powder obtain. We are used Soxhlet apparatus for the extraction of curcumin with help of ethanol as a solvent, for provide a 70 ⁰C. than competed the extraction process evaporate the ethanol by using the simple distillation process or rotary evaporator method.^[23]

Preparation of *Parthenium hysterophorus*-Turmeric IV:

First offal we prepare Turmeric (Curcumin) solution, in this take some amount of turmeric extract powder Dissolve in Ethanol than add some

amount of tween 80 for turmeric powder properly soluble in ethanol than heat this solution for 2 to 3 min. at 50°C to remove small amount of ethanol and then Add Parthenium hysterophorus ethanolic extract (Parthenin) at continues stirring after adding than filter the Solution than add water for injection for volume make up.

This prepared IV solution fill into the stirred injection vial after filling this vial is sterilized into autoclaved.

➤ Research Methodology:

In experimental work we prepared and evaluated the anticancer IV of parthenium hysterophorus and turmeric are show combine effect. They both treat the cancer cell to prevent abnormal cancer cell growth. In this we first prepared parthenium hysterophorus and turmeric ethanolic extract than we prepare the IV formulation they show combine effect on breast cancer (MCF-07). After preparation we perform some analytical study on the prepared formulation. In analytical study we performed some stability testing like Description, Solubility, pH, Identification test, foreign particle test, etc.

Stability Study:

- 1) **Description Study:** In description study we perform on the prepared formulation. In this we check the formulation Colour, Odor, texture, etc.
- 2) **Solubility Study:** The Solubility of the preparation formulation containing parthenium hysterophorus extract and

curcumin was checked in different solvent such as distilled water, ethanol, and phosphate buffer solution (pH 7.4) A small amount of the formulation was added separately into each solvent and mixed properly to observe its solubility behaviour. It was observed that the formulation dissolve well in ethanol, while moderate solubility was seen in phosphate buffer solution. In distilled water, only slight solubility was observed because curcumin naturally has poor water solubility. The presence of Tween80 helped improve the dispersion and overall solubility of the formulation in aqueous medium. This improved solubility may help enhance the adsorption and effectiveness of the formulation.^[17]

- 3) **pH Determination:** The pH of the prepared formulation containing parthenium hysterophorous extract and curcumin was determined using a digital pH meter. Before measurement, the pH meter was calibrated using standard buffer solutions. A small quantity of the formulation was taken in a clean beaker, and the electrode of the pH meter was dipped into the sample. The reading was allowed to stabilized, and pH value was noted the formulation showed a pH near neutral range which indicates that it may be suitable for pharmaceutical application and remains stable without causing major irritation.^[18]

Observation Table:

Table No.3: pH Observation table

Parameter	Observation
pH of Formulation	6.8 – 7.8

- 4) **Identification test:** The identification test of the prepared formulation using suitable

solvent such as ethanol or phosphate buffer solution. The prepared sample was scanned in



the UV-Visible spectrophotometer within the suitable wavelength range. The formulation showed a characteristic absorption peak near 400nm, which confirm the presence of curcumin in the formulation. The observed peak was found to be similar to the standard curcumin spectrum. This study confirmed the successful incorporation of curcumin into the prepared formulation.^[20]

- 5) **Assay:** The assay of the prepared formulation containing curcumin was carried out using UV-Visible spectrophotometric method to determine the drug content present in the formulation. A known quantity of the formulation was accurately measured and diluted using suitable solvent such as ethanol. The sample was analysed using UV-Visible spectrophotometer at 400nm. The absorbance obtained was compared with the standard calibration curve of curcumin to calculate the drug concentration. The assay result showed that the formulation contained drug content within acceptable limits, indicating uniform distribution of the drug in the formulation.^[23]
- 6) **Particle size:** Particle size analysis of the prepared formulation containing parthenium hysterophorus extract and curcumin was carried out to evaluate the size and uniformity

of particles present in the formulation. The particles size was measured using a suitable particle size analyser. A small quantity of the formulation was diluted with distilled water and analysed under controlled condition. The study showed that the formulation possessed practical in nano-size range with uniform distribution. Smaller particles size helps improve drug solubility, stability, absorption, and overall therapeutic effectiveness.

- 7) **Foreign particles test:** The prepared formulation containing parthenium hysterophorus extract and curcumin was examined for the presence of any foreign particles or visible contamination. A small quantity of the formulation was taken in a clean transparent container and observed visually against both light and dark background. The formulation was carefully checked for the presence of dust, fibres, precipitates, or any unwanted particulate matter. No visible foreign particles or contamination were observed in the prepared formulation, indicating that the formulation was clean and properly under suitable laboratory condition.^[18,23]

Observation Table:

Table No.4: Foreign particle test Observation table

Parameter	Observation
Method Used	Visual Inspection
Foreign Particle	Not observed
Appearance	Clear And Homogenous

- 8) **Environmental condition test:** The prepared formulation containing parthenium hysterophorus extract and curcumin was subjected to different environmental condition to study its stability and physical appearance. The formulation was stored at the room temperature and refrigerated condition for a specific period of time. During the study, the

formulation was observed for any changes in colour, Odor, phase separation, precipitation, or clarity, The formulation remained stable under the tested condition with in its physical appearance. No phase separation or precipitation was observed during the study period.



Observation Table:**Table No. 5: Environmental condition Observation table**

Storage Condition	Observation
Room Temperature	Stable, no phase separation
Refrigerated Condition	Stable, no visible change

9) Microbiological test: The microbiological test of the prepared formulation containing parthenium hysterophorus extract and curcumin was performed to evaluate microbial contamination and ensure the microbiological quality of the formulation. The test was carried out using nutrient agar media under aseptic conditions. Sterile agar plates were prepared, and a small quantity surface of the media. The plate was then incubated at suitable temperature for 24-48 hours. After incubation, the plates were examined for the presence of bacterial or fungal colonies. The formulation showed no significant microbial growth, indicating that it was prepared under proper hygienic and sterile condition.

10) Percentage yield of ethanolic extract: The percentage yield of ethanolic extract of parthenium hysterophorus was calculated to determine the efficiency of the extraction process. The dried powdered plant material was extracted using ethanol by Soxhlet method. After completion of the extraction, the solvent was evaporated and the obtained extract was weighed. The percentage yield was then calculated using the following formula. [23]

$$\% \text{ Yield} = \frac{\text{Weight of the Sample extract}}{\text{Initial weight of sample}} \times 100$$

11) Diffusion study: The diffusion study of the prepared formulation containing parthenium hysterophorus extract and curcumin was carried out to evaluate the drug release pattern

from the formulation. The study was performed using a diffusion cell containing phosphate buffer solution (pH 7.4) as the diffusion medium. The membrane was properly fixed between donor and receptor compartment. As measured quantity of the formulation was placed in the donor compartment, while the receptor compartment was filled with buffer solution and maintained under continuous stirring at suitable temperature. At predetermined time intervals, small quantities of the sample were withdrawn from the receptor compartment and replaced with equal amount of fresh buffer solution to sample were analysed using UV-Visible spectrophotometer at 425nm to determine the amount of drug released. The study showed gradual and controlled release of drug from the formulation over a period of time. [15, 17,23]

➤ **Results:**

1) Organoleptic Study: The prepared formulation was evaluated for its physical appearance and organoleptic properties. The formulation showed a uniform appearance with smooth texture and characteristic Odor. No. phase separation, grittiness, or precipitation was observed during the study. The colour of the formulation was found to be yellowish brown due to presence of turmeric and parthenium extract. The formulation is stable and acceptable in appearance.



Table No. 6: Organoleptic Study

Sr No.	Description	Observation
1.	Colour	Yellowish Brown
2.	Odor	Characteristic
3.	Texture	Liquid

2) **Solubility Study:** The parthenium hysterophorus (parthenin) is soluble in organic solvents like ethanol, Hexane, alcohol, etc. and slightly soluble in Aq. Medium. And turmeric (curcumin) is purely soluble in lipophilic medium but slightly soluble in organic solvent and poorly soluble in Aq. Medium.

3) **pH Determination:** The pH of the prepared formulation was determined using a digital pH meter. The pH of the formulation was found to be within the acceptance range and suitable for pharmaceutical application. The observed pH was **7.4**. The formulation showed good stability without any significant change in pH during the study period. The obtained pH

indicates that the formulation is compatible and safe for further application.

4) **UV Spectrophotometer:** The UV Spectrophotometric method was successfully carried out for the analysis of Parthenium hysterophorus and turmeric combine formulation. A calibration curve was prepared using different concentrations ranging from 2-16 g/ml. the absorbance was observed with increasing concentration. The correlation coefficient value was: $R^2 = 0.09978$. The High R^2 value indicates excellent linearity and accuracy of the developed UV spectrophotometric method. Hence the method was found to be suitable for quantitative estimation and further analytical studies of the formulation.

Table No. 7: UV Spectrophotometer Absorbance

Sr. No.	Concentration	Absorbance
1.	2	0.029
2.	4	0.073
3.	6	0.101
4.	8	0.132
5.	10	0.164
6.	16	0.27

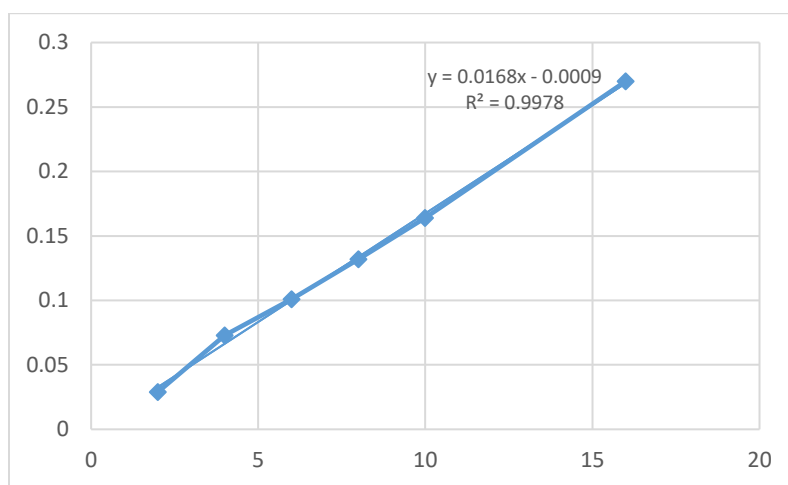


Figure 3:UV Spectrophotometer

- 5) Foreign Particle test:** The prepared formulation was evaluated for the presence of foreign particle by visual inspection under stable lighting conditions. No visible foreign particle, dust, fibres, or other extraneous matter were observed in the formulation. The formulation was found to be clean, uniform, and free from contamination. Hence, the formulation passed the foreign particle test and was considered suitable for further evaluation studies.
- 6) Stability Study:** The prepared formulation was subjected to stability studies under specified storage conditions for the study period. The formulation was evaluated for change in appearance, colour, odour, pH, and phase separation. No significant changes were observed during the stability study. The formulation retained its physical appearance, homogeneity, and stability throughout the storage period. No precipitation, microbial growth, or phase separation was observed. The pH remained within the acceptable range, indicating good stability of the formulation. Therefore, the prepared formulation was found to be stable under the selected storage condition.
- 7) Microbiological Test:** The prepared formulation was evaluated for microbiological contamination using suitable culture media and incubation condition. The study showed no significant microbial growth in the formulation during the observation period. The formulation was found to be free from harmful bacterial and fungal contamination, indicating good microbial quality and proper handling during preparation. The obtained results were within acceptable pharmaceutical limits. Hence, the formulation passed the microbiological test and was considered safe and suitable for further pharmaceutical application.
- 8) Environmental condition study:** The prepared formulation was subjected to different environmental conditions such as room temperature, refrigeration condition, and accelerated temperature condition to evaluate its stability and performance. No significant change in colour, Odor, appearance, pH, or homogeneity of the formulation was observed under the tested environmental conditions. The formulation remained stable without any phase separation, precipitation, or degradation throughout the study period. The result indicated that the prepared formulation passed good environmental stability and can withstand

normal storage condition without affecting its quality and effectivity.

- 9) **Diffusion Study:** The diffusion study of the prepared formulation was carried out using phosphate buffer solution of pH 7.4 at predetermined time intervals. The samples were analysed by UV spectrophotometric

method to determine the percentage cumulative drug release. The formulation showed gradual and sustained drug release throughout the study period. The percentage cumulative drug release increase in time, indicating effective of active constituents from the formulation.

Table No.8: All Sample Absorbance

Time	Absorbance		
	B1	B2	B3
0	0	0	0
30	0.087	0.119	0.111
60	0.112	0.134	0.237
120	0.162	0.147	0.363
180	0.212	0.161	0.422
240	0.522	0.175	0.48
300	0.543	0.203	0.489

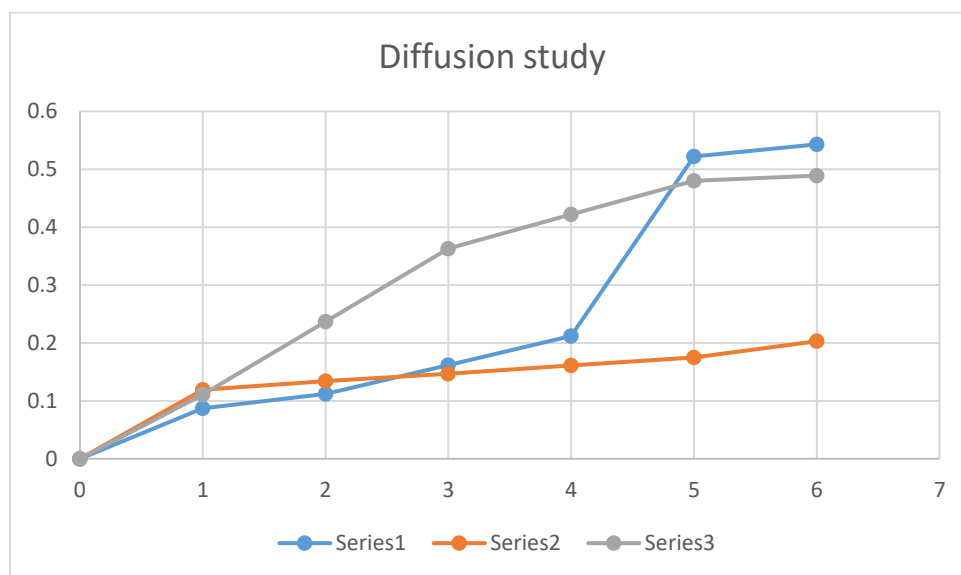


Figure 4: All Sample Drug release

Based on the diffusion study, Batch B3 showed a comparatively better and more consistent drug-release profile than B1 and B2. B3 exhibited a progressive increase in absorbance from 0.111 at 30 minutes to 0.489 at 300 minutes, indicating sustained drug diffusion over the study period.

Therefore, Batch B3 (Series 3) was selected as the optimized batch for further studies due to its desirable and sustained drug-release characteristics.

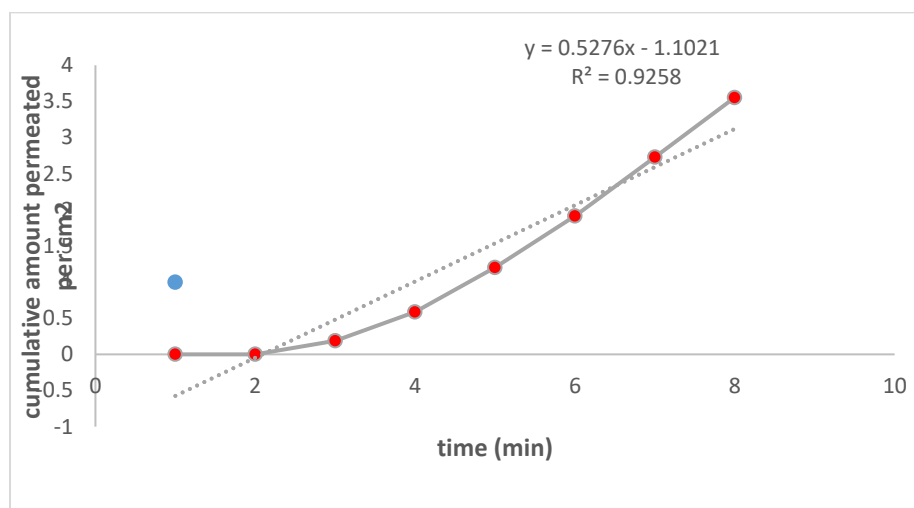
- **Batch 3 Drug Release Study:**

Table No.9: Batch no. 3 Drug release study

Time (Min.)	Absorbance	Concentration	Dilution factor	Concentration in 5ml (in mg)	Cumulative amount (mg)	Cumulative amount permeated per cm ²
0	0	0	0	0	0	0
30	0.111	6.607142857	66.07142857	0.330357143	0.33	0.1875
60	0.237	14.10714286	141.0714286	0.705357143	1.035357143	0.588271104
120	0.363	21.60714286	216.0714286	1.080357143	2.115714286	1.20211039
180	0.422	25.11904762	251.1904762	1.255952381	3.371666667	1.915719697
240	0.48	28.57142857	285.7142857	1.428571429	4.800238095	2.727408009
300	0.489	29.10714286	291.0714286	1.455357143	6.255595238	3.554315476

Table No.10: Diffusion Study

Time (Min.)	% Drug Release
0	0
30	5.27
60	16.55
120	33.82
180	53.9
240	76.74
300	100

**Figure 5: Batch 3 Drug release**❖ **Kinetic Study:**

- **Zero Order:** According, to Zero Order

Table No. 11: Zero Order

Sr. No.	Time	Zero order
1.	0	0
2.	30	6.607142857
3.	60	14.10714286
4.	120	21.60714286



5.	180	25.11904762
6.	240	28.57142857
7.	300	29.10714286

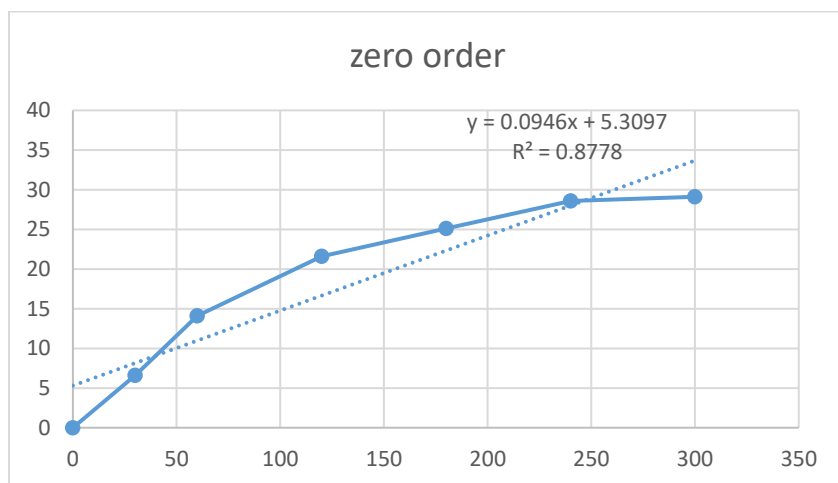


Figure 6: Zero Order

- **First Order:** According, to First Order

Table No. 12: First Order

Sr. No.	Time	First order
1.	0	0
2.	30	0.82001
3.	60	1.14944
4.	120	1.3346
5.	180	1.4
6.	240	1.45593
7.	300	1.464

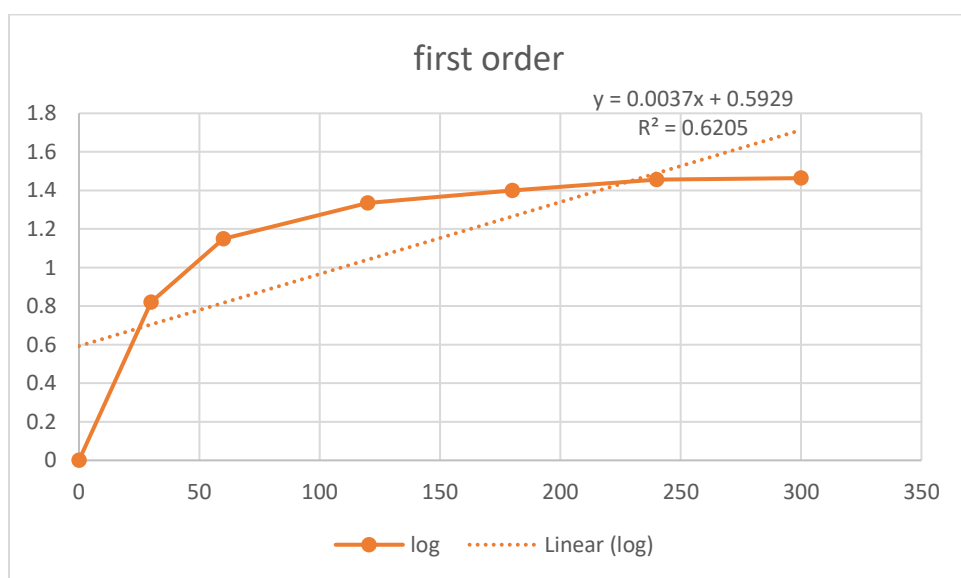


Figure 7: First order

- **Second Order:** According, to Second Order

Table No.13: Second Order

Sr. No.	Time	Second order
1.	0	0
2.	30	0.15135
3.	60	0.07089
4.	120	0.04628
5.	180	0.03981
6.	240	0.035
7.	300	0.03436

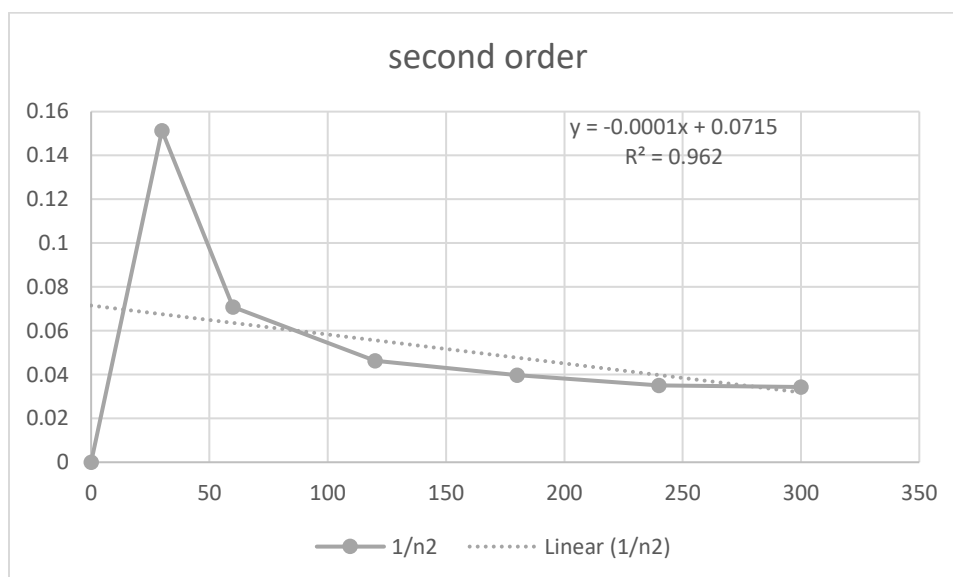


Figure 8:Second order

- **Higuchi Model:** According, to Higuchi Order

Table No.14: Higuchi Model

Time	SQRT	% Drug release
0	0	0
30	5.477226	5.27
60	7.745967	16.55
120	10.95445	33.82
180	13.41641	53.9
240	15.49193	76.74
300	17.32051	100

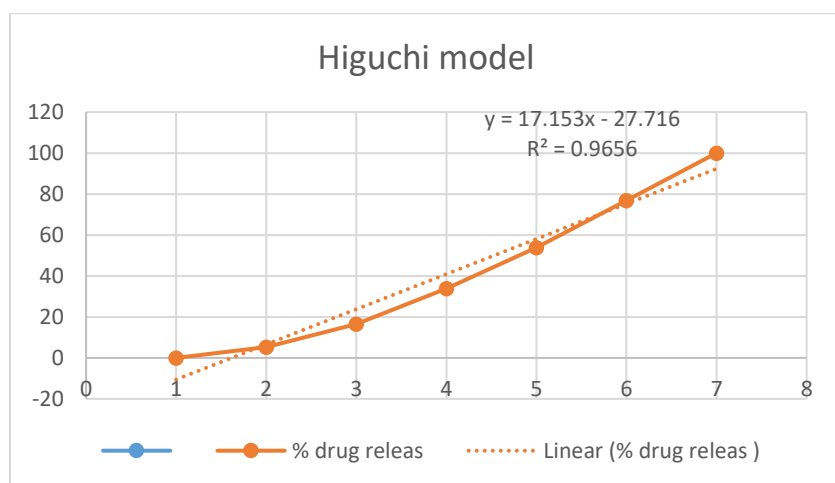


Figure 9: Higuchi model

- **Korsmeyer Peppas:** According to Korsmeyer Order

Table No. 15: Korsmeyer Peppas

Time (min)	concentration(microgram/ml)	Fit	SD
0	0	0	0
30	6.607142857	9.575194436	8.809330177
60	14.10714286	13.66554382	0.195009713
120	21.60714286	19.50321627	4.426507112
180	25.11904762	24.01435046	1.220355805
240	28.57142857	27.8346365	0.542862561
300	29.10714286	31.21165418	4.428967914

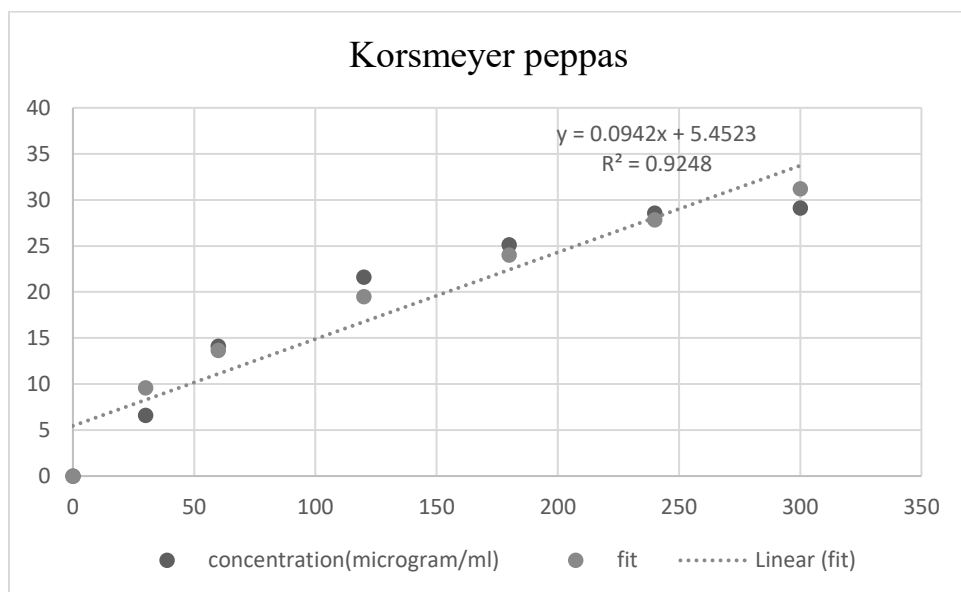


Figure 10: Korsmeyer Peppas

To determine the mechanism of drug release the diffusion data was fitted into different kinetic model such as zero order, first order, second order, Higuchi model and Korsmeyer Peppas. The

regression coefficient (R^2) values obtained from the kinetic study indicated that the formulation followed Higuchi model drug release kinetic with good linearity compared to other kinetic models.

Table No.16: Model fitting of the release profile of optimized formulation

Batch	Kinetic Model				
	R^2				
B3	Zero Order 0.8778	First Order 0.6205	Second Order 0.962	Higuchi 0.9656	Best Fit Model Higuchi
Korsmeyer Peppas Equation					
B3	R2 0.9248	K Value 1.67161	n Value 0.513117	Mechanism Non Fickian Super Case II	

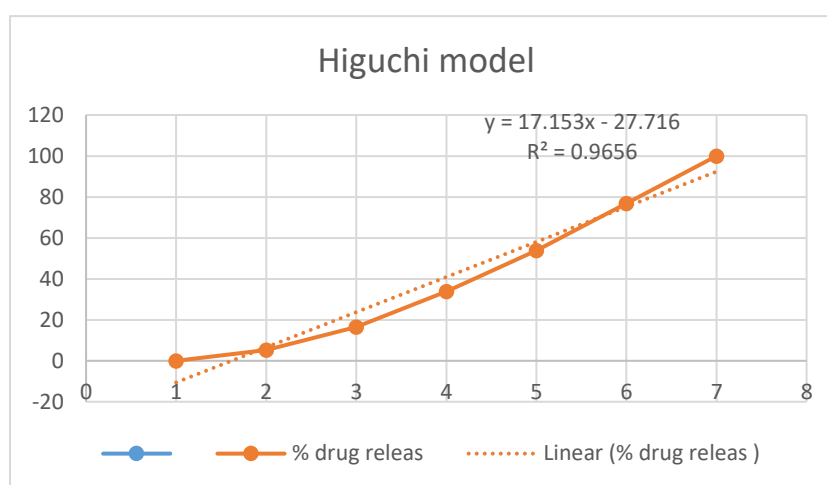


Figure 11: Higuchi Model

The obtained results confirmed that the prepared formulation passed satisfactory diffusion characteristics and controlled drug release behavior suitable for further pharmaceutical and anticancer application.

SUMMARY AND CONCLUSION:

The present study successfully prepared and evaluated an herbal formulation containing Parthenium hysterophorus extract and turmeric. The ethanolic extract showed satisfactory percentage yield and was effectively incorporated into the formulation. Various evaluation parameters including description study, solubility study, pH determination, assay, foreign particle test, microbiological test, environmental condition

test, stability study, and diffusion study were carried out successfully. The formulation showed good physical appearance, acceptable pH, satisfactory drug content, and excellent stability under different storage conditions. No microbial contamination or foreign particles were observed during the evaluation studies. The UV spectrophotometric method showed good linearity and accuracy for quantitative estimation of the formulation. Based on the obtained results, the prepared formulation was found to possess satisfactory pharmaceutical properties and may be considered suitable for further research related to anticancer activity and advanced drug delivery applications



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