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Polyherbal Nanoparticulate Gel Containing Herbal Extracts of Harad, Neem, Liquorice and Turmeric: Characterization and In-vitro Assessment

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ABSTRACT

The creation and characterisation of a polyherbal nanoparticulate gel including extracts of harad (Terminalia chebula), neem (Azadirachta indica), liquorice (Glycyrrhiza glabra) and turmeric (Curcuma longa) is the focus of the current investigation. Due to their well-known strong anti-inflammatory, antioxidant and antibacterial qualities, these plants are excellent choices for topical medicinal uses. To improve the bioavailability and effectiveness of the herbal extracts, a nanoparticulate method was used to make the polyherbal gel. Initially, aqueous extracts of Harad, Neem, Liquorice and Turmeric were evaluated for various pharmacognostical parameters. The aq. Extract & of each plants was lyophilized (60°C) to get powdered forms. The drug content was estimated by HPLC analysis for testing the compatibility of drug with selected excipients used in the formulation. From the outcomes it is concluded that the, Harad, Neem, Liquorice and Turmeric are compatible with the excipients. The prepared gel was characterized for various parameters i.e; Physical appearance, viscosity, spreadability, HPLC, and kinetics release studies. Release data obtained with prepared polyherbal gel was fitted into various kinetics models i.e. Zero & First order, Higuchi and Koresemeyer-peppa models in order to find out the mechanism of drug release from the gel which confirmed zero order kinetics. These results imply that the polyherbal nanoparticulate gel has strong therapeutic potential and might be used to treat a range of skin conditions. To confirm its effectiveness and safety in human patients, more in-vivo research and clinical trials are necessary.

Keywords: *Polyherbal, nanoparticulate gel, harad, neem, liquorice, turmeric*

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1. Introduction

Many plant-derived chemicals have been identified for their therapeutic advantages, and the use of medicinal plants in healthcare has a long history.[1] Known for their diverse range of bioactive ingredients with antibacterial, anti-inflammatory, and antioxidant activities, harad (*Terminalia chebula*), neem (*Azadirachta indica*), liquorice (*Glycyrrhiza glabra*), and turmeric (*Curcuma longa*) are highly valued herbs in traditional medicine.[2-6]

Because of its strong antibacterial and antioxidant properties, harad, often referred to as black myrobalan, has been utilized for a long time. Widely known as the "village pharmacy," neem is well-established for its anti-inflammatory and broad-spectrum antibacterial properties. Liquorice root has strong anti-inflammatory and antibacterial properties due to its high glycyrrhizin and flavonoid content. Turmeric is renowned for its potent anti-inflammatory and antioxidant qualities, and it is mostly recognized for its active ingredient, curcumin.[7-10]

Synergistic preparations of these herbs can greatly boost their medicinal potential, notwithstanding their separate merits. Enhancing the bioavailability and effectiveness of herbal extracts can be achieved through the potential application of nanotechnology. By creating a polyherbal nanoparticulate gel, various herbal extracts may be blended to maximize their combined therapeutic benefits in a single, stable formulation.[11,12]

Formulating and characterizing a polyherbal nanoparticulate gel with extracts of harad, neem, liquorice, and turmeric is our goal in this work. Based on our hypothesis, the herbal extracts will have improved stability, bioavailability, and therapeutic effectiveness thanks to the nanoparticulate

delivery technology. The physical and chemical characteristics of the gel will be assessed. By harnessing the innate healing properties of many conventionally used medicinal herbs, the successful creation of this polyherbal nanoparticulate gel may provide a unique and efficient therapy alternative for a variety of skin conditions.

2. Materials and Methods

2.1 Materials

All chemical and reagent used in the study, including solvents and other materials for the preparation of formulation were obtained from different reputed companies.

2.2 Adopted Method

2.2.1 Pharmacogonostical Studies

2.2.1.1 Collection of the plant material

The plant material (Harad, Neem, Liquorice and Turmeric) was collected from the local market.

2.2.1.2 Extraction

All the plant materials were thoroughly washed with distilled water, dried (60-70°C), crushed in to coarse powder and collected in clean containers individually. A predetermine amount of each crushed material was soaked in sufficient distilled water, set aside for overnight and filtered. Individual filtrate was collected and freeze dried (-60°C) in a lyophilizer. The lyophilized extracts were individually stored in air tight containers and used for further studies.[13]

2.2.1.3 Organoleptic evaluation

It referred to the investigation of coarsely powder drug with reference to shape, color and external surface.[14]

2.2.2 Phytochemical Studies

2.2.2.1 Phytochemical screening

Various phytochemical tests were carried out using each extract powder of selected drugs.[15]

2.2.2.1.1 Test for alkaloids: Included various tests i.e; Wagner's, Mayer's, Hagger's and Dragendroff's tests, according to the official procedures.

2.2.2.1.2 Test for steroids: Presence of steroids was ensured by performing Libermann test as per official procedures.

2.2.2.1.3 Test for glycoside: were identified by performing Keller-Kiliani, Borntrager and modified Borntrager tests as per official methods.

2.2.2.1.4 Tests for carbohydrates: presence of carbohydrates was ensured by Molisch's, Fehling's and Benedict's, Selwinoff's, Bial's, Tollen's, Barford's, Iodine's and Tannic acid tests in accordance with official methods.

2.2.3 Formulation and Development

2.2.3.1 HPLC (High Performance Liquid Chromatography) studies:

The HPLC analysis was performed using a LC-100, CyberlabTM, Salo Torrace, Millburry, MAO 1527, USA with LC-UV-100 UV detector.

Table 1. Chromatographic conditions

S. No.	Parameters	Specifications			
		HARAD	**NEEM**	**LIQUORICE**	**TURMERIC**
1.	**Column**	Phenomenex Luna C18 (4.6 x 250mm, 5µ particle size)	A CAPCELL (C-18) HPLC-packed column (4.6 mm I.D.X 250 mm)	Column (C18, 5µm, 250×4.6 mm)	Reversed-phase column (C-18 -ODS (M) 4.6 mm x 15 cm – particle size 5 µm)

2.	**Mobile phase**	Mobile Phase Water:Acetonitrile (80:20 %v/v)	Mobile phase consisted of solvent A:B [30:70] Solvent A is Acetonitrile and solvent B is water.	Mobile phase consisting of methanol-water (70:30, v/v, and containing 1% acetic acid).	Mobile phase is A:B solvent system (A-Acetonitrile; B- 1.25% H_3PO_4 aqueous)
3.	**Flow rate**	1 mL/min	1.0 ml/min	1.0 mL/min	1.0 mL/min
4.	**Volume of injection**	21 µL	25µl	25 µL	21 µL
5.	**Detection wavelength**	271 nm	218 nm	251 nm	207 nm
6.	**Retention time**	2.344	2.507	5.687	5.735

2.2.4 Formulation of Polyherbal Gel

Table 2. Composition of polyherbal gel

S No.	Ingredients	Quantity
1.	Harad	1gm
2.	Neem	1gm
3.	Liquorice	1gm
4.	Turmeric	1gm
5.	Carbopol 934(gelling agent)	3gm
6.	Polyethylene glycol 400 (plastisizer)	1 gm
7.	Aspartame (sweetner)	1%
8.	Sodium benzoate (preservative)	1%
9.	Distilled water	100gm

An accurately weighed amount (3 gm) of carbopol was dispersed in 100 ml distilled water with continuous heating and mechanical stirring. Predetermined quantity of polyethylene glycol 400

was dissolved. The solution was cooled and calculated amounts of aspartame & sodium benzoate were added. Finally, required quantity of each dried extracts (Harad, Neem, Liquorice and Turmeric), was mixed to above mixture. The content was properly homogenized in the homogenizer followed by pH (6.8) adjustment with sodium hydroxide solution.

3. Evaluation Parameter of Topical Gel Formulations

3.1 Clarity

The prepared gel was observed against a dark and white background to ensure that it was clear.

3.2 pH

The gel compositions pH was measured using a digital pH meter. 2.5g of gel was carefully weighed, combined with 25ml of distilled water, and then stored for two hours. Three measurements of the formulation's pH were made.

3.3 Appearance and homogeneity

Physical appearance and homogeneity of prepared polyherbal gel was tested by visual observation.

3.4 Viscosity

The viscosity of the gel was determined using the Brookfield viscometer. At 25ºC, the rheological characteristics of gel were investigated using a Brookfield viscometer. The measurement was obtained at 100 rpm for 30 seconds using spindle number 60.

3.5 Spreadability

The term "spreadability" describes the area of skin that the gel readily covers after application or affected completely from each other.

$$S=ml/t$$

Where,

m = weight tide to upper slide

l = length moved on the glass slide

t = time taken to separate the slide

3.6 Release kinetics study

The understanding of release kinetics is essential for the formulation of dosage forms, as it allows for the establishment of in vivo in vitro (IVIVC) correlation. One scientific technique for optimizing and assessing the mistake in terms of deviation in the release profiles of developed products during the formulation development stage is the mathematical approach. Due to its simplicity and potential to reduce the number of trials required for ultimate optimization, mathematical models are valuable tools in research and development. This can enhance the formulation development process. The produced formulation's permeation profile was matched to several kinetic models.

3.7 Stability studies

The stability study was performed as per ICH guidelines. The formulated gel was filled in collapsible tubes and stored at accelerated conditions, viz. 40±2 ºC / 75±5% RH for a period of three months and studied for its release pattern. [16,17]

4. Result and Discussion

4.1 Pharmacognostical Studies

4.1.1 Organoleptic evaluation

Characteristics including color, odor, and taste were revealed by the organoleptic research of the plant sections.

4.1.1.1 Harad

Table 3: Organoleptic study of Harad fruit (*Terminalia chebula*)

S. No.	Parameter	Observation
1.	Odour	Weak, characteristic
2.	Color	Brownish-yellow to black
3.	Taste	Astringent, bitter
4.	Size	Typically around 1-2 cm in length
5.	Shape	Oval or slightly elongated

4.1.1.2 Neem (*Azadirachta indica*)

Table 4: Organoleptic study of Neem

S. No.	Parameter	Observation

1.	Odour	Brown
2.	Taste	Bitter
3.	Apex	Ovate, lanceolate, attenuate
4.	Base	Unequal
5.	Shape	Cylindrical

4.1.1.3 Liquorice (*Glycerrhiza glabra*)

Table 5: Organoleptic study of Liquorice

S. No.	Parameter	Observation
1.	Colour	Unpeeled yellowish brown or dark brown externally and yellowish internally while the peeled liquorice is pale yellow in colour
2.	Odour	Faint and characteristic
3.	Taste	Sweet
4.	Size	Length - 20 to 50 cm Diameter - 2 cm
5.	Shape	Cylindrical pieces which are straight may be peeled or unpeeled, peeled liquorice is angular
6.	Fracture	Fibrous in bark and splintery in wood

4.1.1.4 Turmeric (*Curcuma longa*)

Table 6: Organoleptic study of Turmeric

S. No.	Parameter	Observation
1.	Colour	Bright yellow to orange
2.	Odour	Earthy, slightly pungent
3.	Texture	Powdery when dried, tough and fibrous when fresh

4.2 Phytochemical Studies

4.1.1 Phytochemical screening

Table 7: Phytochemical tests for detection of organic constituents present in Harad

Chemical class	Tests	Inference
Alkaloids	Mayer's test	+
	Wagner's test	+
	Hager's test	-
	Dragenderoff's test	+
Steroids	Liebermann burchard test	-
	Liebermann test	-
Glycosides	Keller- killiani test	+
	Borntrager's test	+
	Modified borntrager's test	-
Carbohydrates	Mollish's test	+
	Fehling's test	+
	Bendict's test	-
	Barford's test	+
	Bial's test	-
	Selwinoff's test	+
	Tollen test	-
	Iodine test	-
	Tannic acid test	+

Table 8: Phytochemical tests for detection of organic constituents present in Neem

Chemical class	Tests	Inference
Alkaloids	Mayer's test	+
	Wagner's test	+
	Hager's test	+
	Dragenderoff's test	+
	Dil NH_4OH + Potassium ferricyanide solution	+
Steroids	Liebermann burchard test	-
	Liebermann test	-
Glycosides	Keller- killiani test	+
	Borntrager's test	+
	Modified borntrager's test	-
	Mollish's test	+
	Fehling's test	+
	Bendict's test	-

Carbohydrates	Barford's test	+
	Bial's test	-
	Selwinoff's test	+
	Tollen test	+
	Iodine test	-
	Tannic acid test	+

Table 9: Phytochemical tests for detection of organic constituents present in Liquorice

Chemical class	Tests	Inference
Alkaloids	Mayer's test	+
	Wagner's test	+
	Hager's test	-
	Dragenderoff's test	+
Steroids	Liebermann's burchard test	-
	Liebermann's test	-
Glycosides	Keller- killiani test	+
	Borntrager's test	+
	Modified borntrager's test	+
Carbohydrates	Mollish's test	-
	Fehling's test	+
	Bendict's test	+
	Barford's test	+
	Bial's test	-
	Selwinoff's test	+
	Tollen test	+
	Iodine test	-
	Tannic acid test	+

Table 10: Phytochemical tests for detection of organic constituents present in Turmeric

Chemical class	Tests	Inference
Alkaloids	Mayer's test	-
	Wagner's test	+
	Hager's test	-
	Dragenderoff's test	-
Steroids	Liebermann burchard test	+
	Liebermann test	-

Glycosides	Keller- killiani test	-
	Borntrager's test	-
	Modified borntrager's test	-
Carbohydrates	Mollish's test	+
	Fehling's test	+
	Bendict's test	+
	Barford's test	-
	Bial's test	-
	Selwinoff's test	+
	Tollen test	+
	Iodine test	+
	Tannic acid test	+

4.3 Formulation and Development

4.3.1 HPLC analysis of Harad, Neem, Liquorice and Turmeric

4.3.1.1 Chromatogram of Gallic acid present in Harad

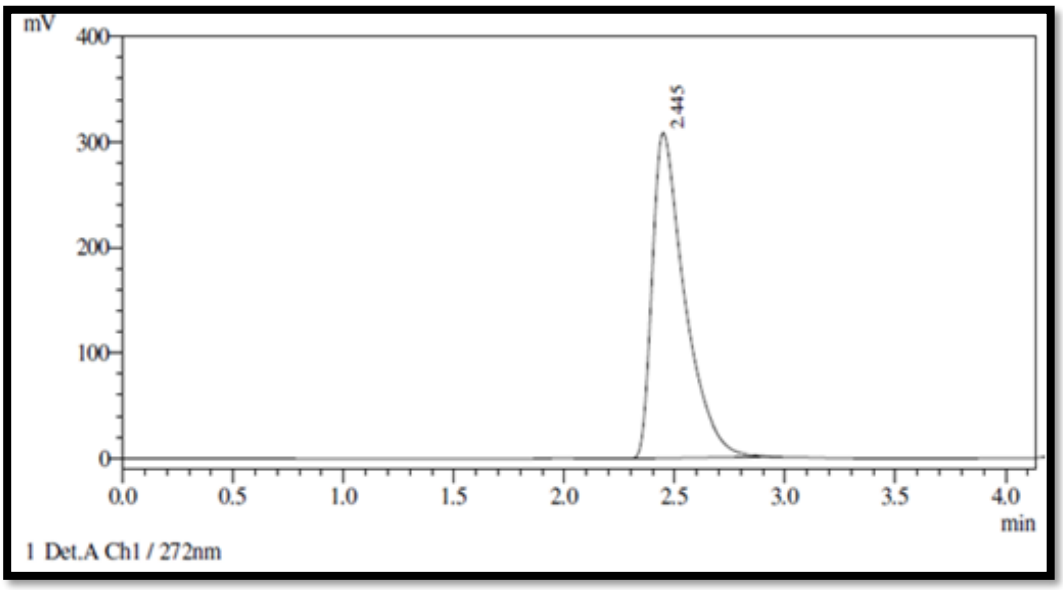

Figure 1. HPLC Chromatogram of Gallic acid present in Harad

4.3.1.2 Standard curve of Gallic acid present in Harad

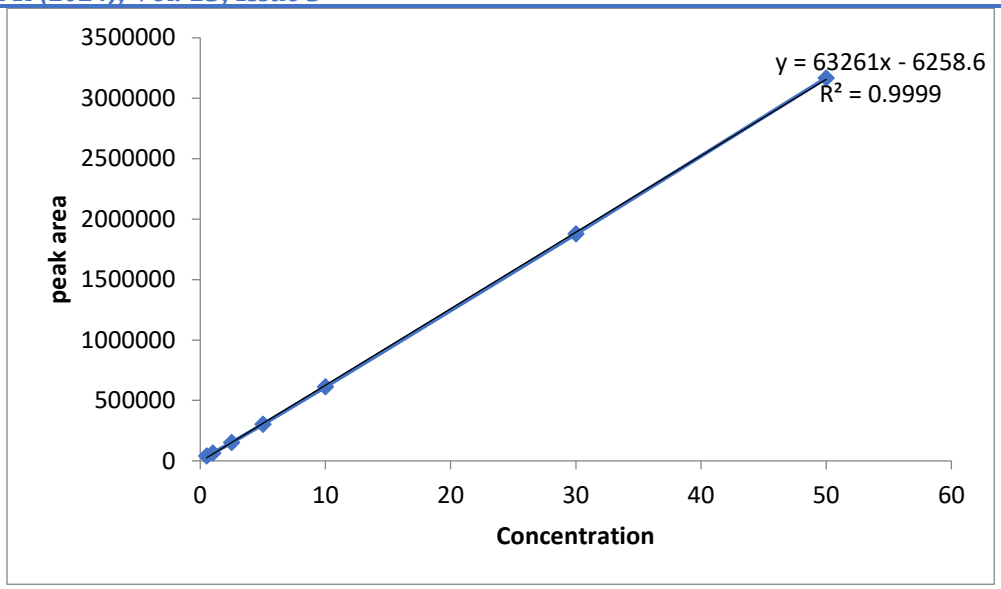

Figure 2: Standard curve of Gallic acid present in Harad

4.3.1.3 Chromatogram of Azadirachtine present in Neem

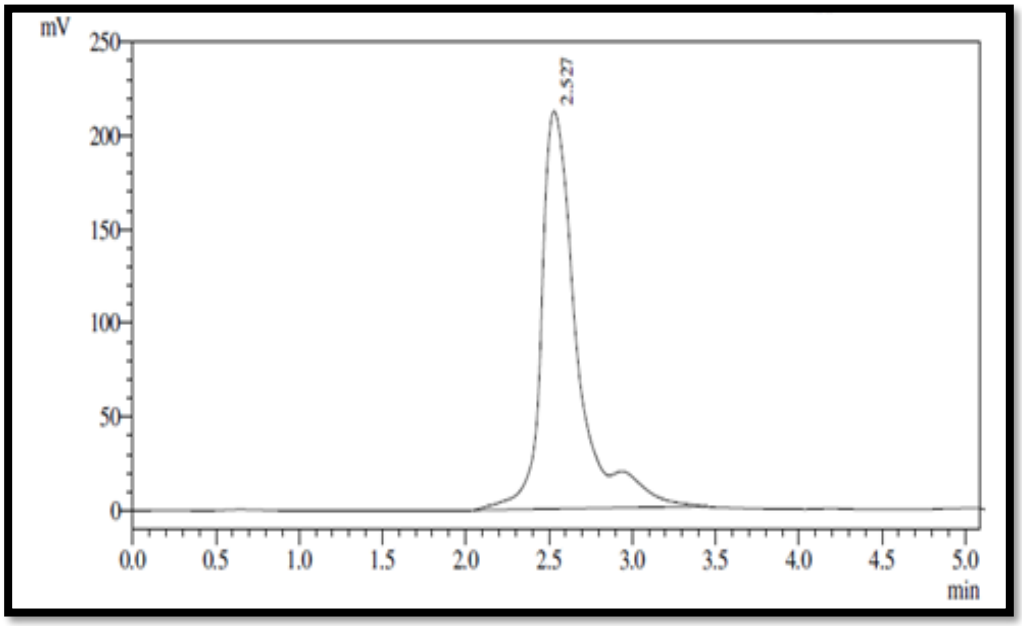

Figure 3: HPLC chromatogram of Azadirachtine present in Neem

4.3.1.4 Standard curve of Azadirachtine present in Neem (Azadirachta indica):

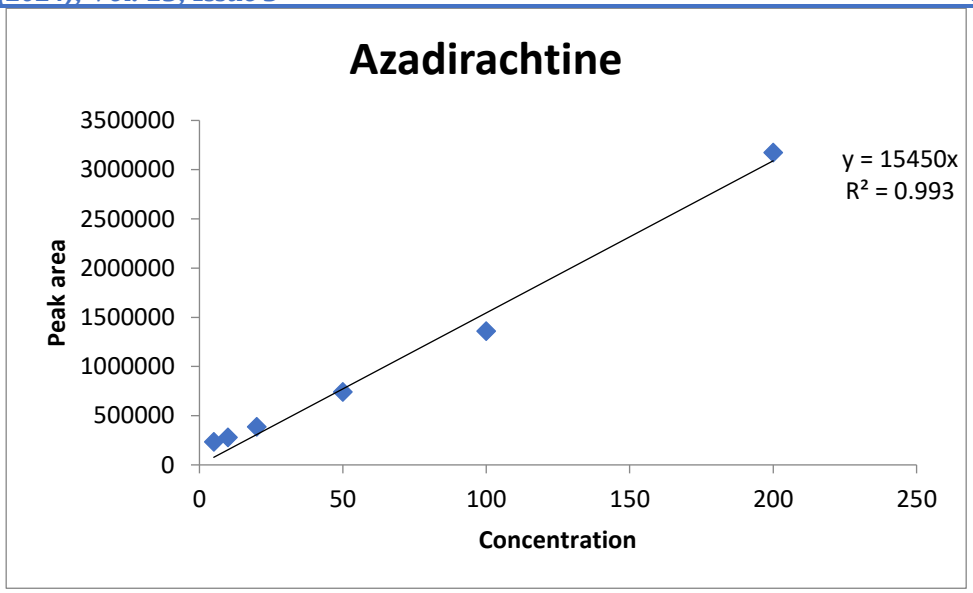

Figure 4: Standard curve of Azadirachtine present in Neem

4.3.1.5 Chromatogram of Glycyrrhizic acid present in Liquorice

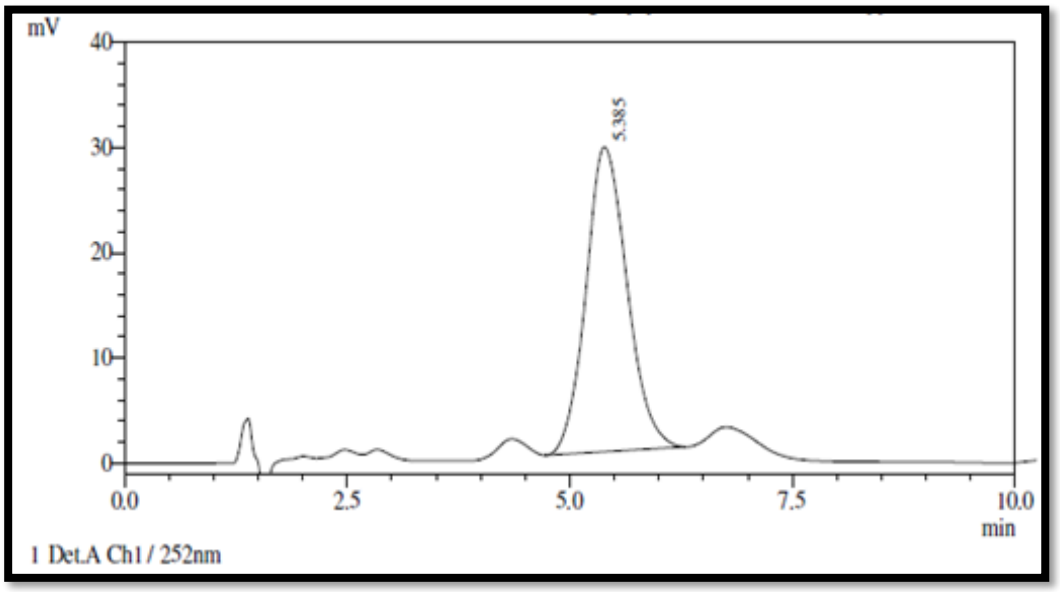

Figure 5: HPLC chromatogram of Glycyrrhizic acid present in Liquorice

4.3.1.6 Standard curve of Glycyrrhizic acid present in Liquorice

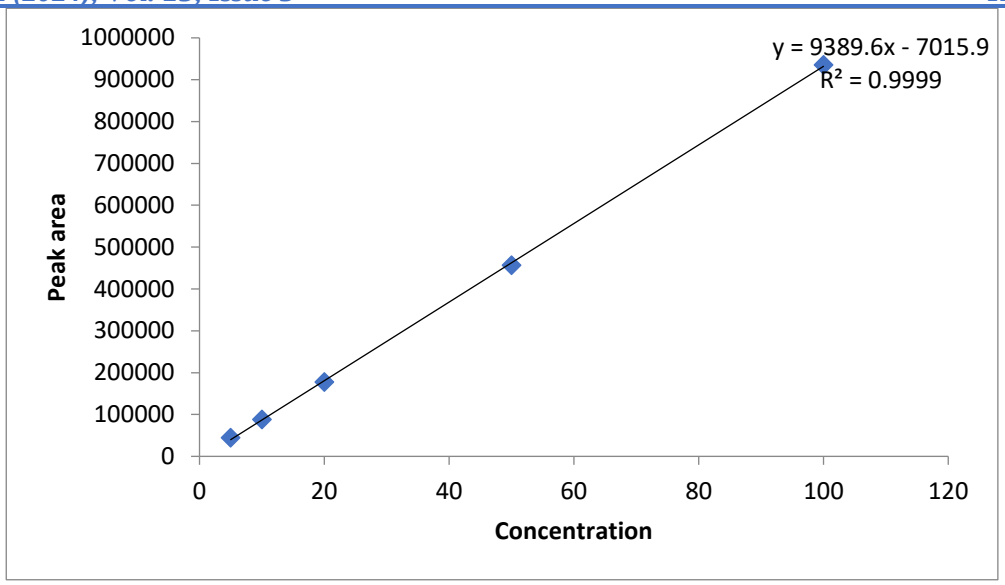

Figure 6: HPLC standard curve of Glycyrrhizic acid present in Liquorice

4.3.1.7 Chromatogram of Turmerone present in Turmeric

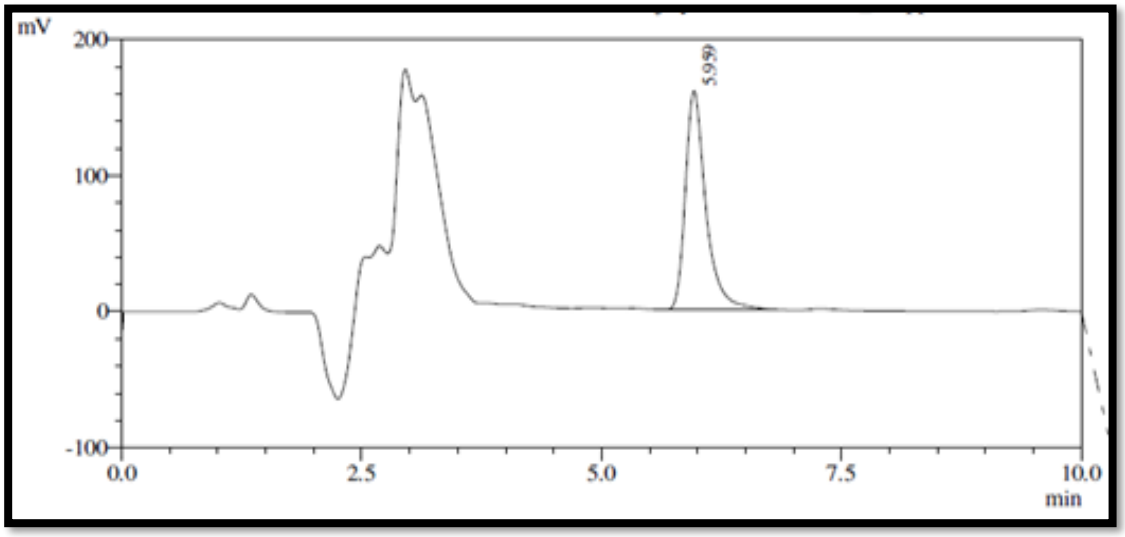

Figure 7: HPLC chromatogram of Turmerone in Turmeric

4.3.1.8 Standard curve of Turmerone in Turmeric

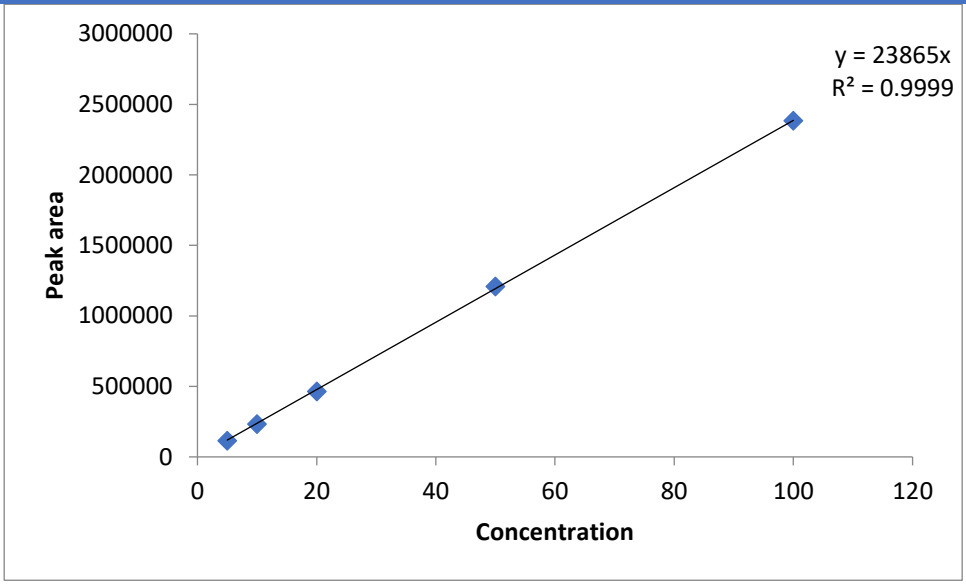

Figure 8: Standard curve of Turmerone in Turmeric

4.4 Evaluation Parameters of Polyherbal gel

4.4.1 Evaluation of Polyherbal gel formulation:

Polyherbal gel thus prepared was subjected to various evaluation parameters such as; clarity, pH, spreadability, viscosity revealed that prepared formulation showed good results and considered as best gel.

a) **Clarity:** Prepared gel was clear.
b) **pH:** pH of the formulation was found to be 6.8 which was similar to salivary pH.
c) **Appearance and Homogeneity:** Formulation was smooth in appearance and had very good homogeneity.
d) **Viscosity:** Viscosity of gel was found to be 6875 cps.
e) **Spreadability:** Spreadability was found to be 9.5 gm.cm/sec.

4.4.2 Release kinetics study:

In vitro release data obtained with each drug was fitted into various kinetic models like Zero order, First order, Higuchi, Korsmeyer- peppas model in order to find out the mechanism of drug release from polyherbal gel.

4.4.2.1 Release kinetic data of Harad

Table 11: Estimated value of R^2 after fitting of dissolution data of polyherbal gel into various release kinetic model in pH 6.8 phosphate buffer

Formulation	Zero order		First order		Higuchi		Korsemeyer peppas	
pH 6.8 phosphate buffer								
Polyherbal gel	Y	R^2	Y	R^2	Y	R^2	Y	R^2
	0.055	0.961	0.001	0.714	0.028	0.934	0.611	0.894

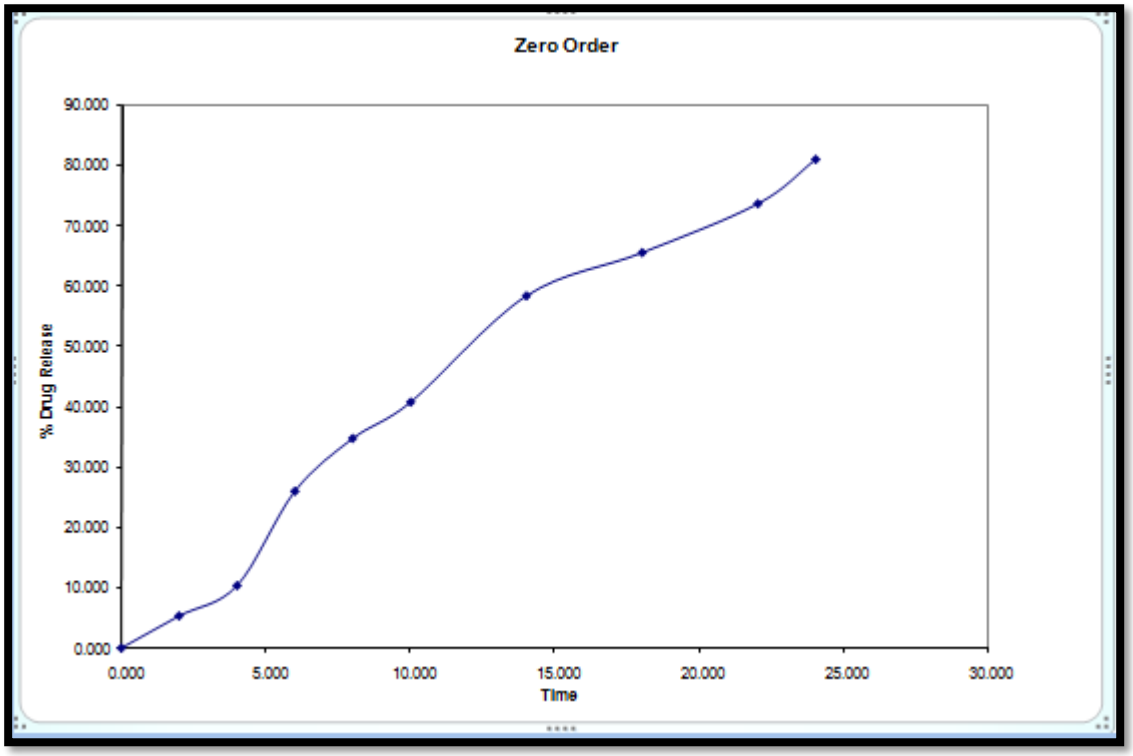

Figure 9: Zero order drug release profile of Gallic acid (Harad) from polyherbal gel in pH 6.8 phosphate buffer

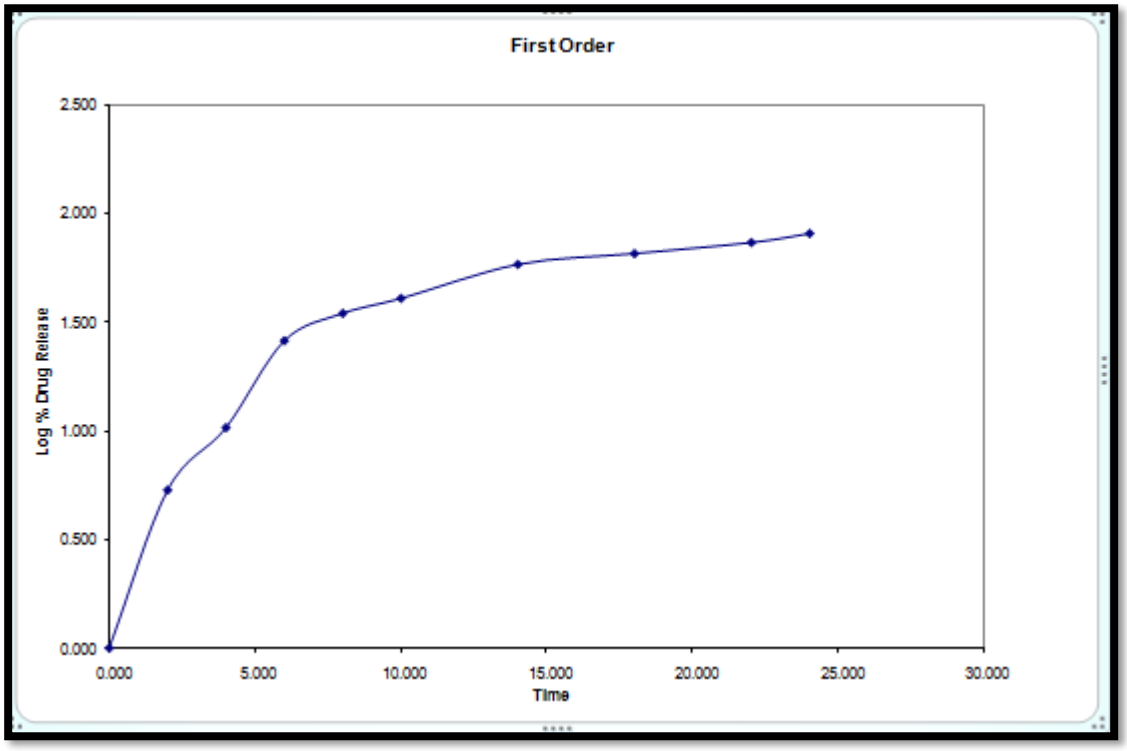

Figure 10: First order drug release profile of Gallic acid (Harad) from polyherbal gel in pH 6.8 phosphate buffer

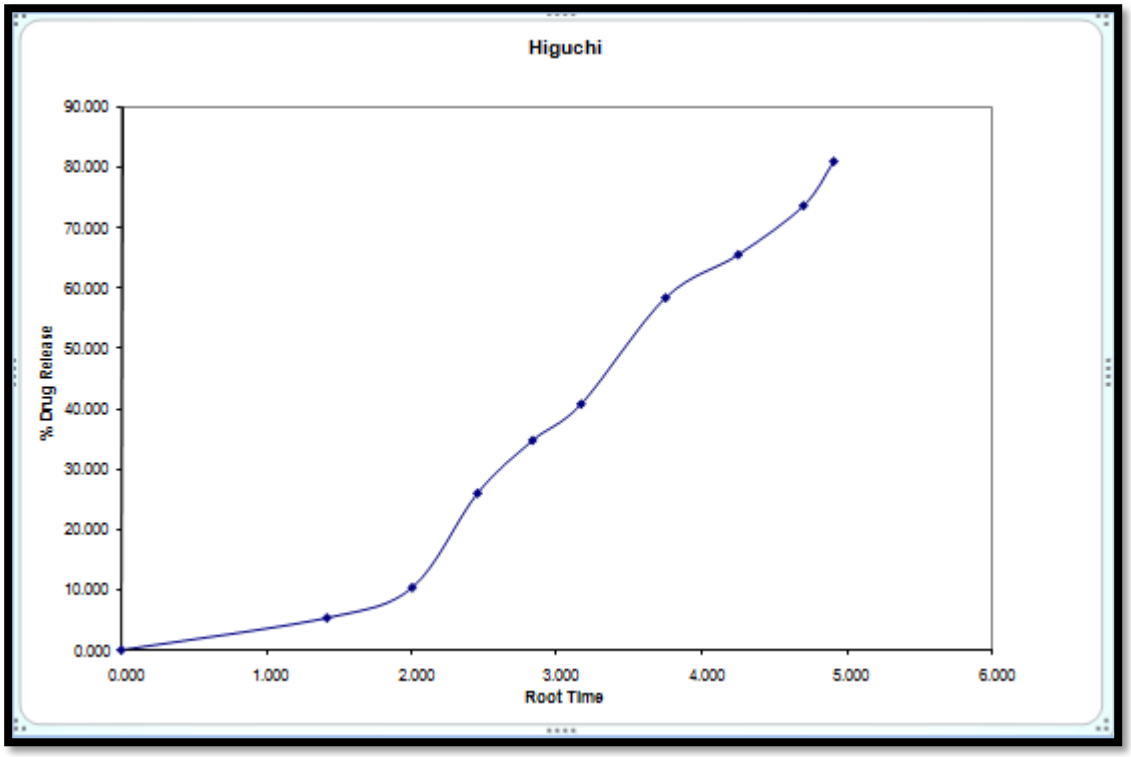

Figure 11: Higuchi drug release profile of Gallic acid (Harad) from polyherbal gel in pH 6.8 phosphate buffer

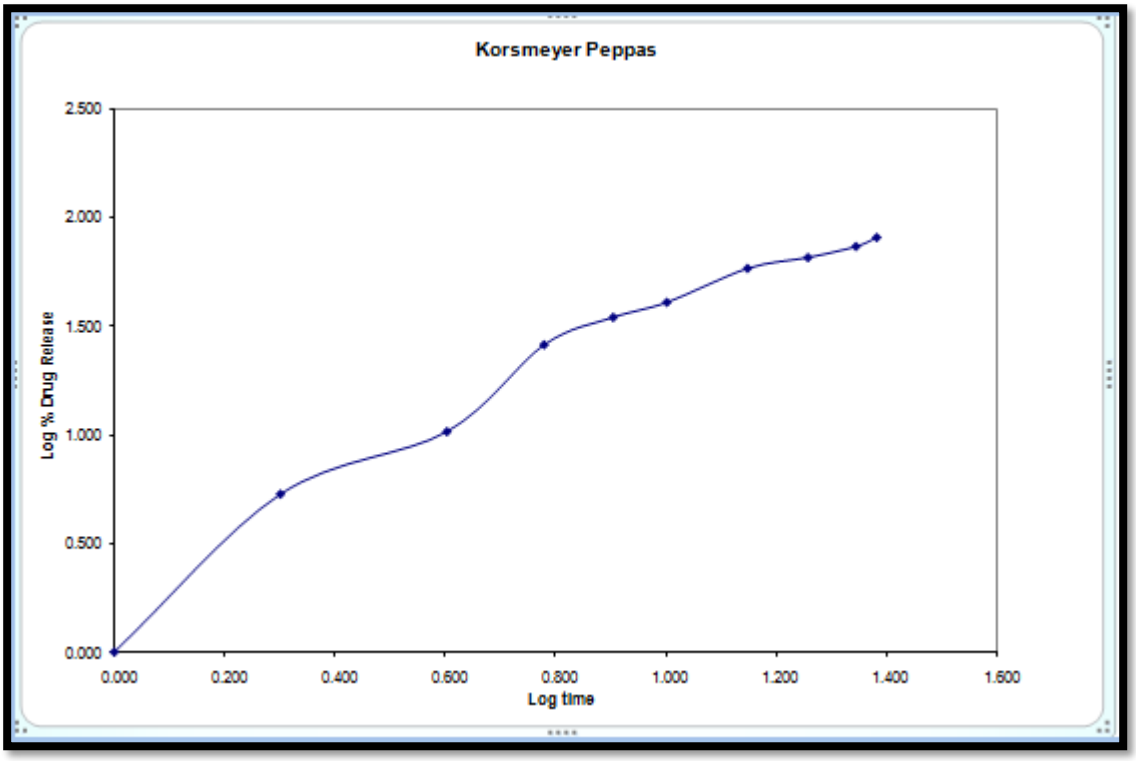

Figure 12: Korsmeyer peppas drug release profile of Gallic acid (Harad) from polyherbal gel in pH 6.8 phosphate buffer

4.4.2.2 Release kinetics data of Neem

Table 12: Estimated value of R^2 after fitting of dissolution data of polyherbal gel into various release kinetic model in pH 6.8 phosphate buffer

Formulation	**Zero order**		**First order**		**Higuchi**		**Korsemeyer peppas**	
pH 6.8 phosphate buffer								
Polyherbal gel	**Y**	**R^2**	**Y**	**R^2**	**Y**	**R^2**	**Y**	**R^2**
	0.058	0.997	0.001	0.708	0.059	0.886	0.565	0.909

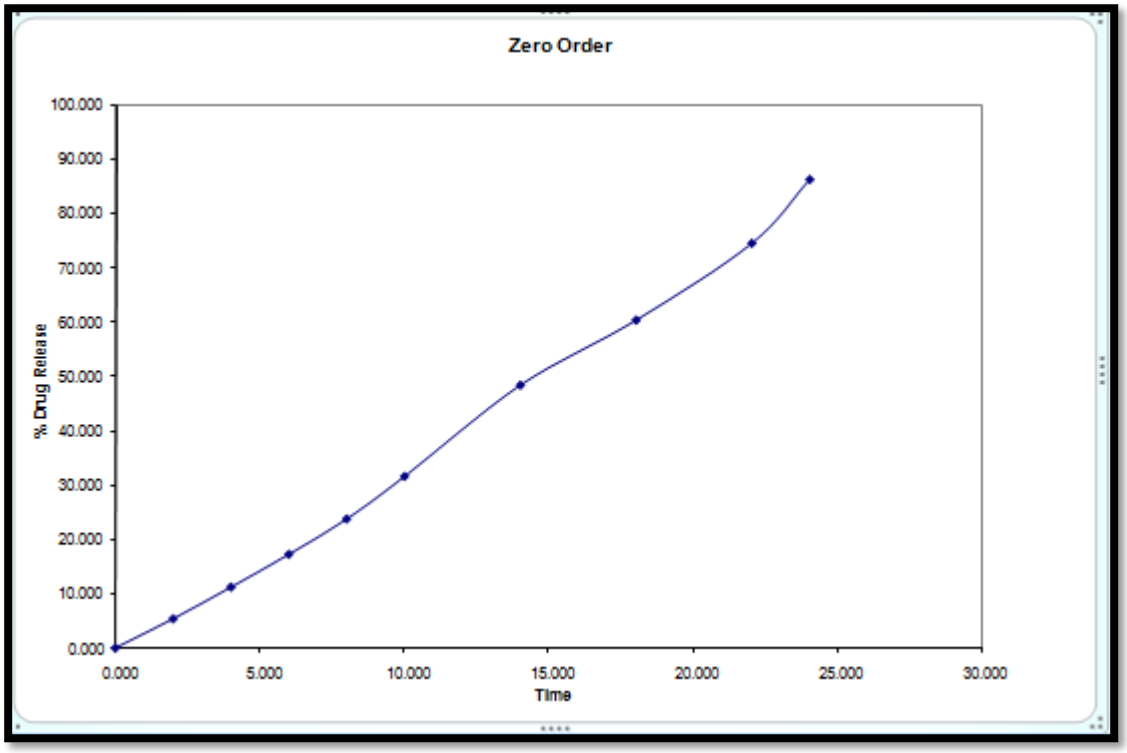

Figure 13: Zero order drug release profile of Azadirachtine (Neem) from polyherbal gel in pH 6.8 phosphate buffer

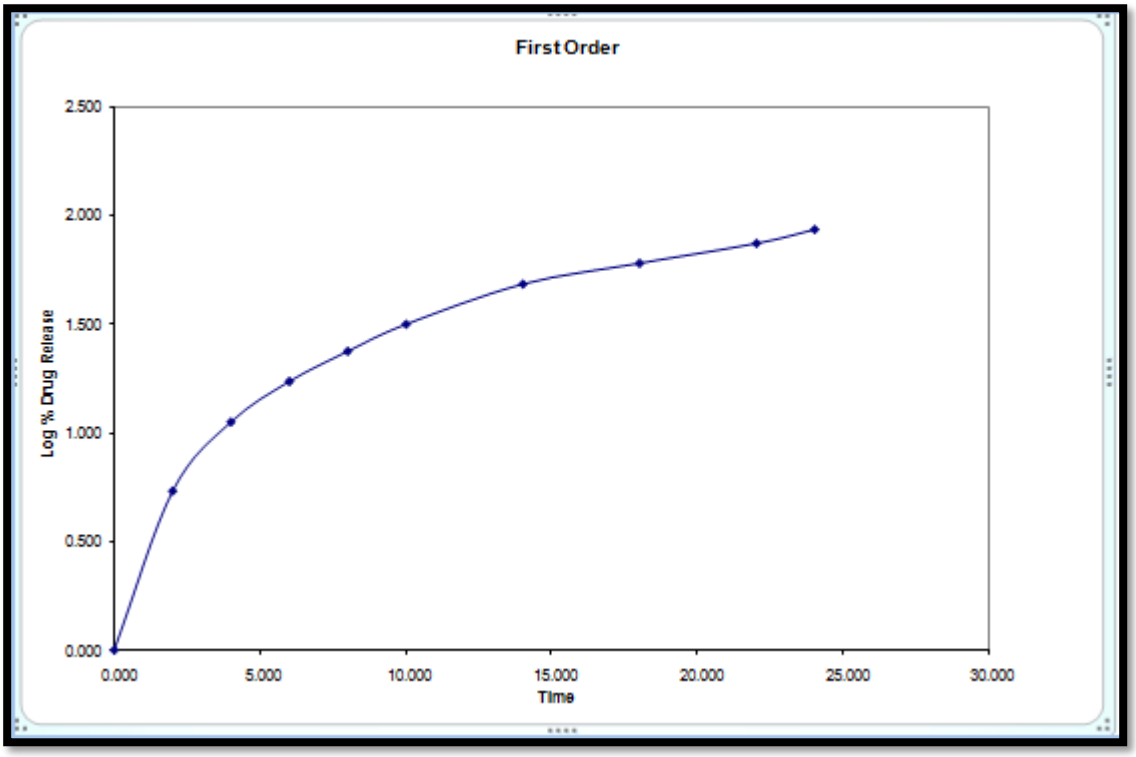

Figure 14: First order drug release profile of Azadirachtine (Neem) from polyherbal gel in pH 6.8 phosphate buffer

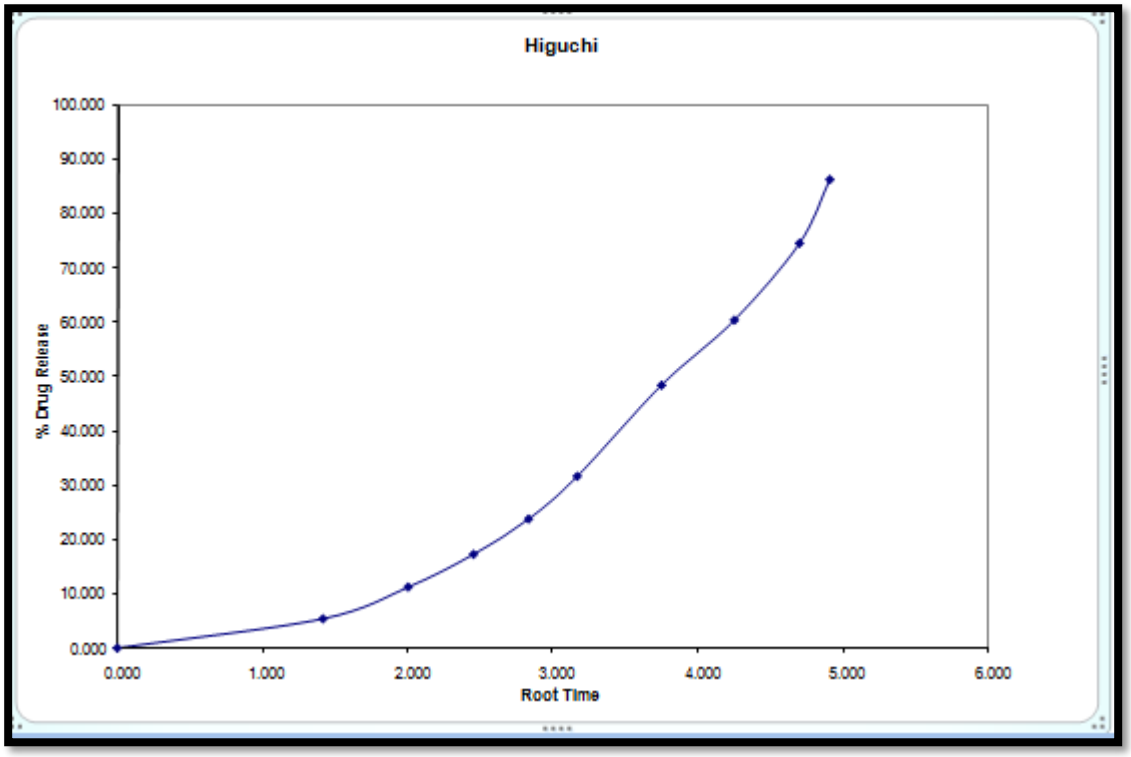

Figure 15: Higuchi drug release profile of Azadirachtine (Neem) from polyherbal gel in pH 6.8 phosphate buffer

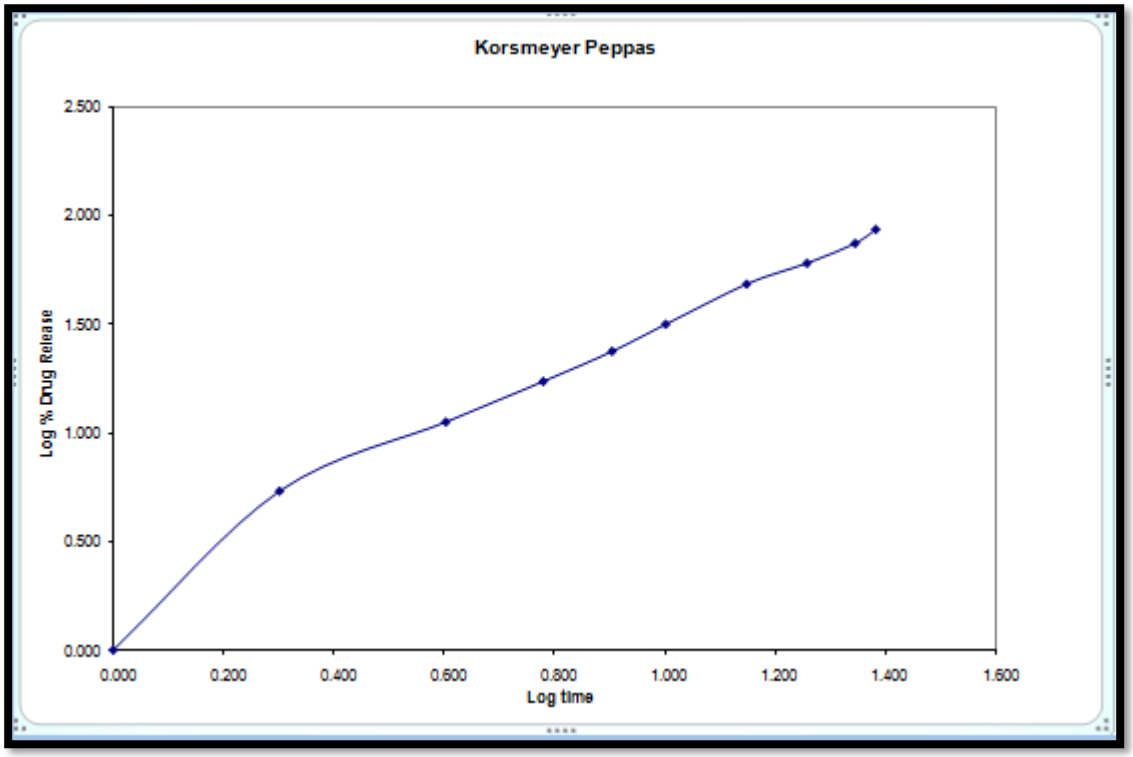

Figure 16: Korsmeyer peppas drug release profile of Azadirachtine (Neem) from polyherbal gel in pH 6.8 phosphate buffer

4.4.2.3 Release kinetic data of Liquorice:

Table 13: Estimated value of R^2 after fitting of dissolution data of polyherbal gel into various release kinetic model in pH 6.8 phosphate buffer

Formulation	Zero order		First order		Higuchi		Korsemeyer peppas	
pH 6.8 phosphate buffer								
Polyherbal gel	Y	R^2	Y	R^2	Y	R^2	Y	R^2
	0.056	0.988	0.001	0.693	0.021	0.940	0.589	0.955

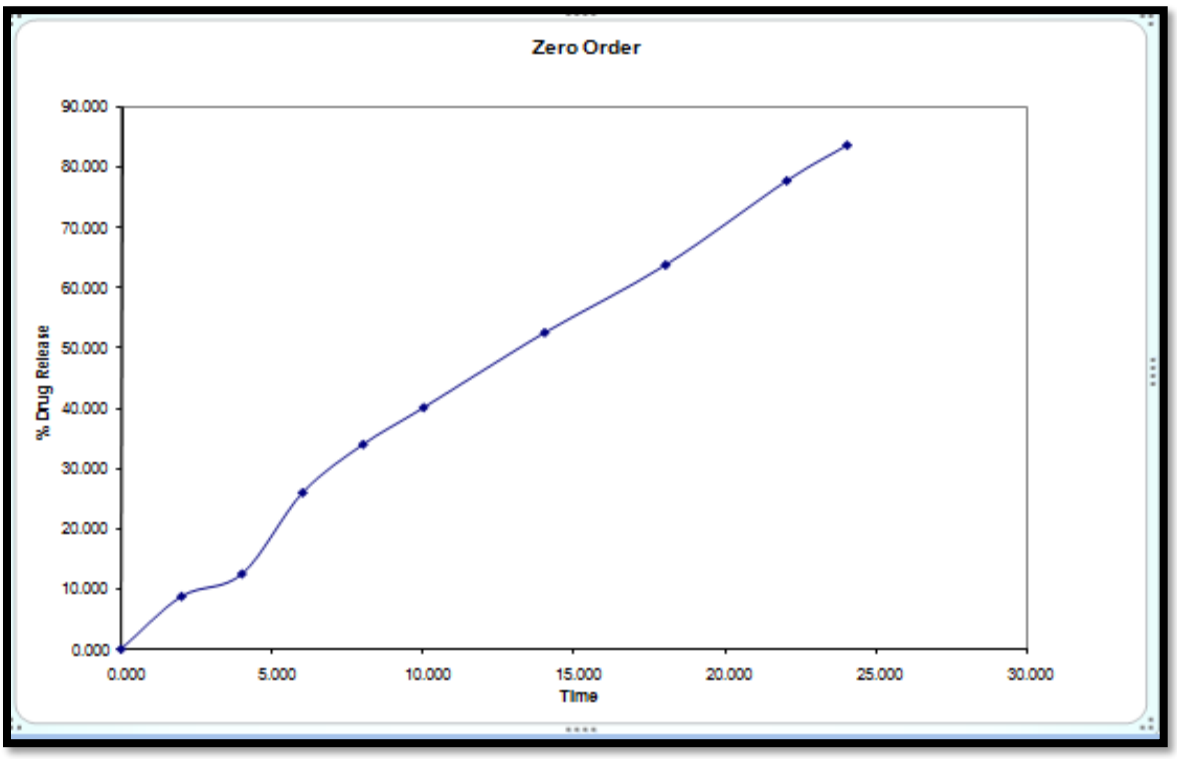

Figure 17: Zero order drug release profile of Glycyrrhizic acid (Liquorice) from polyherbal gel in pH 6.8 phosphate buffer

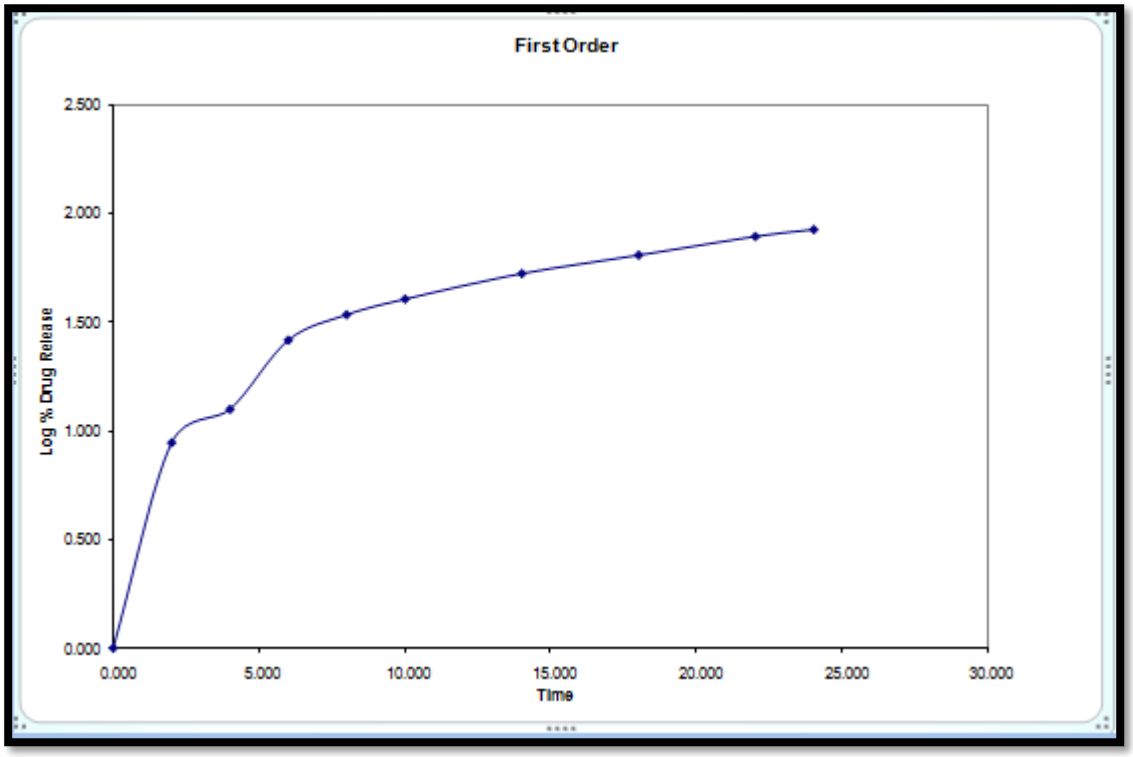

Figure 18: First order drug release profile of Glycyrrhizic acid (Liquorice) from polyherbal gel in pH 6.8 phosphate buffer

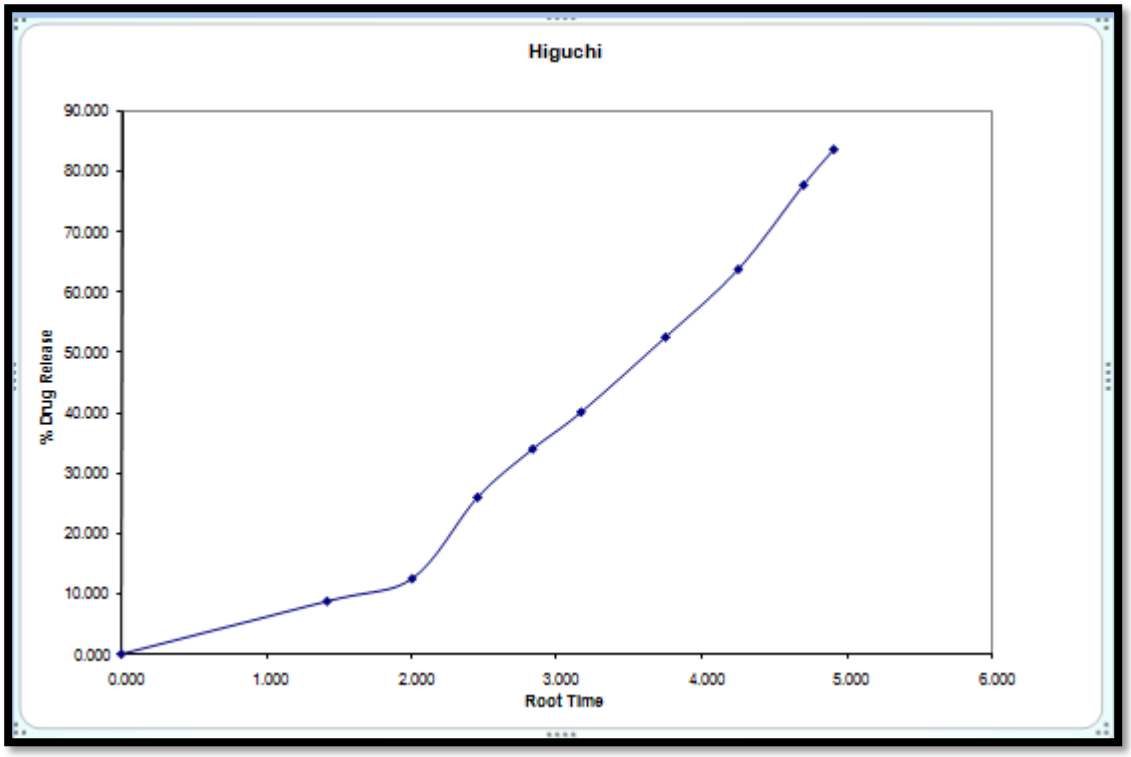

Figure 19: Higuchi drug release profile of Glycyrrhizic acid (Liquorice) from polyherbal gel in pH 6.8 phosphate buffer

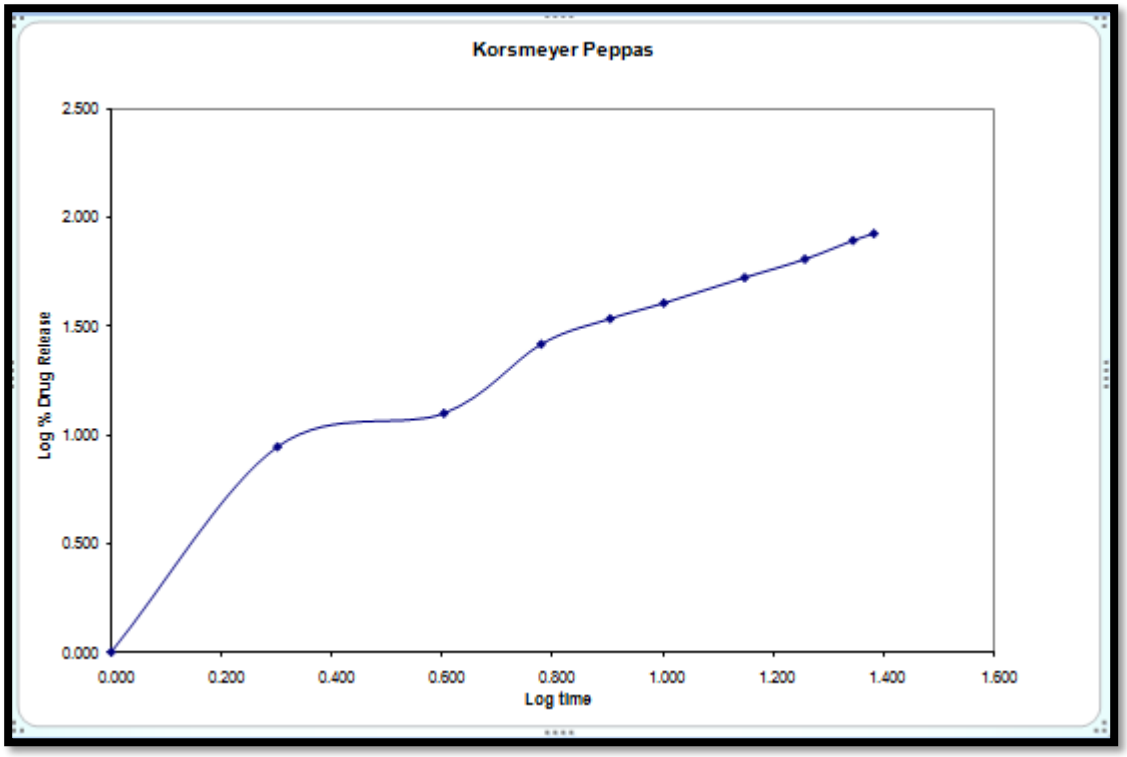

Figure 20: Korsmayer peppas drug release profile of Glycyrrhizic acid (Liquorice) from polyherbal gel in pH 6.8 phosphate buffer

4.4.2.4 Release kinetics data of Turmeric-

Table 14: Estimated value of R^2 after fitting of dissolution data polyherbal gel various release kinetic model in pH 6.8 phosphate buffer

Formulation	Zero order		First order		Higuchi		Korsemeyer peppas	
pH 6.8 phosphate buffer								
Polyherbal gel	Y	R^2	Y	R^2	Y	R^2	Y	R^2
	0.123	0.955	0.002	0.680	0.002	0.934	0.684	0.954

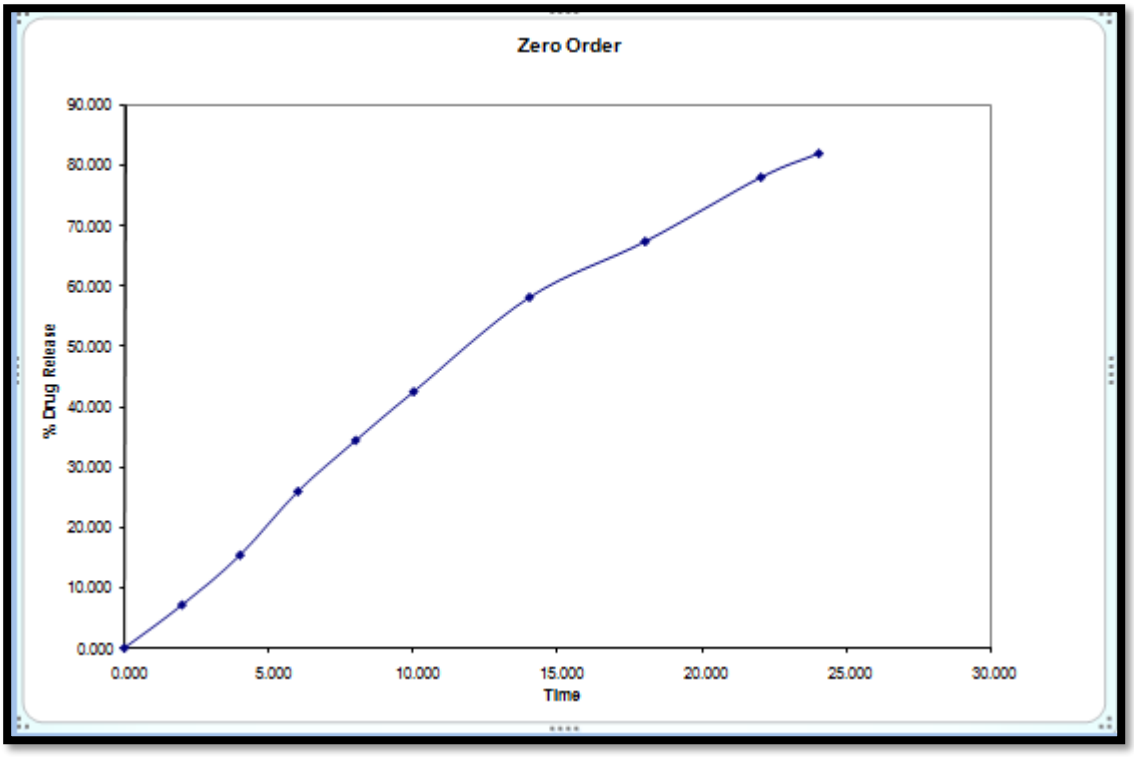

Figure 21: Zero order drug release profile of Turmerone (Turmeric) from polyherbal gel in pH 6.8 phosphate buffer

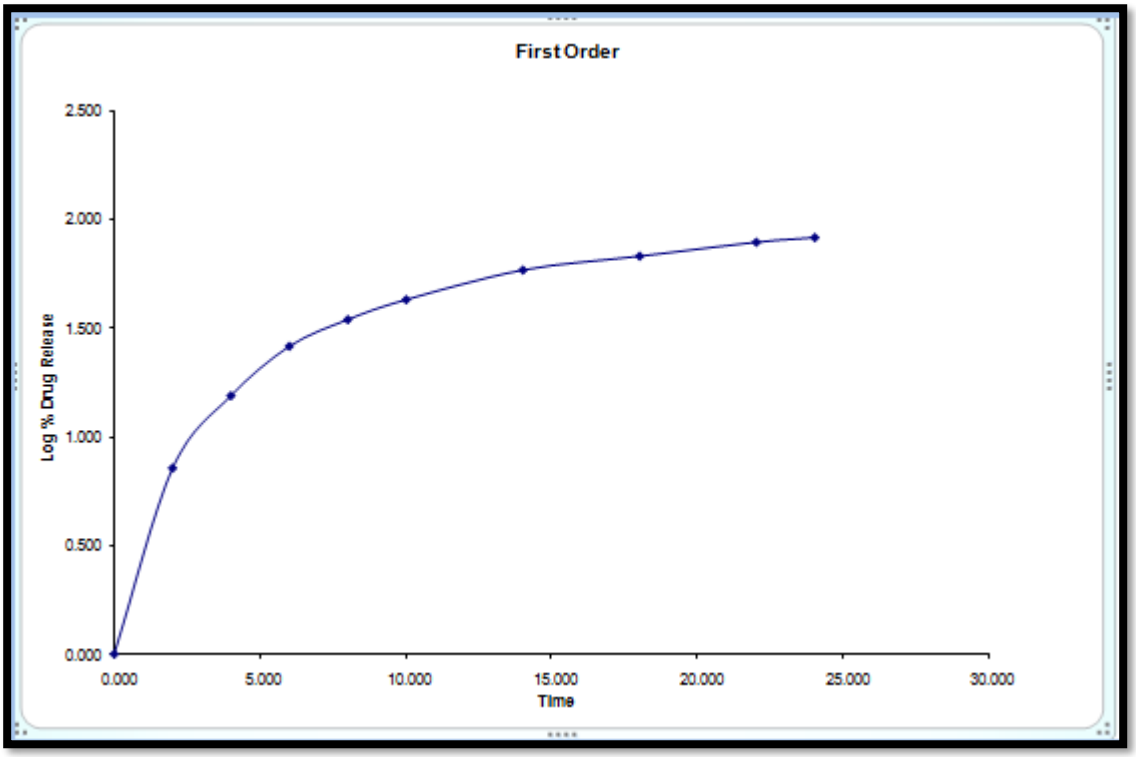

Figure 22: First order drug release profile of Turmerone (Turmeric) from polyherbal gel in pH 6.8 phosphate buffer

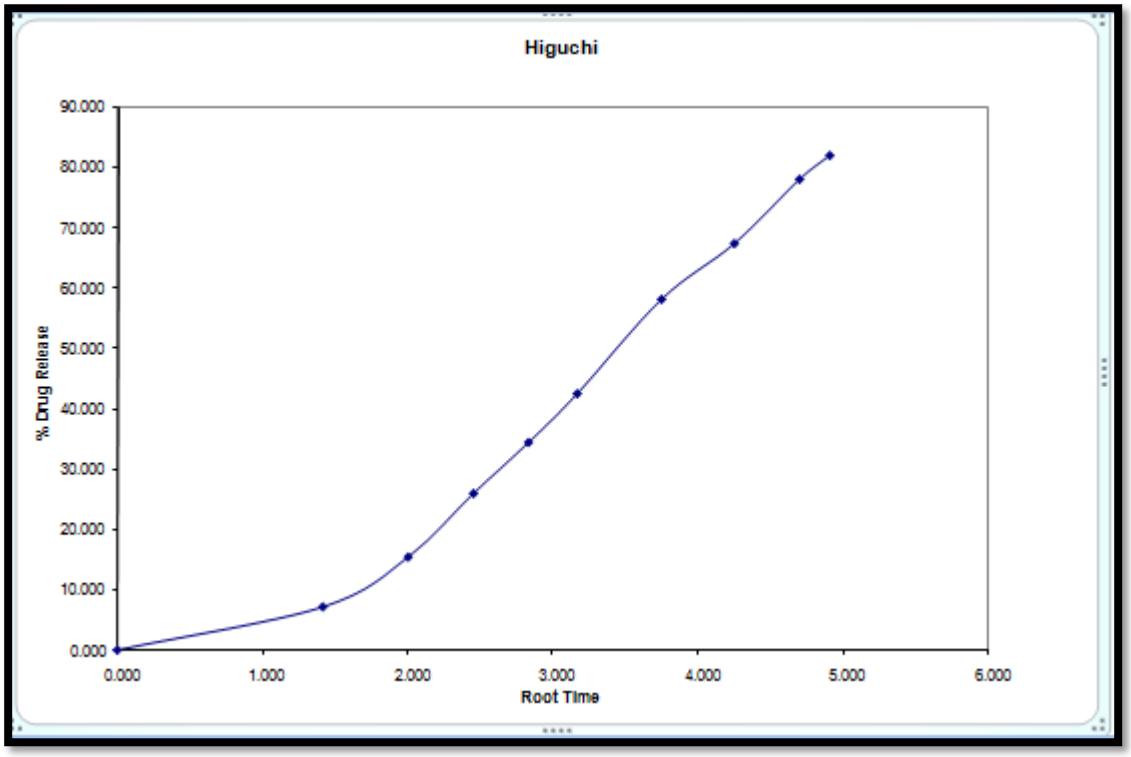

Figure 23: Higuchi drug release profile of of Turmerone (Turmeric) from polyherbal gel in pH 6.8 phosphate buffer

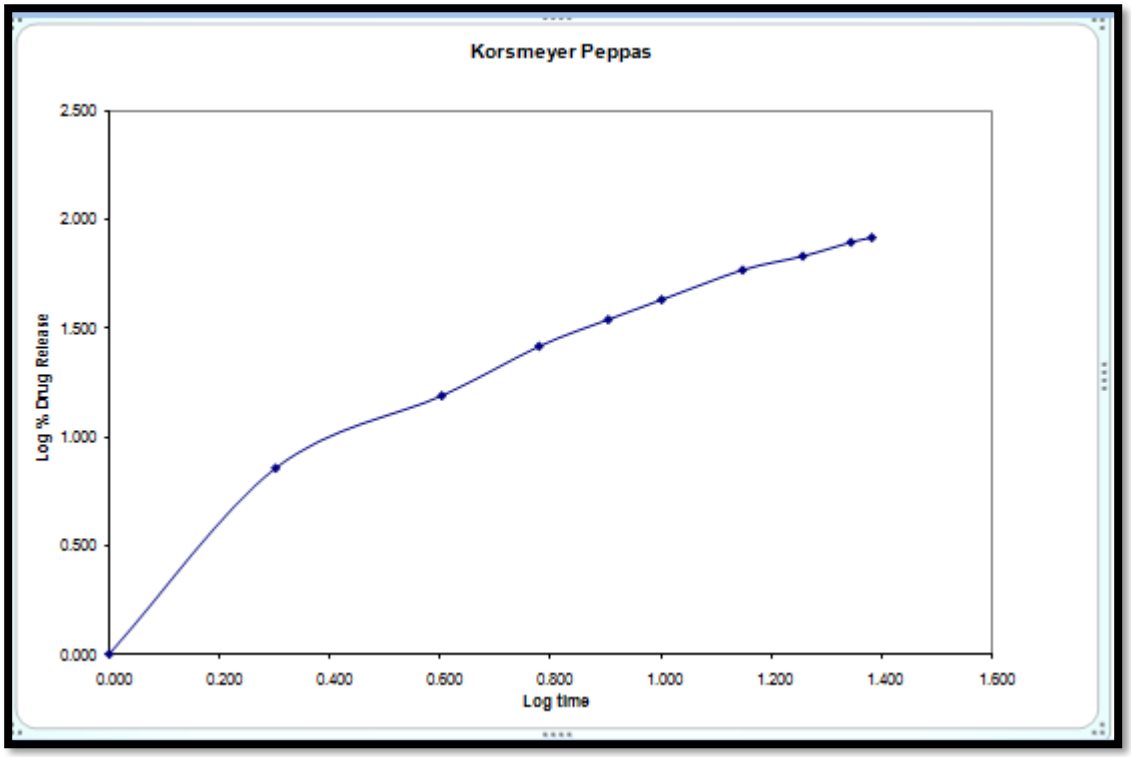

Figure 24: Korsmeyer peppas drug release profile of of Turmerone (Turmeric) from gel in pH 6.8 phosphate buffer

5. Conclusion

The present worker carried out the research work aiming at increasing the residence time of drug at the site of application and preventing severe side effects associated with its topical administration. Considering the local healing the researchers designed an inexpensive formulation for the treatment of mouth ulcers as herbal formulations have growing demand in the world market. In the present work, a good attempt has been made to establish the polyherbal gel consisting of Harad, Neem, Liquorice and Turmeric extracts. Initially, aqueous extracts of Harad, Neem, Liquorice and Turmeric were evaluated for various pharmacognostical parameters. The aq. Extract & of each plants was lyophilized (60°C) to get powdered forms. The drug content was estimated by HPLC analysis for testing the compatibility of drug with selected excipients used in the formulation. From the outcomes it is concluded that the, Harad, Neem, Liquorice and Turmeric are compatible with the excipients. The prepared gel was characterized for various parameters i.e; Physical appearance, viscosity, spreadability, HPLC, and kinetics release studies. Release data obtained with prepared polyherbal gel was fitted into various kinetics models i.e. Zero & First order, Higuchi and Koresemeyer-peppa models in order to find out the mechanism of drug release from the gel which confirmed zero order kinetics. Finally, it was concluded that such novel preparations would be beneficial in the treatment of Mouth ulcer and applicable to other ailments pertaining to the presence of such herbal drug of different characteristics.

6. Conflict of interest

The authors have no conflict of interest.

7. Acknowledgement

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