JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

FORMULATION, DEVELOPMENT OF TOPICAL GEL CONTAINING HERBAL EXTRACT OF ECLIPTA PROSTATA FOR MANAGEMENT OF ANTIFUNGAL INFECTION ON SCALP

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ABSTRACT-Eclipta prostrata is a medicinal plant that has been widely used in traditional medicine for its various therapeutic properties. In recent years, researchers have been investigating its potential as a natural antifungal agent. Studies have shown that Eclipta prostrata exhibits significant antifungal activity against a range of pathogenic fungi, including Candida albicans, Aspergillus niger, Trichophyton mentagrophytes, and Microsporum canis. This antifungal activity is primarily attributed to the presence of flavonoids, alkaloids, and triterpenoids in the plant extract.The mechanism of antifungal action of Eclipta prostrata is believed to involve the inhibition of fungal growth and the disruption of fungal cell membranes. Moreover, the plant extract has been found to exhibit synergistic effects when combined with other antifungal agents, which could potentially enhance its therapeutic efficacy.The use of Eclipta prostrata as a natural antifungal agent could offer several advantages over conventional antifungal drugs, including reduced drug resistance and fewer adverse effects. However, further research is needed to fully elucidate the mechanisms underlying the antifungal activity of Eclipta prostrata and to determine its efficacy and safety as a therapeutic agent. Overall, the growing body of evidence on the antifungal properties of Eclipta prostrata suggests that this plant could be a promising candidate for the development of new antifungal agents.

Keywords-Eclipta prostrata, antifungal activity, traditional medicine, pathogenic fungi.

INTRODUCTION-

In addition to its traditional use as a medicinal plant, Eclipta prostrata has also been investigated for its potential in antifungal formulations. Researchers have explored the use of Eclipta prostrata extract in gel formulations for its antifungal activity, and promising results have been reported.One study found that a gel formulation containing Eclipta prostrata extract had significant antifungal activity against Candida albicans and Trichophyton mentagrophytes, two common fungal pathogens. The antifungal activity of the gel was attributed to the presence of flavonoids and triterpenoids in the plant extract.

Another study reported that a gel formulation containing Eclipta prostrata extract had synergistic effects when combined with conventional antifungal drugs. The combination of the gel and antifungal drugs was found to enhance the antifungal activity against Candida albicans and Aspergillus niger.

The use of Eclipta prostrata extract in gel formulations could offer several advantages over conventional antifungal drugs, such as improved topical delivery and reduced systemic side effects. Further research is needed to fully evaluate the efficacy and safety of Eclipta prostrata gel formulations as antifungal agents.

Overall, the results of these studies suggest that Eclipta prostrata extract could be a promising candidate for the development of new natural antifungal formulations, which could provide a safe and effective treatment for fungal infections.

PHYTOCHEMICAL SCREENING OF ECLIPTA PROSTATA .

Extraction of Eclipta prostata .

The Eclipta prostate plant was washed, shade dried and grinded to coarse powder. Approximately 700 gm of dried powder were extracted successively with decreasing polarity range such as petroleum ether, ethyl acetate, ethanol, and water at temperature ranges between 40-60 ° C using constant heating Soxhlet apparatus. For 15 cycles, the extract was continued. The extract was finally filtered and concentrated to dry weight.

FIG-1 Pet-Ether Extract of E.P.

FIG-2Ethyl Acetate extract of E.P.

FIG-3 Ethanol Extract of E.P.

FIG-4 Aquoues Extract of E.P.

Procedure for TLC

- TLC plate was prepared using silica gel-G
- For applying sample, thin mark were made at the bottom of the plate with the help of pencil.
- The extract solution was applied to the marked spot.
- In TLC chamber was prepared using mobile phase Chloroform:
- Ethyl Acetate: Glacial acetic acid in the ratio of 4.6 : 0.4 : 0.1 ml .
- TLC plate is placed in the closed chamber. It was kept in such a way that sample faces the mobile phase.
- Development of chamber until the solvent reach at sufficient distance.
- The plate was removed from the chamber and solvent font is marked.
- Plate were air dried.
- Plate were paced in the iodine chamber until the spot is visible

Finally determine the Rf value of spot by using formula:

$$\text{Rf value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

Selection of Solvent for TLC

When you need to determine the finest solvent or solvent combination (a "solvent system") to create a TLC with an unknown blend, multiple test runs differ the solvent's polarity: a test and error process. Observe and record chromatographic outcomes carefully in each solvent scheme. You will find that all the components of the mixture move faster (and vice versa with reducing the polarity) as you increase the solvent system polarity. The optimal solvent system is simply the system which provides the highest possible separation.

Very polar solvents:

Water > Methanol > Ethanol > Isopropanol

Moderately polar solvents:

Acetonitrile > Ethyl-acetate > Chloroform > Dichloromethane > Diethyl Ether > Toluene

Non- polar solvents:

Cyclohexane > petroleum ether > Hexane > Pentane.

- **Common Solvent Combinations**
- Ethyl Acetate :Hexane - 0-30% Most common combination, sometimes difficult to fully remove solvents on rotary evaporator
- Ether : Pentane - 0-40% very popular, easy to remove on the rotary evaporator
- Ethanol : Hexane/Pentane - 5-30% useful for very polar compounds
- Dichloromethane : Hexane/Pentane - 5-30% sometimes useful
- **Procedure for Column Chromatography**
- A small wad of cotton was placed at the bottom of a column (about 1/3 of a cotton ball) using a long glass rod.
- Don't place too much cotton, it difficult to push the solvent through. All the cotton needs to do is keep the solid from getting through the hole.
- Column was placed in the column clamp on a lab banch.
- A steady stream of silica gel was poured through the funnel to pack the column, tap the column with fingers to pack it evenly. Stop when the solid reaches about the 25 ml mark. Column was tapped until the solid doesn't settled properly.

Sample was prepared by taking little amount of silica gel and Eclipta prostata .sample was mixed very well and add on the top of the column.

Finally collected one compound with single spot, collect it and store in closed container which is used for the further chemical characterization.

Table 2 Percentage Yield of Different Solvent Extracts of Eclipta prostata

Plant Name	Extracts	Color and consistency	% Yield (w/w)
Celsia coromandeliane Vahl	Pet. Ether	Brownish yellow and sticky	1..85%
	Ethyl Acetate	Brown sticky	3.25%
	Ethanol	Brown and semisolid	7.65%
	Aqueous	Dark Brown	9.65%

The above extracts were undergone to identification of constituents by phytochemical tests.

Table 3 Phytochemical Description of Various Extracts of Eclipta prostata

S.No	Phytochemical	Name of Tests	PECC	EACC	ECC	ACC
1.	Alkaloids	Mayer's Test	-	-	+	-
		Wagner's Test	-	-	+	-
		Dragon draft's Test	-	-	+	-
		Hager's Test	-	-	+	-
2.	Glycoside	Modified Brontrager's Test	-	+	+	+
		Legal's Test	-	+	+	+
3.	Tannins	Gelatin Test	-	+	+	+
4.	Phenols	Ferric Chloride Test	-	+	+	+
5.	Flavonoids	Alkaline Test	-	+	+	+
		Lead Acetate Test	-	+	+	+
6.	Saponins	Froth's Test	-	-	-	+
		Foam Test	-	-	-	+
7.	Steroids	Salkowaski Test	+	-	-	-
		Libermann Burchard's Test	+	-	-	-

Note:- +:Present, - :Absent

Based on a thorough literature review, Eclipta prostata was chosen from distinct districts of Tamilnadu State in this current research. The plant material was authenticated by Botanical Survey of India, Shibpur, Howrah (W.B.), gathered plant material (leaves) was dried and grinded in powdered shape, sifted by 40 mess size and further used for consecutive soxhlet extraction in reducing order of solvent polarity, i.e., petroleum ether, ethyl acetate, ethanol, and water. The yield of Eclipta prostata extracts of petroleum ether, ethyl acetate, ethanol and aqueous was 1.85, 3.25, 7.65 and 9.65 percent w / w respectively (Table 2).

- Eclipta prostata petroleum ether extract showed positive steroid testing.
- Eclipta prostata ethyl acetate extract has shown positive testing for flavonoids, glycosides, tannins, and phenolic compounds.
- Eclipta prostata ethanol extract has shown positive testing for alkaloid, flavonoids, glycosides, tannins, and phenolic compounds.
- Eclipta prostata aqueous extract showed positive testing for flavonoids, glycosides, tannins, phenolic compounds and saponins. (Table 6.2).
- Ethanol extract indicates the largest active phytochemical constituents from the above phytochemical testing of Eclipta prostata extracts. For further thorough characterization and study of pharmacological activity, I chosen ethanol extract.

SOLVENT SYSTEM DEVELOPED BY TLC FOR ETHANOL EXTRACT OF ECLIPTA PROSTATA.

By trial and error method, it was founded that the best solvent system for EECC is

Chloroform : Ethyl Acetate : glacial acetic acid (4.6 : 0.4 : 0.1)

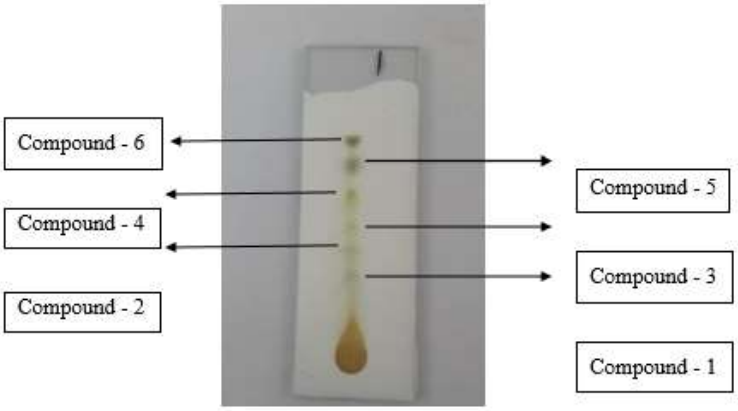

Fig – 5 TLC of ECC

From above TLC, 6 spots are found, whose Rf values are given below Rf values

- **Compound 1 – 0.200**
- **Compound 2 – 0.377**
- **Compound 3 – 0.511**
- **Compound 4 – 0.688**
- **Compound 5 – 0.844**
- **Compound 6 – 0.933**

ISOLATION OF COMPOUND BY COLUMN CHROMATOGRAPHY

One compound was isolated by column chromatography

Compound 1 (Compound A [5]) Rf value : 0.844

Table 4.UV Absorbance

S.No	Wavelength (nm)	Absorbance
1.	668	0.6339
2.	610	0.1226
3.	538	0.1502
4.	508	0.1536
5.	414	1.5349
6.	278	0.4824

FIG 6 UV Spectral Data of Isolated Compound (λ max : 414nm)

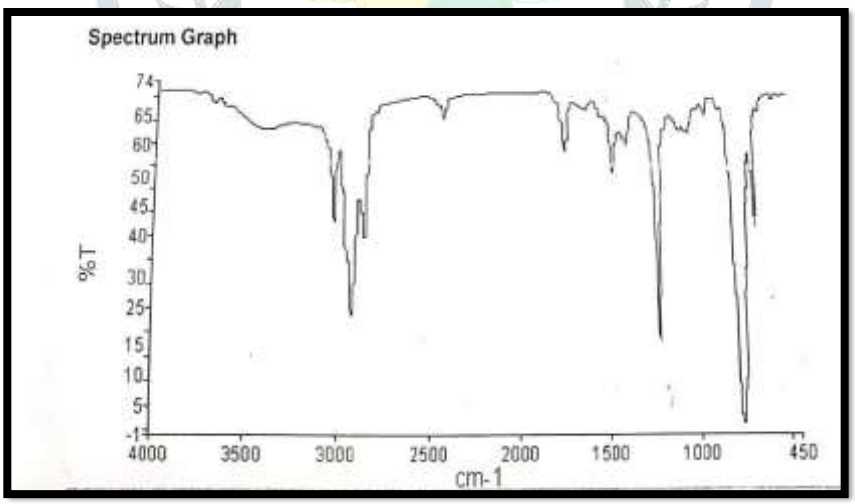

FIG 7– FT-IR Spectral Data of Isolated Compound

PeakName	X	Y
14	669.9	42.35
13	760.53	0.92
12	928.91	67.12
11	1028.85	63.21
10	1215.76	18.45
9	1378.66	60.5
8	1462.3	54.49
7	1608.19	68.09
6	1725.96	58.82
5	2401.25	65.83
4	2855.4	39.6
3	2926.2	23.57
2	3020.65	43.18
1	3398.3	63.38

FIG 8 FT-IR Peaks

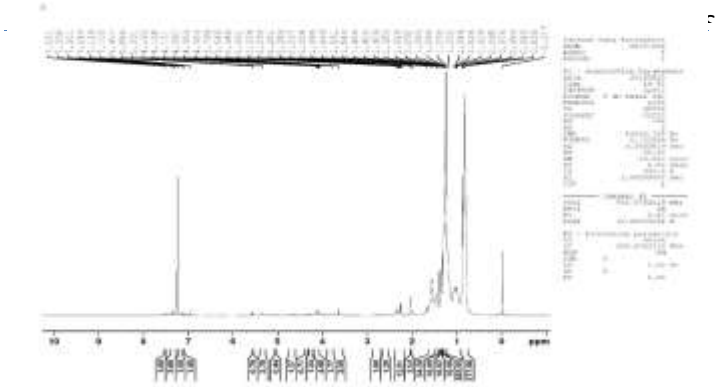

FIG- 9 1H NMR Spectral Data of Isolated Compound

CHARACTERISATION OF ISOLATED COMPOUND (COMPOUND A)

One compound A was isolated from the ethanol extract of aerial parts of Eclipta prostata by gradient column chromatography technique using chloroform and ethyl acetate as solvent system. Compound A having Rf value 0.844, λ max value 414nm and IR spectra with 750.53 cm-1, 1462.3 cm-1 (for aromatic group), 1028.85 cm-1, 1215.76 cm-1, 2401.25 cm-1, 3398.3 cm-1 (for COOH group), 1725.96 cm-1 (for CHO group), 1028.85 cm-1, 1215.76 cm-1 (for OH group), suggest the structural similarity with aromatic acid type of compound. NMR spectra of compound A also indicates the same type of compound. Further investigation is required to conform the structure of compound A.

Determination of Physical Characteristics

The physical characteristic studies were conducted for the compound A as per the method described the results are tabulated in table 3.

Table.5 The physical characteristic studies

S.No	Characteristics	Observation
1	Description	White crystalline powder
2	Solubility at 20°C	Soluble in 1 in 16 parts of methanol.(w/w) Soluble in 1 in 160 parts of water.(w/w)
3	Partition coefficient of isolated compound between octanol and water	2.1
4	Melting Point	204-208 °C

Physical Analysis of The Trail Formulations of 1% Isolated Compound (Compound A)

The physical and mechanical properties like appearance, color, pH, viscosity, spreadability, extrudability, firmness, consistency, cohesiveness, hardness and stickiness of the ointment(O1,O2,O3 and RO), trial formulations of Compound A were analyzed as per the procedure described .Out of various formulations analyzed based on the physical and mechanical properties, the best one of the formulation from ointment, cream and gel was taken for further studies.

Table.6 Physical Analysis of The Trail Formulations of 1% Compound A

Properties	O1	O2	O3	RO
Appearance* (Scores)	8	9	8	7
Color	White	White	White	White
pH	5.7	5.8	6.4	6.3
Viscosity (cps) At 12 rpm at 30 c	26900	22200	28796	28240
Spreadability (g.cm/s)	33	34	36	39
Extrudability (g)	532	513	599	586
Firmness (g)	1149.2	1255.32	1363.328	1359.72
Consistency (g)	2755.61	1836.42	2456.64	2632.42
Cohesiveness (g)	-540.67	-514.50	-735.97	-721.20
Hardness (g)	29.4	25.74	31.12	26.14
Stickiness (g)	-17.25	-16.72	-20.34	-19.73

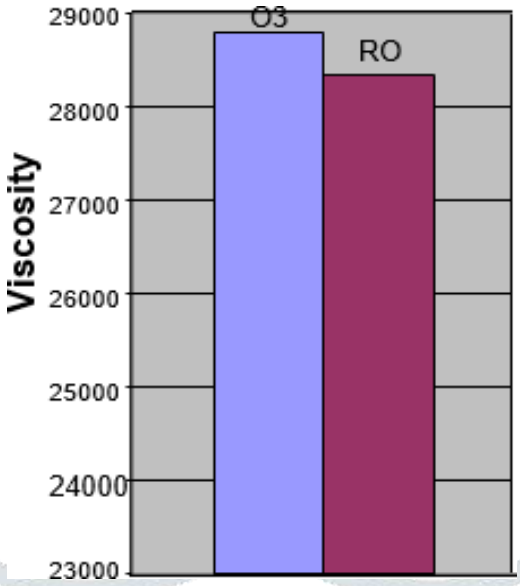

Figure. 10 Comparative viscosity profile of formulations

The viscosity profile of the best formulations was compared with reference and marketed products and given in the figure

Prearation of 1% Isolated Compound (Compound A) of Eclipta prostata Ointments

1%isolated compound (Compound A) ointments pick which one has the correct spreadability and consistency and their formulas are described below.

isolated compound (Compound A) ointment was set up by a combination technique. First hard paraffin (50°C to 57°C) and delicate paraffin (38°C to 56°C) were liquefied together in a china dish over a water shower and the fluid paraffin and propylene glycol containing isolated compound (Compound A) were included and blended well. The liquid blend was detracted from the water shower mixed until cooled, maintaining a strategic distance from air circulation. The substance was mixed successfully to stay away from any crystallization.

Three clusters of isolated compound (Compound A) treatments will plan as referenced above and it will be exposed to physical and substance analysis. Ointment of isolated compound (Compound A) isn't accessible in the market. Subsequently, basic hydrocarbon salve base I shortened as RO was bought and contrasted and three bunches of arranged isolated compound (Compound A) balm for their physical examination. The best plan practically identical with a basic ointment base was picked for additional examination.

Calibration curve for Isolated Compound (Compound A) by UV spectrophotometer

The calibration curve of Isolated Compound (Compound A) was done by UV spectrophotometer according to the method .

Table.7 Standard concentration of Isolated Compound (Compound A) of Eclipta prostata by UV spectrophotometer

S.NO	CONCENTRATION (µg/ml)	ABSORBANCE
1	1.0	0.0421
2	1.4	0.0752
3	1.8	0.1062
4	2.2	0.1413
5	2.6	0.1765

The Std. curve of Isolated Compound (Compound A) was examined by UV spectrophotometer and the curve .

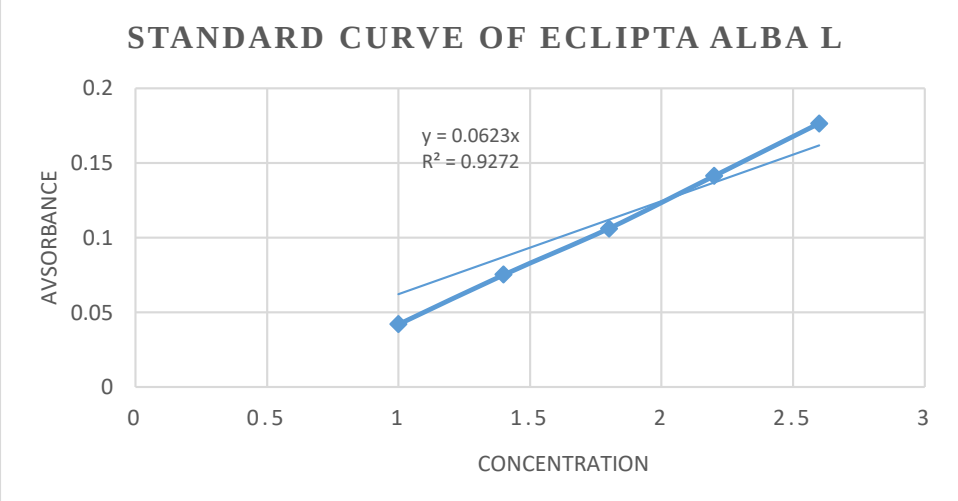

Figure. 11 Standard curve of Isolated Compound (Compound A) by UV spectrophotometer

The data obtained in the in vitro release analysis were analysed using different kinetic models to explain the mechanism of drug release from hydrogels to investigate ,1% Eclipta prostata ointment release kinetics ,The following were added to the release data: four models:

Table No .8 In Vitro % Drug Release Of Prepared Formulations Of 1% Eclipta prostata Ointment

SN.NO	FORMULATION	AMOUNT	%DRUG RELEASE IN MINUTES						
			0	30	60	120	180	360	720
1	(O1)	1	0	16.2	32.6	65.4	78.6	85.4	91.8
2	(O2)	1	0	15.4	30.9	62.8	78.1	84.2	89.7
3	(O3)	1	0	15.2	31.8	63.2	77.8	84.9	90.5
4	(O4)	1	0	16.3	33.2	65.7	78.9	86.7	92.1

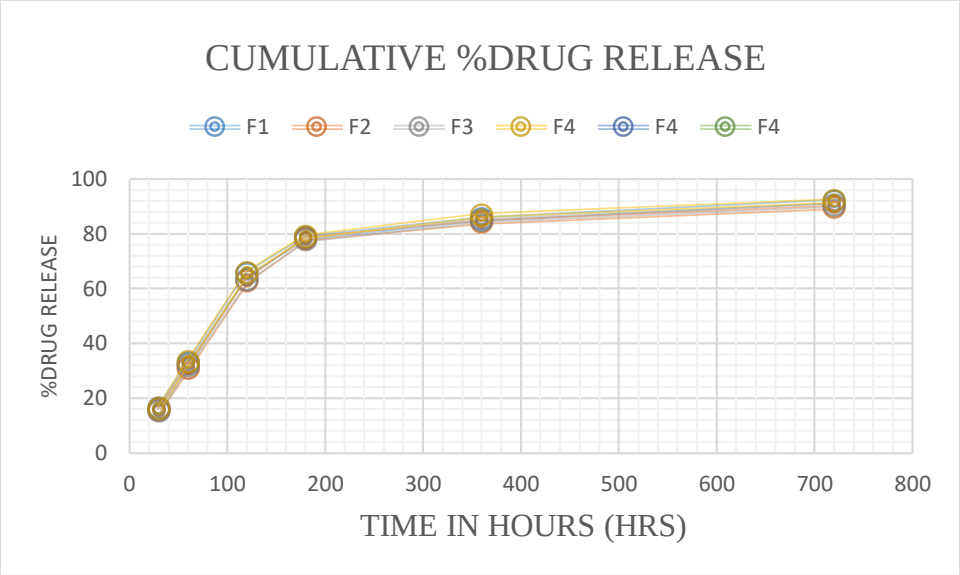

Figure 12 . %cumulative drug release Of Prepared Formulations Of 1% Eclipta prostata Ointment Formulations

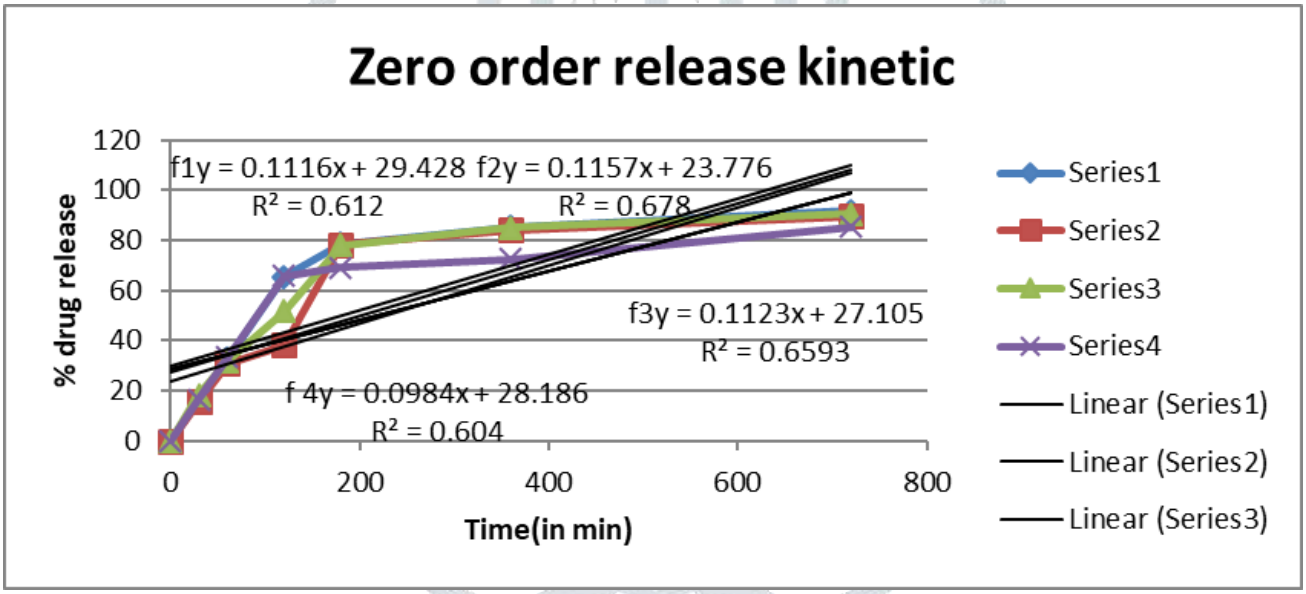

Figure. ZERO ORDER RELEASE KINETICS

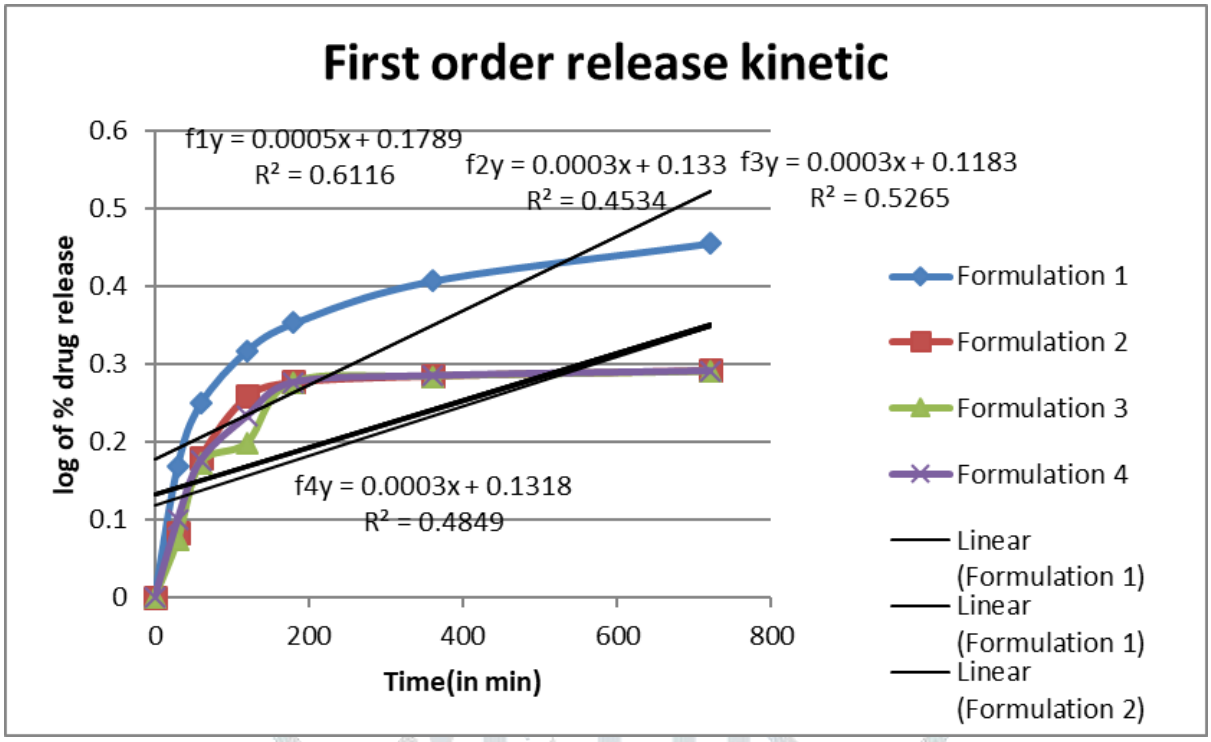

Figure. 13-FIRST ORDER RELEASE KINETICS

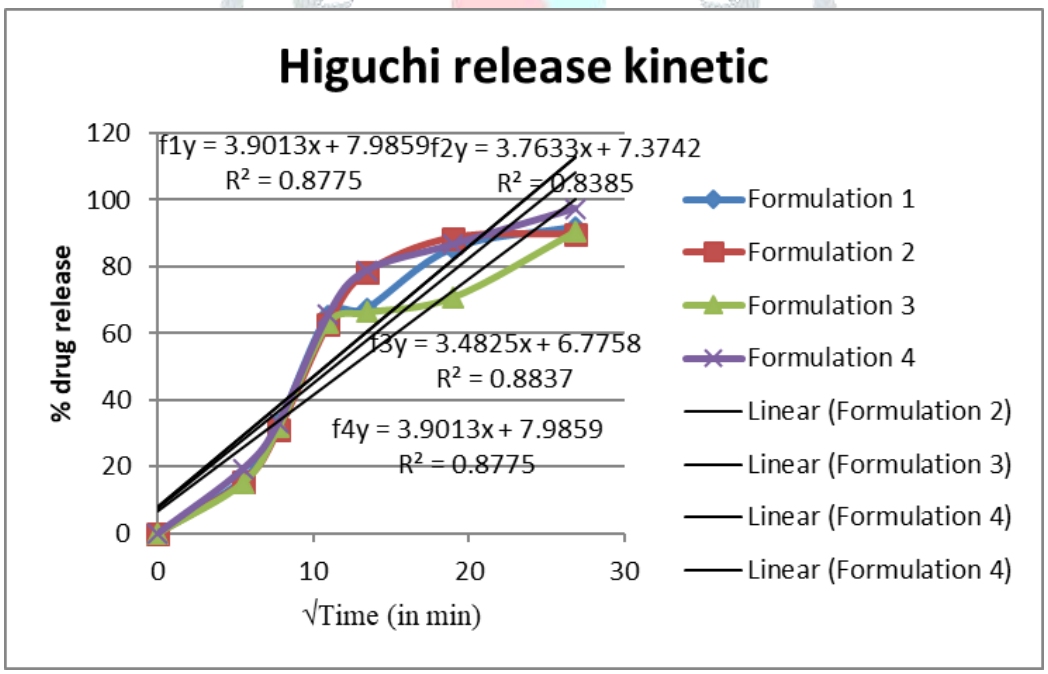

Figure14. HIGUCHI RELEASE KINETIC

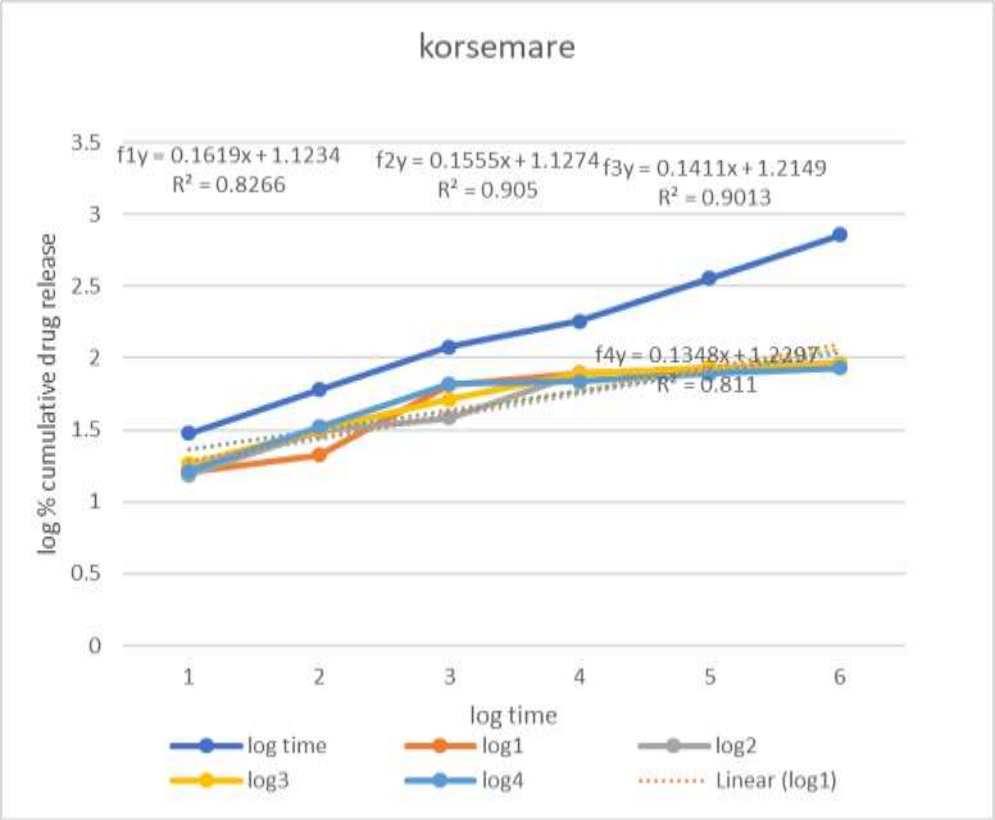

Figure.15 KORSEMARE PEPPAS MODEL

Fig Mathematical models of release profiles of prepared 1% Eclipta prostata ointment using the linearity curve of percent cumulative drug release as a function of time while fig. Korsmeyer-Peppas model represents drug release .

Table.9 In Vitro % Drug Release R2 Values Of Prepared Formulations Of 1% Eclipta prostata Ointment

FORMULATIONS	ZERO ORDER	FIRST ORDER	HIGUCHI	KORSMEYER-PEPPAS
	R2			
F1	0.1858	--0.248	0.8255	0.8266
F2	0.201	-0.415	0.8248	0.905
F3	0.2065	-0.347	0.8295	0.9013
F4	0.6101	0.4589	0.8439	0.811

Formulation 4. Is The Best Formulation Of All That's R2Value

CONCLUSION -

Eclipta prostata shows that it has fungicidal action against a wide range of dermatophytosis, moulds and other dimorphic moulds and other dimorphic fungi. The present work was aimed for the production of 1 per cent topical formulations of Eclipta prostata as an ointment for the treatment of dermatophytosis in a number of human skin conditions. Different kinetic models have been introduced to explain Eclipta prostata Ointment Prepraction release kinetics Figure 33. The plots were shown to be reasonably linear with regard to Higuchi kinetics, as shown by their highest regression. One of the most often employed and most popular controlled release equations is the Higuchi Equation. in a release profile, and an assessment was performed in the graphic presentations of Eclipta prostata Ointment Prepractices (Figure 33) The figure reveals that graphical depiction of the total percent of the time-limited drug release suggests that drug usage of Eclipta prostata from the ointment perfectly matches the Higuchi drug release model because the profile of the drug release is very similar to the pattern or regression axis, with the maximum R^2 coefficient being inside (0.).The kinetic model of Korsmeyer- Peppas releases a drug release from the gel across the membrane to the media of the reception. A curve is presented between the log cumulative percent release of drugs to analyse release kinetics by Korsmeyer – Peppas model. As mentioned above, a (n) in the model was (0,5 <nn< 1) which suggested two drug release mechanisms from the Ointment The 1 percent formulations of the Eclipta prostata ointment trial will evolve with bases of various compounds in the future. Build research products can be evaluated mechanically and compared with the industry and reference substance having the same base. We would always gain recognition. Production formulations must be clinically subject to physiochemical examination and formulations will be tested, including ointment containing 1 per cent precision and efficacy of Eclipta prostata

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