Original Article

Design,Development And Evaluation Of Topical Formulation Contaning Essential Oil As Antidermatophytic Agent

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Abstract

In the present study an attempt was made to formulate gel containing plant extracts and essential oils and was evaluated for antim icrobial activity against different microorganism.

The plants were collected and extracted with suitable solvent. Different gels were formulated by using different concentration of polymers and extracts.

The novel drug delivery system research area of herbal drugs is an innovative work that target for phytoconstituents and plant extracts regarding the therapeutic and cosmetic usefulness of plant products particularly containing flavonoids and poly phenolic compounds. However, due to its poor lipid solubility and larger molecular size limiting their ability to pass across the lipid-rich biological membranes, resulting poor bioavailability. Different reports show a promising future of gel as an advanced form of herbal products that are better absorbed, utilized, and as a result produce better results than conventional herbal extracts. It was confirmed that Ocimum Americanum gel showed a better diffusion as well as stability profile, hence providing an attractive carrier for the delivery of various phytoconstituents present in it. The application of gel formulation as topical pharmaceutical agent and cosmetics with improved safety and efficacy results in proper utilization of herbal drugs and cost-effective pharmaceutical product.

The evaluation was done using cup plate method for zone of inhibition and two fold dilution method for MIC (Minimu m Inhibitory Concentration). Minimum Bactericidal concentration was also calculated. Sensitivity of microorganism to markete d products was also studied. MIC for antimicrobial activity of plant extracts and essential oils were studied prior to gel for mulation to compare the changes in activity after incorporation in polymer gel.

The gel showed promising antibacterial and antifungal activity against other strains used for the stuy. The gel was stable at room t emperature.

Key words: Ocimum Americanum, Carbopol 934, Triethanolamine, Propyl paraben, Ethanol and Distilled Water.

INTRODUCTION

Herbal medicine is one of the oldest and most universal systems of health care system. The advancement in the field of herbal drug delivery started recently with the aim to manage human diseases efficiently. World Health Organization (WHO) estimates that 80% of the world populations presently use herbal medicine for primary health care. Every

nation is seeking health care beyond the traditional boundaries of modern medicine; turning to self medication in the form of herbal remedies. [1] Modern herbal medicine is based upon the combination of traditional knowledge, clinical experience, and understanding of medicinal science and scientific evidence of herbal medicine. People are slowly and gradually switching to alternative forms of medicine. One of these many alternative therapies includes herbal system of medicine. It is made of from an extract taken from the plant parts (leaf, root, flower and bark). They are absolutely natural and safe form of curing illness form occurring repeatedly. They help in curing the ailment and are also known to prevent the illness from occurring repeatedly. Herbal medicines may have long curing periods, but they eradicate the illness from it and prevent any future episodes of the same.[2]

Despite criticism of herbal medicine among mainstream medical professionals , it is wise to remember that many common drugs we use today were derived from plant based sources .Many of the pharmaceuticals currently available to physicians have a long history of use as herbal remedies , including opium , aspirin , digitalis and quinine . According to World Health Organization (WHO) approximately 25% of modern drugs used have been derived from plants. At least 7000 medical compounds in modern pharmacopoeia are derived from plants .Among the active compounds currently isolated from the higher plants and which are widely used in modern medicine today show a positive correlation between their modern therapeutic use and the traditional use of the plants from which they are derived.[3]

Advantages of herbal system of medicines[4]:

- Lower risk of side effects
- Widespread availability
- Effectives with chronic medicine
- Low cost effectiveness make them all the more alluring
- Efficacious for life style diseases for prolonged period of time
- Natural detoxification process of the body is effectively enhanced by herbal medicine.
- These types of formulation are best for the people who are allergic to various types of drugs.
- These types of medicines do not have any types of side effects as they are free from chemicals.

MATERIALS AND METHODS

Table 1: List of Excipients used and their Sources

Sl. No	MATERIAL	SOURCES
1	Ocimum Americanum	Local Market, Barpali, Bargarh.
2	Carbopol 934	SD Fine chemicals, Mumbai
3	Triethanolamine	SD Fine chemicals, Mumbai
4	Propyl paraben	SD Fine chemicals, Mumbai
5	Ethanol	SD Fine chemicals, Mumbai
6	Distilled Water	SD Fine chemicals, Mumbai

Table 2: List of Equipments used

Sl. No	EQUIPMENTS	SUPPLIERS / MANUFACTURES
1	Rotary vaccum evaporator	Superfit, India
2	Weighing balance	Sartorious
3	Heating mantle	Sunbim, India
4	Viscometer	Brookfield Viscometer , USA
5	Sonicator	Life Care
6	Franz diffusion cell	Borosil Glass Works Ltd
7	Magnetic Stirrer	Remi ,Mumbai
8	Centrifuge Apparatus	Remi , Mumbai
9	Stability chamber	Technico, Chennai, India
10	Digital pH meter	Lab India
11	FT-IR spectrophotometer	Bruker, Germany
12	UV-Spectrophotometer	Lab India 3000^{+}

PREPARATION OF PLANT EXTRACTS

The leaves of plant were air-dried until dryness at room temperature and under shade. The dried leaf was then powdered to a fine grade by using laboratory scale mill. Further it was sequentially extracted successively with ethanol using soxhlet apparatus. The solvent was removed and concentrated in a rotary evaporator and water bath. The dried extracts were stored in refrigerator for further studies.

Distillation of essential oil

The essential oil was obtained by the hydro distillation of fresh leaves (7.5 kg) using a Clevenger-type apparatus for 4 hr. The resulting oil/water mixture obtained was extracted using dichloromethane. The organic layer was then separated, dried over Na_2SO_4, filtered and the solvent removed by means of vacuum evaporation at room temperature, resulting Ocimum americanum essential oil. The dried leaves of Ocimum americanum were extracted using a Soxhlet apparatus for 8 h in ether.

Determination Of λmax

The stock solution of 1000µg/ml was prepared by dissolving approximately100mg of pure Ocimum americanum extract in 100ml of pH 7.4 phosphate buffer. From the stock solution, 10ml was taken and was further diluted to 100ml with the buffer solution. The prepared solution was then scanned in a wavelength range of 200-400nm, to find the maximum absorbance. The maximum wavelength was found to be 385 nm and was used for further studies.

DETERMINATION OF STANDARD CURVE

The serially diluted stock solution was obtained in the range of 2- 10µg/ml by taking 0.2, 0.4, 0.6, 0.8 and 1 ml from the stock solution, into 100ml volumetric flask.

- The final solution is made by using phosphate buffer of pH 7.4.
- The serially diluted solutions were measured in a UV spectrometer at 285nm of the drug.
- The calibration curve was plotted by taking absorbance on Y-axis and concentration in µg/ml on X-axis, to find the slope.

FORMULATION OF GELS

- **Preparation of gel:** Gel bases were prepared by separately dispersing Carbopol 934 in distilled water with constant stirring at a moderate speed using mechanical shaker. The pH of all the formulations was adjusted to 5.5 - 6.5 using triethanolamine
- **Incorporation of Phytosomal complex into the gel:** The solution of phytosome complex was prepared in 0.1 ml of ethanol in another beaker and was added to the Carbopol base. Different formulations were prepared using varying concentration of gelling agent. Prepared gels were stored in suitable containers at room temperature for further studies.

Table No 3: Formulation of Gels Complex

Ingredients	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**
Ocimum Americanum : Carbopol 934	1:1%	1:1.5%	1:2%	1:2.5%	1:3%	1:3.5%
Triethanolamine	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S
Propyl paraben	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%
Ethanol	1%	1%	1%	1%	1%	1%
Distilled Water	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S

EVALUATION OF GELS

1.Homogeneity

All developed gels were tested for homogeneity by visual inspection after the gels have been set in the container .They was tested for their appearance and presence of any aggregates.

2. Measurement of pH

The pH of the gels was measured with the help of digital pH meter. 0.5 g of gel was dissolved in 50 ml of distilled water and stored for two hrs. The measurement of pH of each formulation was determined.

3.Drug content

1 g of the prepared gel was mixed with 100ml of suitable solvent. Aliquots of different concentration were prepared by suitable dilutions after filtering the stock solution and absorbance was measured at 285 nm.

4.Rheological study

The measurements of viscosity of prepared gels were carried out with Brookfield Viscometer (spindle type S–96). The readings of each formulation were taken.

5.Spreadability

On a glass plate of 10×5cm, 350mg gel was taken and another plate of same sized was dropped from a distance of 5cm. After 1 minute the diameter of the circle spread was measured.

6.Extrudability

In the present study, extrudability was determined by measuring the weight (in grams) required to extrude at least 0.5cm gel from lacquered aluminum collapsible tube in 10 sec. The extrudability was then calculated by using the following equation:

$$\text{Extrudability} = \frac{\text{Applied weight to extrude gel from tube (in gram)}}{\text{Area (in cm}^2\text{)}}$$

7.In-vitro drug release study

The in-vitro rug release studies were carried out using a modified Franz diffusion (FD) cell. The formulation was applied on egg membrane which was placed between donor and receptor compartment of the FD cell. Phosphate buffer pH 5.5 was used as diffusion media. The temperature of the cell was maintained at 37˚C. The whole assembly was kept on a magnetic stirrer and the solution was stirred continuously using a magnetic bead. One ml of aliquots were withdrawn from the diffusion medium at specific time interval for 12 hours and same quantity of fresh , pre-warmed diffusion medium was replaced for the amount withdrawn. The samples withdrawn were analyzed spectrophotometrically at 385 nm and the cumulative % drug release was calculated.

8.Drug Release Kinetics

To know the release kinetics, the data obtained from the in-vitro release profile was fitted into various models like :

- Zero order kinetic model: cumulative percent drug release v/s time
- First order kinetic model: log cumulative percent drug remaining v/s time
- Higuchi's model: cumulative percent drug release v/s square root of time
- Korsmeyer - Peppas model: log cumulative percent drug release v/s log time

Zero order kinetics:

It describes the system in which the drug release rate is independent of its concentration.

$$\mathbf{Q_t = Q_0 + K_0 t}$$

Where,

Q_t = Amount of drug dissolved in time t

Q_0 = Initial amount of drug in the solution, which is often 0 K_0 =Zero order release constant

If the release pattern obeys zero order, then the plot of Q_t v/s t will give a straight line with a slope of K_0 and an intercept at 0.

First order kinetics

It describes the drug release from the systems in which the release rate is concentration dependent.

$$\mathbf{\log Q_t = \log Q_0 + k\, t/2.303}$$

Where,

Q_t = Amount of drug released in time t
Q_0 = Initial amount of drug in the solution
K = First order release constant
If the release pattern obeys first order, then the plot of log (Q_0-Q_t) v/s t will be straight line with a slope of kt/2.303 and an intercept at t = log Q_0.

Higuchi model

According to this model, the fraction of drug from the system is proportional to the square root of time.

$$M_t/M_\alpha = kHt^{1/2}$$

Where,
M_t & Mα = Cumulative amounts of drug release at time t and at infinity
kH = Higuchi dissolution constant (reflects formulation characteristics)
If the Higuchi model of drug release is obeyed, then a plot of Mt/M ∞ v/s t ½ will be straight line with slope of kH.

Korsmeyer – Peppas model (power law)

The power law describes the drug release from the polymeric system in which the release deviates from Fickian diffusion. It is expressed using the following equations :

$$M_t/M_\alpha = k_t n$$

$$\log [M_t/M_\alpha] = \log k + n \log t$$

Where, M_t & Mα = Cumulative amounts of drug release at time t and at infinity
k = Constant incorporating structural and geometrical characteristics of the system n
= Exponent determining the mechanism of drug release
To characterize the release mechanism, the dissolution data (M_t/M_α < 0.6) are evaluated.

A plot of M_t/M_α v/s log t will be linear with slope n and intercept value of log k. Antilog of k gives the value of k. Peppas used the n value in order to characterize different release mechanisms as shown below:

Table 4: Release Mechanisms

'n' value	**Drug Release**
<0.5	Fickian
0.5<n<1	Non – Fickian
>1	Case 2 transport

STABILITY STUDIES

Stability of a drug in a dosage form at different environmental conditions is important, because it determines the expiry date of that formulation. Hence, the stability of the prepared formulation was studied. Stability studies were conducted according by storing the gel formulation at $40^oC \pm 2^0C$, 70% RH ±5% for 45 days. The samples were withdrawn at initial, 30^{th} & 45^{th} day and analyzed suitably for the physical characteristics, drug content and cumulative drug release.

Drug-excipient compatibility studies

Fourier Transform Infrared spectroscopic studies

A Fourier Transform – Infra Red spectrophotometer was used to study the non-thermal analysis of drug-excipient (binary mixture of drug: excipient 1:1 ratio) compatibility. The spectrum of each sample was recorded over the 450-4000 cm^{-1}. Pure drug of Ofloxacin with physical mixture (excipients) compatibility studies were performed.

Determination of antibacterial and antifungal activity

Microorganisms

Microorganisms used were,

Fungi: A. varis, A. niger, P. notatum,

Bacteria: E. coli, S. aureus, P. aeruginosa, B. subtilis.

Preparation of inoculums

For evaluation of antifungal activity, 24 hours fresh culture of fungi and bacteria were suspended in sterile water to obtain a uniform suspension of microorganism.

Determination of zone of inhibition

Antifungal and Antibacterial activity was checked by agar well diffusion method. In this method a previously liquefied medium was inoculated with 0.2 ml of Fungal and Bacterial suspension having a uniform turbidity at temperature of 400C. 20 ml of culture medium was poured into the sterile petridish having an internal diameter of 8.5 cm. Care was taken for the uniform thickness of the layer of medium in different plates. After complete solidification of liquefied inoculated medium, the wells were made aseptically with cork borer having 6mm diameter. In each of these plate extract and gel solution was placed carefully. Plates were kept for pre diffusion for 30 mins. After it normalized to room temperature; the plates were incubated at 37ºc for 24 hrs in case of bacteria and at 27ºc for 48 hrs in case of fungi. After incubation period was over, the zone of inhibition was measured with help of Hi-media.

RESULTS AND DISCUSSION

Calibration Curve of Ocimum americanum Extract

The λ of Ocimum americanum Extract was determined by scanning the prepared solution in the max wavelength range of 200-400 nm. The maximum wavelength was found to be 385nm. The calibration curve of Ocimum americanum extract was constructed by dissolving the drug in pH 7.4 phosphate buffer. The linearity of the curve was found in the concentration range of 2-10µ g/ml. A regression coefficient (R^2) value of 0.9989 was obtained.

Table 5: Calibration curve data of Ocimum americanum extract

Concentration(µg/ml)	Absorbance
0	0

2	0.101
4	0.181
6	0.268
8	0.347
10	0.432

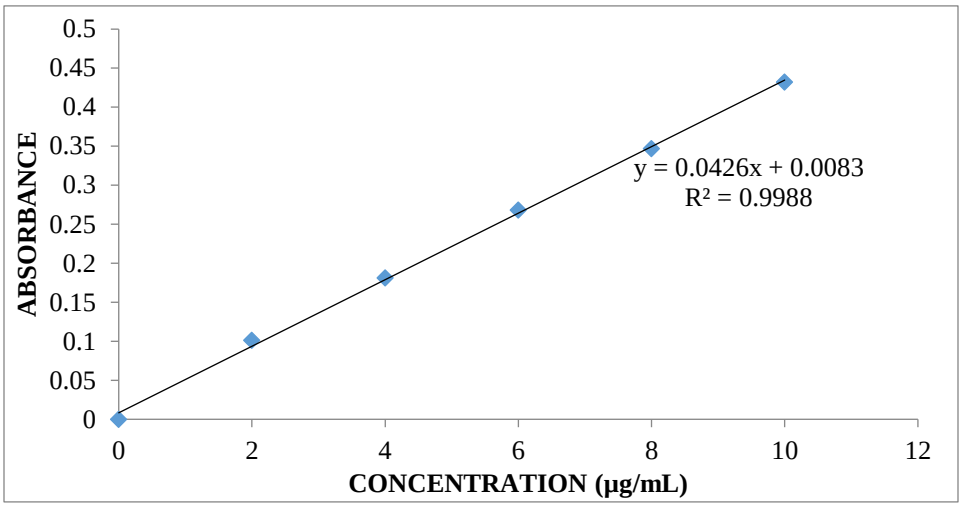

Figure No:1 Calibration curve of Ocimum americanum extract

EVALUATION OF GELS

1. Homogeneity

Table No: 6 Results of Homogeneity of Different Gel Formulations

Formulation	Homogeneity
F1	Good
F2	Good
F3	Good
F4	Good
F5	Good
F6	Good

The visual inspection of all the prepared gel formulations were carried out and it was concluded that all the gel formulations showed good appearance and homogeneity.

2. Measurement of pH

Table No 7: Results of pH of different gel formulations

Formulation	pH

F1	5.2
F2	5.3
F3	5.6
F4	5.4
F5	5.1
F6	5.6

The pH of the gel formulations was in the range of 5.1 to 5.6, which lies in the normal pH range of the skin and would not produce any skin irritation.

3. Drug Content

Table No 8: Results of Drug Content of different Gel Formulation

Formulation	Drug Content (%)
F1	87.14
F2	91.34
F3	97.81
F4	85.93
F5	90.24
F6	94.62

The drug content of the formulated gels was estimated spectrophotometrically at 385nm. The drug content of all the formulation was found to be in the range of 85.93 % to 97.81% in which the best formulation F3 contained 97.81 % of the Ocimum Americanum.

4. Rheological study

Table No 9: Results of Viscosity of Different Gel Formulations

Formulation	Viscosity (Centipoise)
F1	5564
F2	11210
F3	9564
F4	13241
F5	14210
F6	15021

The gel was rotated at 50 rpm for 10 minutes with spindle 64. The corresponding reading was noted. The viscosity of the formulations increases as concentration of polymer increases. The viscosity of the best formulation was found

to be 9564 centipoise.

5. Spreadability

Table No 10: Results of Spreadability of Different Gel Formulations

Formulation	Spreadability (cm)
F1	2.1
F2	3.8
F3	4.3
F4	3.5
F5	3.0
F6	2.8

Spreadability denotes the extent of area to which the gel readily spreads on application to skin or the affected part. The spreadability of the prepared gel formulations was carried out and it was concluded that all the formulation showed acceptable spreadability. The spreadability coefficient of the best formulation F3 was found to be 4.3cm. The value of spreadability indicates the gel was easily spreadable by small amount of shear.

6. Extrudability

Table No 11: Results of Extrudability of Different Gel Formulations

Formulation	Extrudability (gm/cm^2)
F1	10.1
F2	11.9
F3	9.6
F4	14.0
F5	15.1
F6	16.4

It was found that extrudability of the prepared gel formulation was a function of concentration of gelling agents. Extrudability was decreased with increase in concentration of gelling agents. The extrudability of the best formulation F1 was found to be 9.6 gm/cm^2. Thus the prepared gel posses optimum extrudability.

7. In-vitro drug release study

The in-vitro permeation studies of all the formulations were carried out using Franz diffusion cell with egg membrane as a diffusion membrane for the study as described in the methodology section.

The comparative data of cumulative % drug permeation of the formulations were shown in table and the figure. Cumulative % drug release of the prepared gel formulations after 12 hours was found to be in the range of 72.39% to

98.10%. The drug release from F3 was found to be higher due to the lower concentration of the gelling agent and F6 showed lower drug release due to the higher concentration of the gelling agent.

Table 12: Results of In-vitro drug release study of Gel Formulations

Time in hours	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
0.5	16.19	21.5	14.62	12.41	16.76	10.20
1	22.95	28.5	19.86	18.66	20.89	15.89
2	31.78	36.28	21.35	28.59	29.24	20.64
3	43.81	41.90	29.45	31.84	35.32	25.11
4	56.76	46.27	39.8	40.35	42.75	28.96
5	59.21	52.86	46.25	45.78	48.09	38.30
6	65.42	60.25	55.73	52.60	56.16	40.56
7	71.56	67.83	62.26	60.71	61.36	45.31
8	84.39	73.40	73.24	68.54	67.12	53.78
9	96.12	82.34	77.59	72.28	73.78	57.41
10		90.27	81.70	76.12	78.79	62.02
11		97.52	89.62	85.36	82.31	66.46
12			98.10	96.85	89.45	72.39

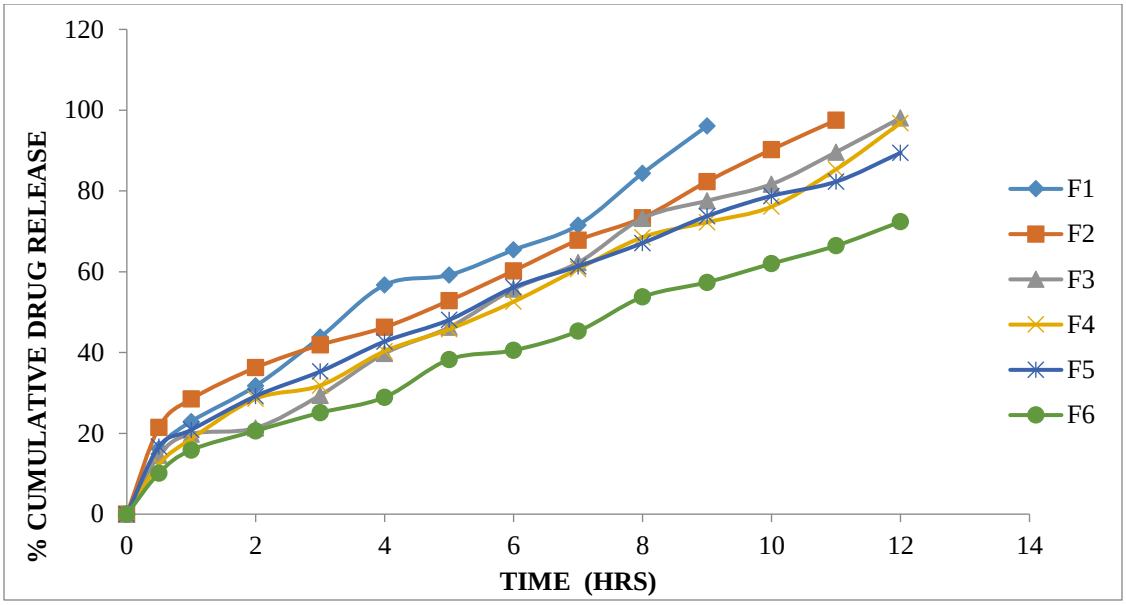

Figure 2: In-vitro Drug Diffusion Profile of Gel Formulation

8. Drug Release Kinetics

Based on the data obtained from the in-vitro drug release studies the best formulation F_3 was analyzed for the release kinetic studies. The cumulative release of drug was fitted into various plots like Zero order, First order and Higuchi model to know the pattern of release and Korsmeyer-Peppas model in order to find out the mechanism of release from the prepared gel. The model that best fits the release data is selected based on the regression coefficient value of various models.

CUMULATIVE (%) RELEASE Q	TIME (T)	ROOT (T)	LOG(%) RELEASE	LOG (T)	LOG (%) REMAIN	RELEASE RATE (CUMULATIVE % RELEASE / t)	1/CUM% RELEASE	PEPPAS log Q/100	% Drug Remaining	Q01/3	Qt1/3	Q01/3-Qt1/3
0	0	0			2.000				100	4.642	4.642	0.000
14.62	0.5	0.707	1.165	-0.301	1.931	29.240	0.0684	-0.835	85.38	4.642	4.403	0.238
19.86	1	1.000	1.298	0.000	1.904	19.860	0.0504	-0.702	80.14	4.642	4.311	0.330
21.35	2	1.414	1.329	0.301	1.896	10.675	0.0468	-0.671	78.65	4.642	4.284	0.357
29.45	3	1.732	1.469	0.477	1.848	9.817	0.0340	-0.531	70.55	4.642	4.132	0.510
39.8	4	2.000	1.600	0.602	1.780	9.950	0.0251	-0.400	60.2	4.642	3.919	0.722
46.25	5	2.236	1.665	0.699	1.730	9.250	0.0216	-0.335	53.75	4.642	3.774	0.868
55.73	6	2.449	1.746	0.778	1.646	9.288	0.0179	-0.254	44.27	4.642	3.538	1.104
62.26	7	2.646	1.794	0.845	1.577	8.894	0.0161	-0.206	37.74	4.642	3.354	1.287
73.24	8	2.828	1.865	0.903	1.427	9.155	0.0137	-0.135	26.76	4.642	2.991	1.651
77.59	9	3.000	1.890	0.954	1.350	8.621	0.0129	-0.110	22.41	4.642	2.819	1.822
81.7	10	3.162	1.912	1.000	1.262	8.170	0.0122	-0.088	18.3	4.642	2.635	2.006
89.62	11	3.317	1.952	1.041	1.016	8.147	0.0112	-0.048	10.38	4.642	2.181	2.460
98.1	12	3.464	1.992	1.079	0.279	8.175	0.0102	-0.008	1.9	4.642	1.239	3.403

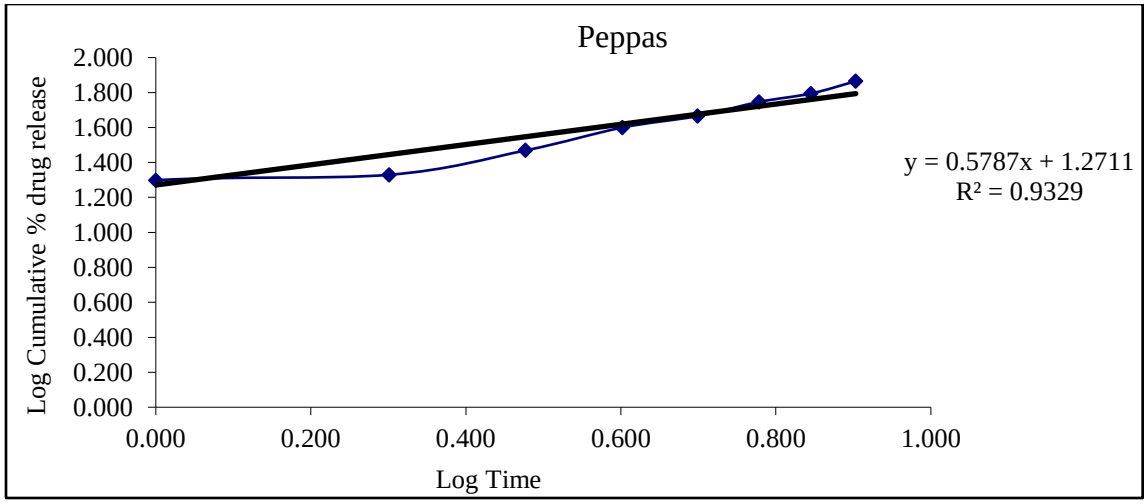

Figure No 3: Korsmeyer-Peppas Plot

Table No 13: Results of kinetic analysis

Formulation	Zero order	First order	Higuchi model	Korsmeyer-Peppas model
	R^2	R^2	R^2	R^2
F_3	0.980	0.955	0.942	0.932

This formulation was following Zero order release mechanism with regression value of 0.980.

9. Stability Studies

Table No 14: Stability Studies For F3 Formulation

Sl.No	Parameters	Initial	th 30 Day	th 45 Day
1	Homogeneity	Good	Good	Good
2	Drug Content (%)	97.81	97.24	97.02
3	pH	5.6	5.6	5.5
4	Spreadability(cm)	4.3	4.2	4.2
5	Extrudability(gm/cm)	9.6	9.5	9.5
6	Viscosity (cps)	9564	9554	9551
7	% cumulative release	98.10	98.01	97.99

The purpose of the stability testing is to provide evidence on how the quality of a drug substance or drug varies with time under the influence of variety of environmental factors like temperature, humidity and light and to establish a

test period for the drug substance or a shelf life for the drug and recommended storage conditions. Here the gel are packed in collapsible aluminum tubes and were loaded at accelerated condition at 40 ± 2°C/ RH 70 ± 5% RH in a stability chamber. Samples were withdrawn at initial 40 ± 2°C/ RH 70 ± 5% and days and evaluated for homogeneity, drug content, pH, spreadability, extrudability, viscosity and in-vitro diffusion profile. The results showed that the storage at these conditions had no effect on those parameters.

FTIR

FTIR spectra of the drug and the optimized formulation were recorded. The FTIR spectra of pure Ocimum Americanum, drug with polymers (1:1) shown in the below figures respectively. The major peaks which are present in pure Ocimum Americanum are also present in the physical mixture, which indicates that there is no interaction between Ocimum Americanum and the polymers, which confirms the stability of the drug.

There was no disappearance of any characteristics peak in the FTIR spectrum of Ocimum Americanum and the polymers used. This shows that there is no chemical interaction between the drug and the polymers used. The presence of peaks at the expected range confirms that the materials taken for the study are genuine and there were no possible interactions.

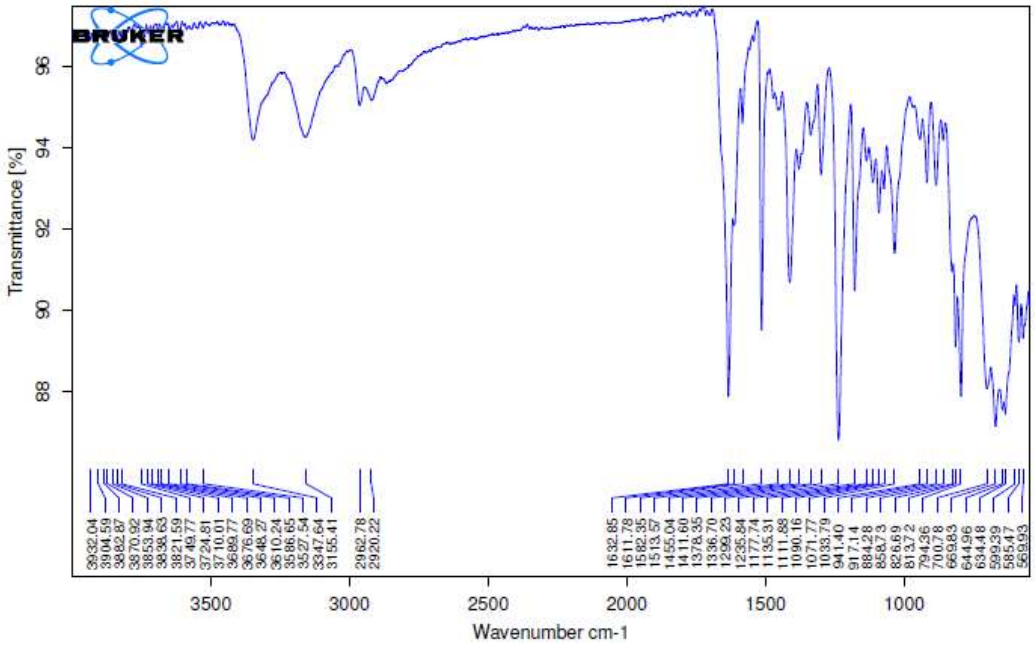

Fig 4: FTIR Peak of pure Ocimum Americanum

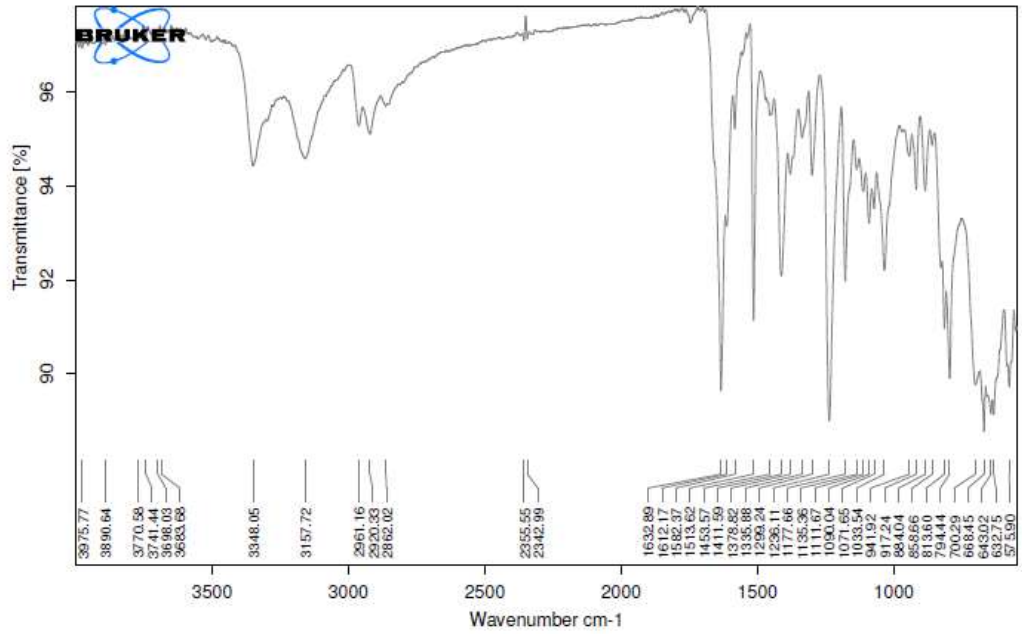

Fig 5: FTIR Peak of Optimised formulation

DETERMINATION OF ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY

Table 15: Zone of inhibition of plant extracts

Microbial strains	**Zone of Inhibition (mm)**
	Ocimum Americanum
S. aureus	11
E. coli	10
B. subtilis	8
P. aeruginosa	7
A. varies	3
P. notatum	9
A. niger	-

Table 16: Zone of inhibition of individual gel formulations

Microbial strains	**Zone of Inhibition (mm)**
	Ocimum Americanum

S. aureus	10
E. coli	9
B. subtilis	7
P. aeruginosa	5
A. varies	2
P. notatum	8
A. niger	-

Zone of inhibition of gel formulation of same plant extracts was almost similar to that of isolated plant extract activity. This shows that incorporation of extracts into polymeric extracts does not decrease its activity.

MIC of most of the extracts was between 0.5-10 mg/ml. Ocimum Americanum showed antimicrobial activity although. Observed that essential oil of Ocimum Americanum inhibited microorganism.

Table 17: Zone of inhibition of Gel formulations

Microbial strains	**Zone of Inhibition (mm)**
	Ocimum Americanum gel
S. aureus	12
E. coli	11
B. subtilis	12
P. aeruginosa	10
A. varies	8
P. notatum	8
A. niger	5

The gel containing plant extracts showed synergistic effect against most of the strains. Most of the extract showed little or no activity against fungal strains but the gel showed activity against all the fungal strain used.

CONCLUSION

Ocimum Americanum have been used for thousands of years for the treatment of many health problems including cancer, cold, diabetes, flu, hypertension and pain. Scientific investigations reported its important properties like wound healing effects, antioxidant, anti fungal, anti bacterial, anti-inflammatory, Antioxidant, Immunomodulatory and Cardiac stimulant activities. Eventhough extracts have reported several therapeutic benefits, but extraction of individual compound from it often exhibits limited clinical utility. As the synergistic effect of various natural ingredients gets lost and the various phytoconstituents present in it are poorly absorbed either due to their large

molecular size, which cannot be absorbed by passive diffusion or due to their poor lipid solubility, thus severely limiting their ability to transport across lipid-rich biological membranes, resulting in their poor bioavailability.

Gel technology has proved to be beneficial in providing better absorption and bioavailability of polar biomolecules over conventional herbal extracts. Hence the present study was aimed to prepare and evaluate topical phytosomal gel of Ocimum Americanum with an objective to increase its bioavailability and therapeutic efficacy.

Formulation

Ocimum Americanum gel was prepared by the anti solvent precipitation technique using different ratios of drug and Carbopol polymer. A total of 6 formulations were prepared. From the prepared formulation the best formulation which contained drug extract: Carbopol in the ratio 1:2% was on various evaluation parameters a gel base of different concentration using carbopol 934 as a polymer.

UV spectrophotometric method was developed for determining λ_{max} of Ocimum Americanum extract

Evaluation

- The prepared gel formulations was evaluated for pH, spreadability coefficient, extrudability, drug content, rheological studies and in-vitro drug diffusion study.
- Release kinetic data revealed that the gel followed Zero order model kinetics with non- Fickian diffusion of drug.
- Stability studies were carried out at accelerated temperature $40^oC \pm 2^0C$, 70% RH ±5% for 45 days. There were no significant changes in the homogeneity, drug content, pH, spreadability, extrudability, viscosity and in-vitro diffusion profile.
- The gel showed promising antibacterial and antifungal activity against other strains used for the study. The g el was stable at room temperature.
- In conclusion, topical formulations of Ocimum Americanum essential oil can be alternative topical agents with safe broad-spectrum activity for the treatment of skin disorder. Further studies should focus on clinical study of the product.

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