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FORMULATION AND EVALUATION OF CURCUMINOIDS- CAPSAICINOIDS-EUDRAGIT® EPO TRANSDERMAL FILM FOR ANTI-INFLAMMATORY ACTIVITY IN WISTAR RATS

Hulaswar R. Preeti [*1], Patil S. Kalpana [2], Pratibha Khot [3] and Harshada Suryavanshi [4]

Department of Pharmacognosy [1], Department of Pharmaceutical Chemistry [4], Y. D. Mane Institute of Pharmacy, Kagal, Kolhapur - 416216, Maharashtra, India.
Department of Pharmacognosy [2], Tatyasaheb Kore College of Pharmacy, Warananagar - 416113, Maharashtra. India.
Department of Pharmaceutical Chemistry [3], KLE'S College of Pharmacy, Nipani - 591237, Karnataka, India.

Keywords:

Curcuminoids, Capsaicinoids, Transdermal Polyherbal film, Franz diffusion cell, Anti-inflammatory activity in Wistar rats

Correspondence to Author:
Hulaswar Preeti R.

Assistant Professor,
Department of Pharmacognosy,
Y. D. Mane Institute of Pharmacy,
Kagal, Kolhapur - 416216,
Maharashtra, India.

E-mail:hulaswarpreeti10@gmail.com

ABSTRACT: Background: This study presents the extraction, fractionation, formulation, and evaluation of transdermal polyherbal films containing Curcumin and Capsaicin for potential anti-inflammatory use. *Capsicum annuum* extract underwent ethanol-based extraction and fractionation, isolating alkaloids for further study. **Results:** Phytochemical screening revealed the presence of alkaloids, flavonoids, polyphenols, and terpenoids in both *Curcuma longa* and *Capsicum annuum* extracts. Chemical fractionation was done for *Capsicum annuum* extract, Dichloromethane fraction given the best results for alkaloids. Compatibility studies employing FTIR and DSC demonstrated the compatibility of Curcumin and Capsaicin with excipients, crucial for film formulation. *In-silico* molecular docking studies elucidated interactions between Capsaicin/Curcumin and Cox-2 protein, hinting at potential anti-inflammatory activity. The films were formulated using chitosan and solid dispersions of Curcuminoids/Capsaicinoids with Eudragit® EPO. Evaluation encompassed various parameters like weight variation, thickness, folding endurance, moisture content, water uptake, drug content, and *in-vitro* drug release. Further assessment involved a Carrageenan-induced paw edema model in rats, comparing different film formulations with a standard Diclofenac patch for their anti-inflammatory efficacy. **Conclusion** This comprehensive approach involving extraction, fractionation, formulation, evaluation, and efficacy testing demonstrates the potential of these polyherbal films for anti-inflammatory applications, offering a promising avenue for transdermal drug delivery systems.

INTRODUCTION: Traditional medicines, particularly herbal remedies, are gaining attraction for their historical relevance and therapeutic potential. Inflammation, a complex biological response, is mediated by key factors like Prostaglandin and Cyclooxygenase-2.

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Capsaici exhibits Capsicum and Curcumin from Curcuma exhibit potent anti-inflammatory properties [1, 2]. Skin, previously believed impermeable, is now recognized as a viable route for drug delivery *via* Transdermal Drug Delivery Systems, offering advantages like sustained drug action. The other advantages of transdermal patches are it provides maintenance of constant and prolonged drug action, self medication, minimizes the dosing frequency and easy and painless medication [5]. This study focuses on designing and evaluating a pharmaceutical formulation containing Curcuminoids and Capsaicinoids, harnessing their synergistic anti-inflammatory potential.

Capsicum and Curcuma's compounds hold promise for an innovative pharmaceutical formulation combating inflammation.

MATERIALS AND METHODS:

Materials: *Curcuma longa* extract: gifted by natural remedies. *Capsicum annum* extract: obtained via soxhlet extraction method.

Markers: Curcumin from Sigma-Aldrich, Germany; Capsaicin from Global HERBO TECH, Secunderabad. Standard drug formulation: Diclofenac Patch (NuPatch). Chemicals and reagents: Analytical grade; excipients sourced from our institute.

Methods: Collection and Authentication of plant**.** The *Capsicum annum* plant was collected from district of Belagavi (Chikkodi) region and authenticated in NITM (ICMR), Belagavi.

Extraction, Fractionation and Isolation of Capsaicinoids: Dried Capsicum annum powder extracted using ethanol in a Soxhlet apparatus. The extract was concentrated and fractionated using various solvents (Dichloromethane, 5% Citric acid solution, Methanol, Petroleum ether, etc.). Alkaloidal fraction selected for further investigation **Fig. 1.**

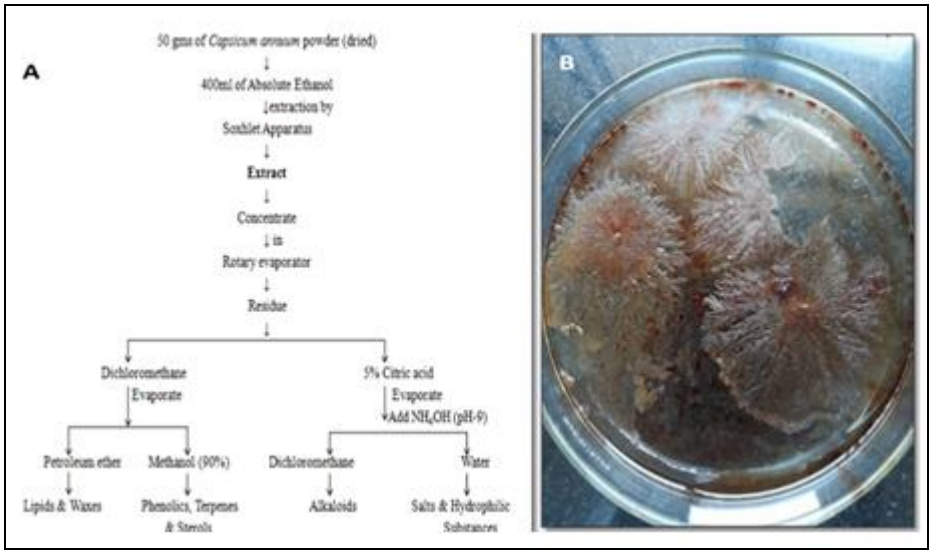

FIG. 1: FRACTIONATION OF *CAPSICUM ANNUM* EXTRACT, B. DICHLOROMETHANE FRACTION OF *CAPSICUM ANNUUM* EXTRACTS (CAPSAICINOIDS)

Phytochemical Screening of *Curcuma longa* and *Capsicum annum* Extract [6, 7]: *Curcuma longa* and *Capsicum annum* extracts underwent qualitative chemical composition assessment using standard phytochemical tests.

Pre- Formulation Studies (Compatibility Study) [8, 9, 10]: Compatibility between the drugs and excipients was evaluated using Fourier Transform Infrared Spectroscopy (FTIR) and Differential Scanning Colorimetry (DSC). For FTIR, the IR spectra of pure Curcumin and Capsaicin were studied individually, alongside combinations of respective drugs with excipients using FT-IR 200 from Shimadzu Corporation, Japan. DSC analysis, performed with a DSC60 detector, involved approximately 2mg samples of pure Curcumin and Capsaicin, as well as mixtures of Curcumin, Capsaicin, and Excipients. Heating was conducted from 30 to 400°C at a rate of 10°C/min under constant nitrogen flow. Furthermore, an *in-silico* molecular docking study was executed using Autodock 4.0 and PyRx 0.8 software to predict the interaction between Curcumin and Capsaicin with Cox-2 protein, focusing on ligand-protein interactions [11].

Preparation of Transdermal Chitosan Film [20]: Chitosan solutions of varying concentrations (1%, 2%, and 3%) were prepared using Glacial acetic acid and HCl. Dissolving chitosan in 5% HCl, followed by addition of 0.5 ml Glacial acetic acid with continuous stirring using a Magnetic Stirrer. Propylene glycol acted as a plasticizer, and pH adjustment was done with NaOH to neutralize the solution.

After 24 hours of stirring, the solution was left to settle for another 24 hours, filtered through muslin cloth, and poured into glycerin-coated Petri plates. These plates were then air-dried for at least 24 hours at room temperature to obtain the optimized chitosan film suitable for subsequent studies. [16]

Preparation of Solid Dispersions using Curcuminoids and Capsaicinoids: Due to observed difficulties in dissolving Curcuminoids and Capsaicinoids in the Chitosan solution, solid dispersions were attempted to enhance solubility. For Curcuminoids, solid dispersions with Eudragit® E PO were prepared using methanol in varying ratios of 1:1, 1:2, and 1:3.

Initially, Eudragit® E PO was dissolved in methanol with continuous stirring, followed by the combination of both solutions and subsequent evaporation to obtain dry solid dispersions. The same procedure was applied for Capsaicinoid solid dispersion which is mentioned above.

Formulation of Transdermal film using both solid dispersions of Curcuminoids-Eudragit® EPO and Capsaicinoids and Eudragit ®E PO: For the formulation of polyherbal film, 2% Chitosan was dissolved in 5% HCl, 2-3 drops of Propylene glycol and 0.5 ml of Acetic acid. This solution was stirred for 48 hours continuously by using magnetic stirrer.

Then kept aside for 24 hours and filtered. To the filtrate, the prepared solid dispersions of Curcuminoids-Eudragit® EPO and Capsaicinoids and Eudragit ®E PO was added in the ratio of 25:75, 50:50 and 75:25 respectively. The obtained mixture was poured into petri plate (previously smeared with glycerol) and kept aside for drying.

Evaluation of Polyherbal Transdermal Films [4, 12, 13]:

Weight Variation: Six films from each batch were individually weighed to assess weight variation. Results of each film were recorded, and the average weight with ± standard deviation (SD) was calculated.

Thickness Measurement: The film's thickness was randomly measured using a Vernier caliper at various points: corners and the center. Average thickness along with standard deviation (SD) was recorded.

Folding Endurance Test: 2x2 cm sections were repeatedly folded at the middle to assess folding endurance. The films were folded until breakage or until showing signs of curve/crack.

Moisture Content Analysis: Films were individually weighed and placed in a Desiccator with Silica desiccant at room temperature for 24 hours. Weights were recorded until constant. Moisture content percentage was calculated based on initial and final weights.

Water Uptake: Films were immersed in phosphate buffer (pH 5.5) for 24 hours at room temperature. After removing excess buffer solution, the weight was recorded. This process was repeated at least three times for each sample, and water uptake percentage was calculated based on initial and final weights.

Drug Content Determination: 2x2 cm sections of the film were placed in phosphate buffer (pH 5.5) and sonicated for an hour. The resulting solution was filtered and analyzed using a UV-Spectrophotometer at 424 nm for curcumin and 280 nm for capsaicin to determine drug content.

***In-vitro* Drug Release Studies:** For the *in-vitro* drug release assessment, the Franz Diffusion Cell served as the experimental setup **Fig. 2**.

Phosphate Buffer (pH 5.5) was used to fill the reservoir, and the assembly was placed on a magnetic stirrer for continuous stirring. A predetermined surface area of the films was cut and applied on the top of the diffusion cell, which contained a dialysis membrane (pore size 0.4µm).

The assembly was covered with a glass lid and clamped together. The temperature was carefully maintained at 32°C throughout the study. At predetermined time intervals of 15 minutes, 1ml of the solution was collected and replenished with buffer. These collected samples were analyzed simultaneously using a UV-Visible Spectrophotometer at 424 nm for Curcumin and 280 nm for Capsaicin, allowing for the observation and quantification of drug release kinetics [8, 9, 10].

FIG. 2: FRANZ DIFFUSION CELL APPARATUS

Anti-Inflammatory Activity by Carrageenan Induced Paw Edema [10]: The evaluation of anti-inflammatory activity in Wistar rats involved employing the Carrageenan-induced Paw Edema method using prepared Polyherbal films containing Curcumin and Capsaicin, comparing their effects with a standard Diclofenac Patch. The permission was given by Institutional Animal Ethical Committee having the Resolution No. KLECOP/CPCSEA- Reg. No.221/Po/Re/S/2000/CPCSEA. A 1% w/v Carrageenan suspension was induced in the rats' right hind paw, and after observing inflammation, the paw volume was measured initially and after treatment using a Plethysmometer for each rat. The study design, as presented in **Table 1**, delineated the groups and their respective treatments: Group I received 1% w/v Carrageenan, Group II was administered 1% w/v Carrageenan along with the Diclofenac Patch (Standard), Group III received 1% w/v Carrageenan along with Film-A (75:25 ratio), Group IV received 1% w/v Carrageenan along with Film-B (50:50 ratio), and Group V received 1% w/v Carrageenan along with Film-C (25:75 ratio). Each group consisted of six rats, forming the basis for assessing the anti-inflammatory effects of the prepared polyherbal films compared to the standard Diclofenac Patch.

TABLE 1: STUDY DESIGN FOR ANTI-INFLAMMATORY ACTIVITY IN WISTAR RATS BY CARRAGEENAN INDUCED METHOD

S. no.	Groups (n=6)	Treatment
1	Group-I	1%w/v of CI
2	Group-II	1%w/v of CI + Diclofenac Patch (Standard)
3	Group-III	1%w/v of CI + Film -A (75:25)
4	Group-IV	1%w/v of CI + Film -B (50:50)
5	Group-V	1%w/v of CI + Film –C (25:75)

RESULTS:

Extraction and Fractionation of Capsaicinoids: In the fractionation of Capsicum annuum extract, the dichloromethane fraction was isolated for subsequent evaluation and the formulation of a film. **Fig. 2** presents the dichloromethane fraction obtained from the *Capsicum annuum* extract (Practical yield-41gm and percentage yield- 82%), showcasing its properties and characteristics crucial for further analysis and application in film formulation.

Phytochemical Screening of *Curcuma Longa* and *Capsicum annum* Extract: The ethanolic extract of *Curcuma longa* and *Capsicum annum* extract shows following results, described in **Table 2.**

TABLE 2: PHYTOCHEMICAL SCREENING OF *CURCUMA LONGA* AND *CAPSICUM ANNUM*

Phytochemical Test		Observation	*Curcuma longa* extract	*Capsicum annum* extract
Alkaloids	Dragendorff's test	Orange brown ppt	+++	+++
	Mayer's test	Gives ppt	++	++
	Hager's test	Yellow ppt	++	++
	Wagner's test	Reddish- brown ppt	++	+++
Flavonoids	Lead acetate test	Yellow ppt	+	++
	Shinoda test	Orange, yellow, pink, purple colour	++	++
	NaOH + conc. H_2SO_4	NaOH-colouration H_2SO_4- decolouration	+++	+++
Carbohydrates	Molisch's test	Violet ring at the junction of 2 liquids	-	++
	Fehling's test	Brick red ppt	-	+
	Benedict's test	Green, Yellow or Red	-	+
	Bromine water test	Decolouration	++	-
	5% $FeCl_3$ test	Deep blue-black ppt	++	-

Tannins	Acetic acid test	Red colour	+++	-
	Dil. HNO_3 test	Reddish – yellow colour	+	+
Polyphenols	Lead acetate test	White ppt	++	+
Terpenoids	Salkowski test	Chloroform layer- red Acid layer-greenish yellow fluorescence	++	++
	Biuret test	Violet/Pink colour	++	-
Proteins	Millon's test	Red/brick red ppt	++	-

Phytochemical Screening of Ethanolic *Curcuma longa* Extract and Ethanolic *Capsicum annum* Extract: *Curcuma longa* extract showed positive presence for alkaloids, flavonoids, tannins, polyphenols, terpenoids, and proteins, while *Capsicum annum* extract exhibited alkaloids, flavonoids, tannins, and polyphenols. *Capsicum annum* extract also displayed the presence of carbohydrates, but terpenoids were not detected Shown in **Table 2.**

Pre-formulation Studies:

Compatibility Study:

Differential Scanning Colorimetry (DSC): The Differential Scanning Colorimetry (DSC) analysis of pure Curcumin and Capsaicin revealed distinct endothermic peaks at 177.02°C and 63.24°C, respectively.

Fig. 3 Interestingly, when incorporated into the polyherbal film, both compounds maintained analogous peak temperatures of 174.58°C and 62.15°C, respectively, suggesting their stability within the film matrix.

Additionally, an extra peak at 124°C in the polyherbal film's DSC thermogram possibly signifies the influence of the polymer/excipients used in the formulation.

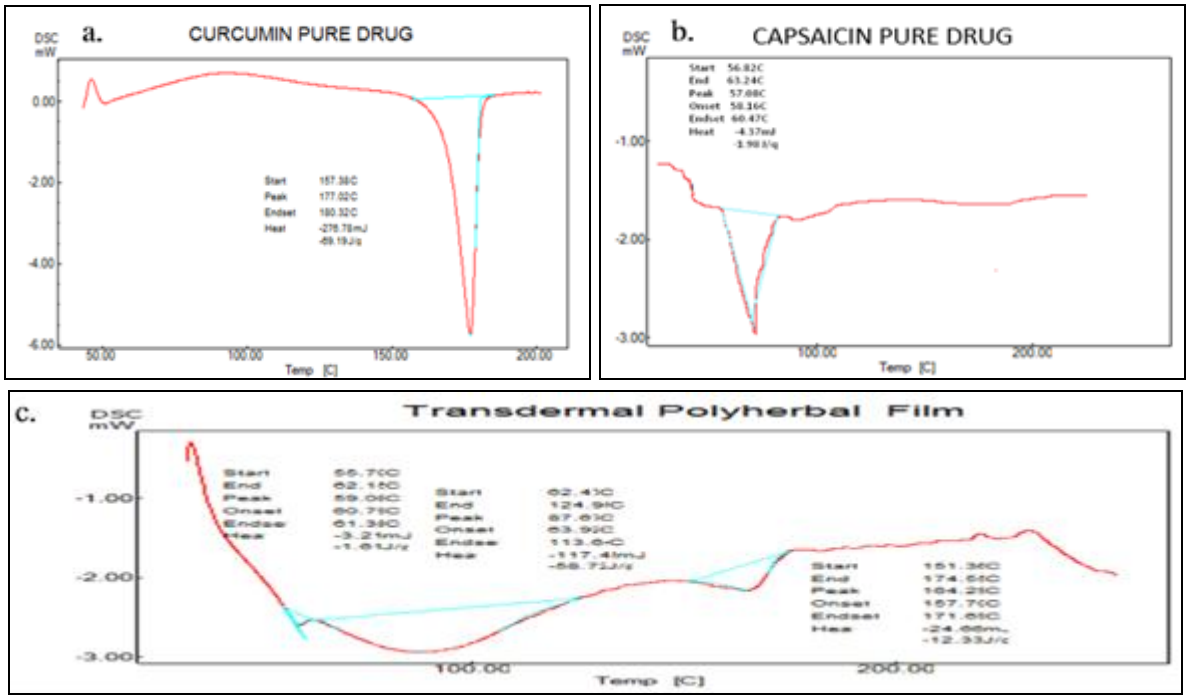

FIG. 3: (A) DSC THERMOGRAM FOR PURE CURCUMIN, (B) DSC THERMOGRAM FOR PURE CAPSAICIN & (C) DSC THERMOGRAM FOR TRANSDERMAL POLYHERBAL FILM

Fourier Transform infrared spectroscopy (FTIR): The Fourier Transform Infrared spectroscopy (FT-IR) investigations underscored the compatibility of Curcumin and Capsaicin with the employed excipients. Comparison between the pure drugs and their combinations with the polymer revealed no substantial alterations in functional groups. **Table 3** The FT-IR spectra showcased consistent profiles, affirming the absence of significant interactions between the drugs and the excipients, thereby supporting their compatibility within the formulation **Fig. 4.** This comprehensive analysis highlights the thermal stability of Curcumin and Capsaicin within the polyherbal

film, reinforcing their compatibility with the matrix components. The findings from both DSC and FT-IR analyses collectively indicate the promising suitability of these compounds within the formulated polyherbal transdermal film, fostering potential applications in pharmaceutical formulations.

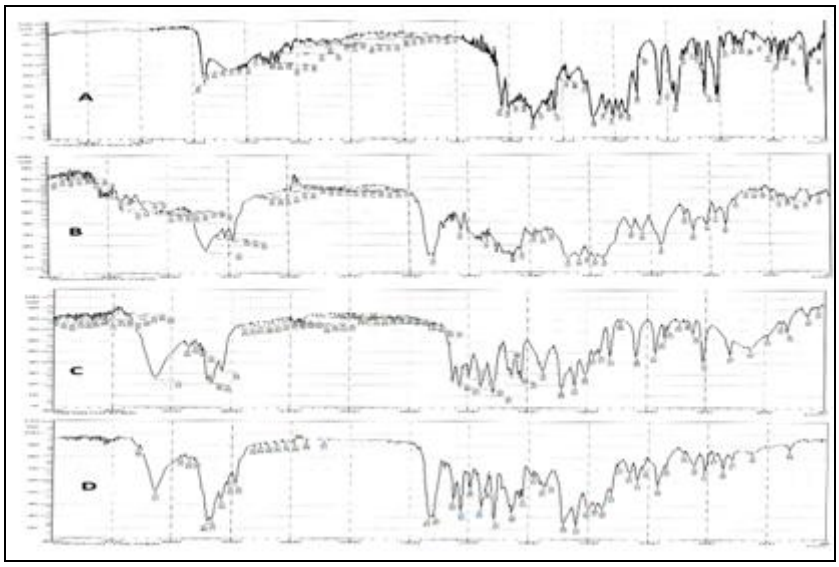

FIG. 4: (A) FT-IR SPECTRA OF PURE CURCUMIN, (B) FT-IR SPECTRA OF CURCUMIN + POLYMER/ EXCIPIENTS, (C) FT-IR SPECTRA OF PURE CAPSAICIN, (D) FT-IR SPECTRA OF CAPSAICIN + POLYMER/EXCIPIENTS

TABLE 3: DATA OF FT-IR SPECTRA

Functional Group	IR-Range	Curcumin	Curcumin + Polymer	Capsaicin	Capsaicin + Polymer
O-H Stretch (Alcohols & Phenols)	3500-3200 cm^{-1}	3236 cm^{-1}	3246 cm^{-1}	-	-
O-H Stretch (Carboxylic acid)	3300-2500 cm^{-1}	-	-	3084.18	3080 cm^{-1}
C=C Stretch	1512-1640 cm^{-1}	1544.49 cm^{-1}	1541 cm^{-1}	1597 cm^{-1}	1597 cm^{-1}
C=O Stretch		1718.58 cm^{-1}	1758.18 cm^{-1}	1657 cm^{-1}	1732 cm^{-1}

***In-silico* Molecular Docking Study:** In the realm of molecular docking studies, the interaction dynamics between compounds and specific biological targets offer critical insights into their therapeutic potential. Evaluating the binding affinities of Capsaicin and Curcumin with Cox-2, the data revealed intriguing nuances. Capsaicin exhibited a varied binding affinity range of -5.2 kcal/mol to -4.5 kcal/mol, demonstrating its potential interactions with Cox-2. Visual representations in both 3D and 2D interactions illustrated key bonding with essential residues NH-GLU: 465 and NH-SER: 463. Figure 6: Conversely, Curcumin showcased a binding affinity range from -8.8 kcal/mol to -7.2 kcal/mol, suggesting robust interactions with the Cox-2 enzyme. Detailed analysis through 3D and 2D representations highlighted binding interactions with crucial residues like OH-ALA: 385. These findings underscore the potential therapeutic relevance of both Capsaicin and Curcumin, showcasing their ability to interact with Cox-2, thus opening avenues for further exploration in pharmaceutical research aimed at Cox-2 targeted therapies **Table 4.**

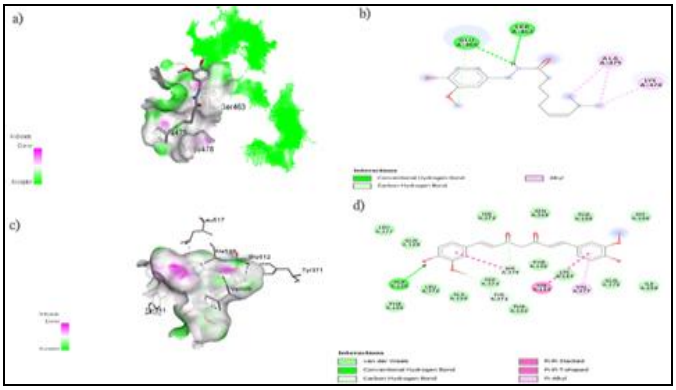

FIG. 5: (A) 3D INTERACTION OF CAPSAICIN WITH COX-2, (B) 2D INTERACTION OF CAPSAICIN WITH COX-2 (CAPSAICIN – NH- - - -GLU: 465, CAPSAICIN – NH - - - -SER: 463) (C) 3D INTERACTION OF CURCUMIN WITH COX-2 & (D) 2D INTERACTION OF CURCUMIN WITH COX-2 (CURCUMIN- OH- - - - ALA; 385)

TABLE 4: BINDING AFFINITY (KCAL/MOL) OF CAPSAICIN AND CURCUMIN WITH COX-2 (PDB ID: 3LN1)

Ligand	Binding Affinity (kcal/mol)	Rmsd/ub	Rmsd/lb
3ln1_capsaicin	-5.2	0	0
3ln1_capsaicin	-5	7.674	5.289
3ln1_capsaicin	-5	2.26	0.98
3ln1_capsaicin	-4.9	8.712	5.436
3ln1_capsaicin	-4.9	8.062	5.554
3ln1_capsaicin	-4.8	2.16	1.506
3ln1_capsaicin	-4.6	7.871	5.471
3ln1_capsaicin	-4.5	7.838	5.177
3ln1_capsaicin	-4.5	7.993	5.55
3ln1_curcumin	-8.8	0	0
3ln1_curcumin	-8.8	5.226	1.156
3ln1_curcumin	-8.7	6.274	1.015
3ln1_curcumin	-8.4	5.33	1.323
3ln1_curcumin	-8	2.53	1.201
3ln1_curcumin	-7.7	19.23	15.223
3ln1_curcumin	-7.7	20.101	15.272
3ln1_curcumin	-7.4	19.945	15.119
3ln1_curcumin	-7.2	19.448	15.35

Preparation of Transdermal Polyherbal Film and Preparation of Solid dispersions using Curcuminoids -Eudragit ®EPO & Capsaicinoids - Eudragit ®EPO: Different concentrations like 1%, 2% and 3% of Chitosan were used for preparation of film. 2% of Chitosan gave better results than other. The prepared solid dispersions were used in the formulation of film and we were observed that film B **Fig. 6** shown the best result compared to other two films.

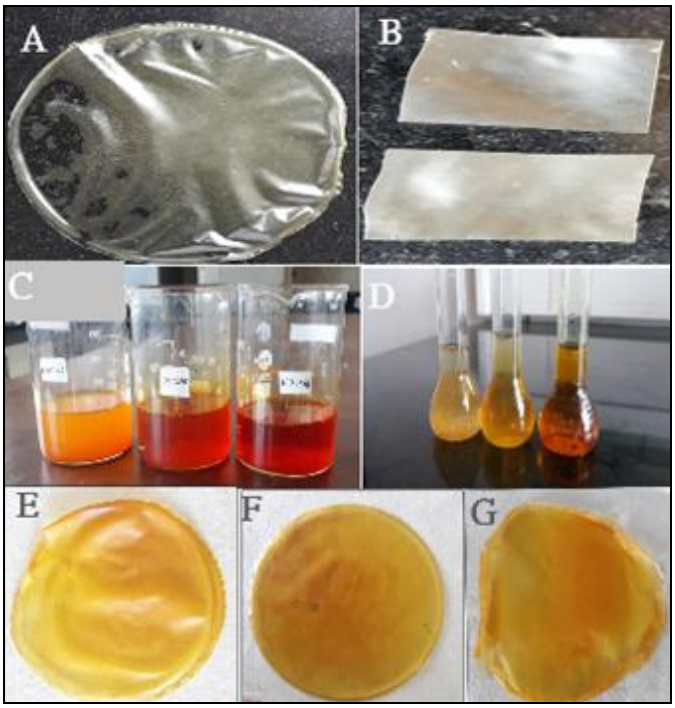

FIG. 6: (A & B) 2% CHITOSAN FILM, (C) PREPARATION ® OF SOLID DISPERSION OF CURCUMINOIDS: EUDRAGIT EPO (1:1, 1:2 & 1:3), (D) PREPARATION OF SOLID DISPERSION OF CAPSAICINOIDS: EUDRAGIT ®EPO (1:1, 1:2 & 1:3), (E), (F) & (G) FORMULATION OF CURCUMINOIDS- EUDRAGIT® EPO: CAPSAICINOIDS- EUDRAGIT® EPO OF FILM A (75:25), FILM B (50:50) & FILM C (25:75) RESPECTIVELY

Evaluation of Polyherbal Transdermal Films: The prepared polyherbal film were evaluated for weight variation, thickness, folding endurance, moisture content, moisture uptake and drug content results are described in **Table 5.** After the analysis of all data we were found that Film B was passed the criteria.

TABLE 5: EVALUATION OF POLYHERBAL FILMS

Film	Weight of the film (in mg)	Average weight	Thickness of the film (in mm)	Average weight	Folding endurance	Average	Initial weight (mg)	Final weight (mg)	Moisture Content (%)	Initial weight (mg)	Final weight (mg)	Moisture Uptake (%)	Curcumin Y= (Absorbance-0.004)/(0.154) X100	Drug Content (%)	Capsaicin Y= (Absorbance-0.005)/(0.007) X100	Drug Content (%)
A	90.2	80.1	0.14	0.15	247	247	90	81	11.11	82	85	3.75	2.28	91.20	71.57	95.42
B	97.2	89.8	0.12	0.17	254	254	95	87	9.19	88	91	3.40	4.83	97.73	49.57	99.14
C	87.4	91.7	0.11	0.13	271	271	87	79	10.12	80	84	5.00	7.79	90.53	23.28	93.12

***In-vitro* Drug Release Studies: Fig. 7A, B:** Describes the % cumulative drug release of Curcumin and Capsaicin respectively from the film by utilizing the Franz Diffusion Apparatus and obtained results were mentioned in **Table 6.**

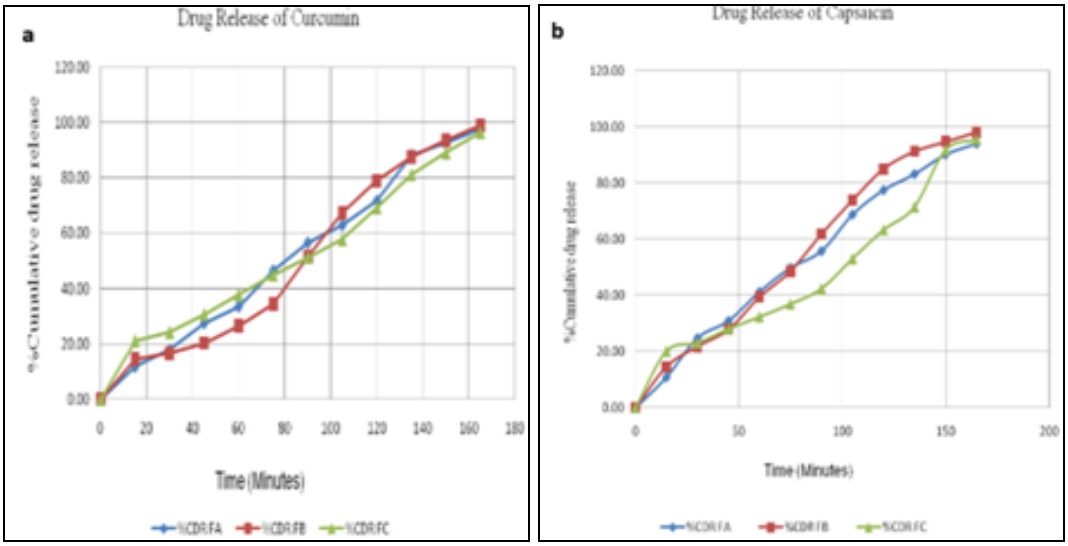

FIG. 7 (A) *IN-VITRO* CUMULATIVE DRUG RELEASE OF CURCUMIN IN FILM A, B & C AND (B) *IN-VITRO* CUMULATIVE DRUG RELEASE OF CAPSAICIN IN FILM A, B & C

TABLE 6: *IN –VITRO* CUMULATIVE DRUG RELEASE OF CURCUMIN AND CAPSAICIN FROM FILM A, B & C

Time	Curcumin (424nm)			Capsaicin (280nm)		
	%CDR FA	%CDR FB	%CDR FC	%CDR FA	%CDR FB	%CDR FC
0	0.00	0.00	0.00	0.00	0.00	0.00
15	11.88	14.73	21.27	10.94	14.77	20.21
30	17.91	17.78	24.34	24.89	21.87	23.12
45	27.72	20.39	30.77	31.00	27.91	28.09
70	33.77	27.73	37.88	41.35	39.47	32.40
75	47.58	34.41	44.97	49.90	48.74	37.97
90	57.73	51.50	51.28	55.91	71.91	42.47
105	72.98	77.29	57.71	78.88	73.94	53.08
120	71.72	78.94	79.15	77.59	85.12	73.33
135	88.14	87.52	81.10	83.30	91.29	71.51
150	92.59	93.49	88.95	90.24	94.77	91.78
175	97.78	99.01	97.35	94.13	98.17	95.54

Anti-Inflammatory Activity by Carrageenan Induced Paw Edema of Formulation A, B and C: Carrageenan induced rat paw edema test was employed in this study to evaluate the anti-inflammatory activity of formulation A, B and C. The paw volume was measured for Test and Standard groups by using Plethysmometer. The obtained results described in **Table 7** and % inhibition of paw edema shown in **Fig. 8.**

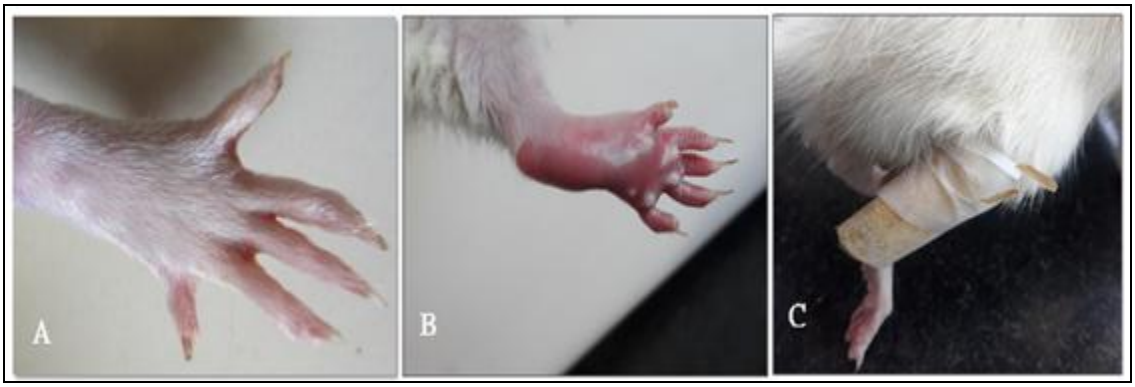

FIG. 8: NORMAL PAW OF RAT, B. PAW EDEMA OF RATS AFTER CARRAGEENAN INDUCTION & C. APPLICATION OF FILM ON RAT

TABLE 7: % INHIBITION OF EFFECT POLYHERBAL FILM (CURCUMIN AND CAPSAICIN) ON RAT PAW EDEMA

Time (min)	Diclofenac Patch (STD)	Curcumin: Capsaicin (75:25)	Curcumin: Capsaicin (50:50)	Curcumin: Capsaicin (25:75)
0	0	0	0	0
30	20.7	9.7	14.8	13.2
70	39.8	13.4	20.9	18.7
90	72.7	21.9	32.7	27.9
120	77.8	31.8	48.7	34.8
150	53.7	38.7	57.7	42.7
180	44.2	43.4	40.8	47.7
210	39.3	39.7	32.9	45.8
240	24.7	31.8	33.4	39.4

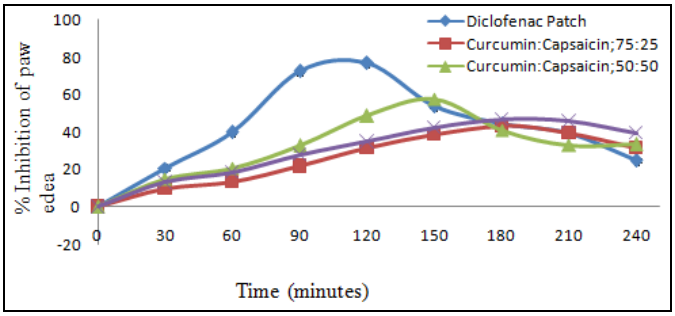

FIG. 9: % INHIBITION OF PAW EDEMA OF POLYHERBAL FILM IN WISTAR RATS. "Two- way Analysis of Variance (ANOVA) followed by *** = P < 0.001 compared with Standard group**.** # = P < 0.05 compared with Standard group"

DISCUSSION: In the present work attempt has been made to formulate and evaluate a transdermal polyherbal film of Curcuminoids and Capsaicinoids and to study its anti-inflammatory activity in Wistar rats. The transdermal polyherbal film (A, B & C) was prepared by using Curcuminoids and Capsaicinoids in three different ratios (75:25, 50:50 and 25:75) respectively by Solvent evaporation technique. The formulations were prepared by using 2% Chitosan and solid dispersion of Curcuminoids- Capsaicinoids and Eudragit® EPO. Utilization of Eudragit® EPO enhanced the solubility of Curcumin and Capsaicin from the film in buffer solution as well as through the skin. DSC and FT-IR were used to determine compatibility between the drug and excipients. We were found that there is no interaction between the drug and polymer used. The result showed compatibility between the drug and excipients used. *In-silico* molecular docking was done to predict the interaction of Curcumin and Capsaicin with Cox-2 protein, and it shows the good binding with Cox-2 Protein. The prepared polyherbal transdermal film was subjected to various quality control parameters. The *in-vitro* drug releases were performed by Franz Diffusion Cell by using Phosphate Buffer (pH-5.5). *In-vivo* anti –

inflammatory studies were done by Carrageenan induced paw edema method in Wistar rats. The prepared polyherbal films passed the entire QC test. Among 3 formulation batches; Film B has shown a good drug release pattern at 175 minutes of Curcumin and Capsaicin with 99.01 % and 98.17 % respectively. The *in-vivo* studies revealed significant reduction of paw edema in animal groups treated with Film B.

CONCLUSION: In the present research work it was found that the Capsicum *annuum* ethanolic extract gives good yield. The obtained extract was fractionated with Dichloromethane, citric acid, methanol and petroleum ether. Dichloromethane fraction showed the presence of alkaloid which identified as Capsaicin. The phytochemical screening of *Capsicum annuum* extract and *Curcuma longa* extract showed the presence of alkaloid, phenolic contents, flavonoids and terpenoids. The physicochemical compatibility studies were characterized by Differential Scanning Colorimetry, FTIR and it was found that there was no interaction between the drug and excipients.

The *in-silico* molecular docking was carried out by using autodock 4.0 to predict the interactions between the Curcumin and Capsaicin with Cox-2 protein. The pose having minimum binding affinity was chosen ligand- protein interaction and it was found Curcumin and Capsaicin binding affinity which ranges from -8.8 kcal/mol to -7.2 kcal/mol and -5.2 kcal/mol to -4.5 kcal/mol respectively. The 1, 2, and 3% of Chitosan were used for formulation of film; 2% of Chitosan film passed all quality control parameters. Formulation of Curcuminoids and Capsaicinoids film (film A, B and C) in three different ratios that is 75:25, 50:50 and 25:75 used respectively. The *in-vitro* studies were done by using Franz diffusion cell in Phosphate Buffer (pH-5.5). The prepared polyherbal film showed greater drug release due to solid dispersion of drug with Eudragit ®EPO. The UV Spectrophotometric technique was developed for the simultaneous estimation of Curcumin and Capsaicin in bulk and validated according to ICH guidelines. The developed method was found to be new, simple, selective, specific, precise, reliable and reproducible. All obtained results were found to be within the acceptance limit as per ICH guidelines. Hence it can be easily used for the routine quality control analysis of combined Curcumin and Capsaicin in bulk. The transdermal polyherbal film (A, B and C) showed Anti-inflammatory activity by inhibiting Carrageenan induced paw edema in Wistar rats. As per the obtained results from *in-vitro* drug release and *in-vivo* anti-inflammatory activity it was found that; polyherbal film of formulation B showed good results than A and C.

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Animal Ethics Approval: Applicable. (This work was permitted by Institutional Animal Ethical Committee. Resolution No. KLECOP/CPCSEA-Reg. No.221/Po/Re/S/2000/CPCSEA)

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