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Formulation And Evaluation Of Polyherbal Anti-Inflammatory Gel

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ABSTRACT

Inflammation is a very complex process and its pain affects millions of people worldwide. Approximately, 1-2% of the world population is affected by various inflammatory disorders and among them, women are three times more prone to the disease as compared to men. The present study was carried out to formulate and evaluate polyherbal gel formulation containing extracts of *Curcuma amada* and *Nyctanthes arbortristis* using carbopol-980 as gelling agents. Total six batches (F1to F6) were prepared and evaluated for physicochemical characteristics such as pH, viscosity, spreadability and drug content which were found within the range. All prepared formulations indicated good physical stability. *In-vitro* drug diffusion studies of formulations were performed by using Franz diffusion cell which shown percentage of total flavonoid release was 35.84% and percentage of total phenol release was 38.25 % for F5 batch formulation in five hours. The *ex-vivo* permeation study was carried out for optimized formulation using goat abdomen skin as barrier and shown percentage flavonoid release of 35.86 % and percentage phenol release of 27.32 % in F5 batch formulation in five hours. The anti-inflammatory activity was conducted by using carrageenan induced rat paw edema model. The prepared formulation shown good inhibitory effect. From the results, we concluded that the polyherbal gel formulation of herbal drug *Curcuma amada* and *Nyctanthes arbortristis* have been found to be effective for treating inflammation.

Keywords: *Curcuma amada, Nyctanthes arbortristis*, Carbopol 980, Anti inflammatory, Herbal gel, Spreadability.

1. Introduction

Herbal medicine is the oldest form of healthcare known to mankind. The first type of healthcare that humans have ever used is herbal medicine. Many of the pharmaceuticals that are currently in the market have either been directly or indirectly produced from plants, which have long been a great source of medication[1,2]. Approximately 2000 medications with a natural origin are included in Indian material, practically all of which are derived from various ancient systems and folkloric activities[3]. In the past, natural products particularly those made from higher plants have been crucial in the development of new pharmaceuticals[4]. According to estimates from the World Health Organization (WHO), traditional medicine serves as the major source of care for about 80% of the population in poor nations[5,6].

A perennial, rhizomatous herb with physical similarities to the turmeric-producing Curcuma longa species, Curcuma amada Roxb. grows up to 80 cm in height[7]. Due to the flavor of the rhizome resembling raw mango, this plant is also called as "Amahaldi," "Amba ada," and "Mango ginger" in common usage. It originated in the Indo-Malayan region and was then spread from Asia's tropics to Africa and Australia[8]. Since ancient times, C. amada has been used in Ayurveda and Unani, two traditional systems of medicine, as a coolant, appetiser, alexteric, antipyretic, aphrodisiac, diuretic, emollient, expectorant, and laxative, as well as to treat biliousness, itching, skin conditions, bronchitis, asthma, hiccups, and inflammation caused by injuries[9]. Because of its bitter, fragrant, cooling, astringent, and carminative properties, the C. amada rhizome is regarded as a potent stomachic. Paste made from rhizomes has also been used traditionally to treat cuts, wounds, and itching [12].

Nyctanthes arbortristis Linn. (NAT) is well known Indian medicinal plant. It is a strong traditional Indian medicine that is utilized extensively in the Ayurvedic, Siddha, Unani, and homoeopathic healing systems[10]. A widely distributed plant from Thailand, Nepal, South and North India, and Northern Pakistan is called *Nyctanthes arbortristis* Linn [13]. Children with roundworms and threadworms are treated with leaf juice to get rid of them. The leaf juice is also used as a diuretic and to treat piles, chronic fever, malarial fever, stubborn sciatica, rheumatism, and loss of appetite. When combined with honey and table salt, fresh leaf juice has been proposed as a safe purgative for infants [14, 15].

A topical drug delivery system allows for the administration of medication to treat a variety of conditions by applying it to a specific area of the body, usually the skin[16–18]. Since the skin is simple to approach, has a big surface area with extensive exposure to the circulatory and lymphatic networks, and the route is non-invasive, drug delivery through the skin has long been considered a promising idea. A natural or synthetic polymer that forms a three-dimensional matrix inside of a dispersion medium or hydrophilic liquid is what makes gel. When a substance is applied, the liquid evaporates, leaving the medicine bound in a thin layer of the gel-forming matrix that physically covers the skin[19].
The rigidity of a gel is caused by the presence of a network created by the interlocking of gelling agent particle particles. The nature of the particles and the kind of form that creates the links dictate the network's structure and the gel properties[17]. The steroidal and non-steroidal anti-inflammatory medications that are readily available have a variety of negative effects. In order to find natural anti-inflammatory drugs, numerous studies are being conducted.

2. Materials

Chemicals used during the present study were Petroleum ether, Ethyl acetate, Acetone, Methanol, Glacial acetic acid, Ethanol, Dragendroff's reagent, Folin Ciocalteu reagent, Sodium carbonate, Toluene, Chloroform, Formic acid, Acetic acid, Rutin, Stigmasterol, Hydrochloric acid, Sodium Hydroxide, Nitric acid, silver nitrate, and Magnesium stearate from LOBA chemicals, Mumbai, India.

3. Methods

3.1. Plant collection and authentication

Fresh rhizomes of *Curcuma amada* Roxb. and leaves of *Nyctanthes arbortristis* Linn. were collected from local areas of Nagpur. The collected rhizomes and leaves were authenticated by Dr. N.M. Dongarwar, Department of Botany. Nagpur University, Nagpur and the voucher specimen numbers were 10602 & 10603. The rhizomes and leaves were then washed with running water, dried under shade conditions and dried leaves are used for further processing.

3.2. Extraction of *Curcuma amada* Roxb.[20]

The continuous hot extraction process was used for the extraction. Dried rhizome was grinded into coarse powder. Approximately 250 g of dried *Curcuma amada* were taken into a thimble and placed in a Soxhlet apparatus. The process of defatting was done in a Soxhlet extractor with petroleum ether for 30 min to extract the fatty material. The defatted material was extracted in a Soxhlet apparatus for 8 hrs. separately with ethanol. The extract was filtered and evaporated finally to obtain a crude dried extract. The extracts were weighed and stored in a desiccator for further evaluation.

3.3. Extraction of *Nyctanthes arbortristis* Linn.[21]

The continuous hot extraction process was used for the extraction. Approximately 250 g of dried leaf powder were taken into a thimble and placed in a soxhlet apparatus. The process of defatting was done in a Soxhlet extractor with petroleum ether for 30 min to extract the fatty material. The defatted material was extracted in a Soxhlet apparatus with methanol for 8 hrs. The extract was filtered and evaporated finally to obtain a crude dried extract. The extracts were weighed and stored in a desiccator for further evaluation.

3.4. Phytochemical screening

3.4.1. Alkaloids[21]

A small portion of the solvent free extract were stirred separately with 5ml of 1.5% v/v of hydrochloric acid and filtered. The filtrate was tested with various test reagents for the presence of alkaloids

Mayer's test : 2-3 ml extract with few drops of Mayer's reagent gave precipitate.
Hager's test : 2-3 ml extract with few drops of Hager's test reagent gave yellow precipitate.
Wagner's test: 2-3ml extract with few drops of Wagner's reagent gave reddish brown precipitate.
Dragendroff's test : 2-3 ml extract with few drops of Dragendroff's reagent gave Orange brown precipitate.

3.4.2. Phenolic compounds and tannins[22]

Small quantities of the extract were taken separately in water then warm and filtered and test for the presence of phenolic compounds and tannins were carried out with the following reagents.

Lead acetate solution: White precipitation was observed
Acetic acid solution: Red color observed.
Potassium dichromate: Red precipitation was observed.
Dil. Iodine solution: Transient red color was observed.

Dil. Potassium permanganate solution: discoloration was observed.

3.4.3. Flavonoids[23]
With con. Sulphuric acid: To 2 ml of extracts, few drops of sulphuric acid were added. Yellow orange color was observed.
Shinoda's test: To dry powder of extract, 5ml of 95% ethanol, few drops of conc. HCl and 0.5 g magnesium turnings were added. Green color was observed.

3.4.4. Steroids[21]
Salkowski reaction: To 2 ml of extract, 2 ml of chloroform and 2ml conc. H_2SO_4 was added, chloroform layer appeared red and acid layer was observed with greenish yellow florescence.
Libermann – Burchard reaction: To 2 ml extract with chloroform, 1-2 ml acetic anhydride and 2 drops conc. H_2SO_4 were added from the side of the test tube. First red then blue and finally green color was observed.

3.4.5. Carbohydrate[24]
Molisch's test: To 2-3 ml of aqueous extract, few drops of alpha- napthol solution in alcohol and conc. H2SO4 were added from side of the test tube. Violet ring was formed at the junction of two liquids.
Fehling's test: 1ml of Fehling's A and Fehling's B solution, boiled for one min., then equal volume of extract was added. In boiling water bath for 5-10 min. first yellow, then brick red precipitate was observed.
Benedict's test: Mixed equal volume of Benedict's reagent and extract solution in test tube. In boiling water bath for 5 min. solution was observed green, yellow or red depending on amount of reducing sugar present in test solution.

3.4.6. Proteins[25]
Biuret test: To 3 ml of test solution 4% NaOH and few drops of 1% CuSO4 solution were added. Violet or pink color was observed.
Millon's test: To 3 ml of test solution 5ml of Millon's reagent was added. White precipitate turns brick red or the precipitate dissolved gave red color solution.
Xantho-protein test: To 3 ml of test solution with 1ml of conc. H_2SO_4. White precipitate is formed. Solution turns yellow. On addition of NH_4OH, precipitate turns orange.

3.5. TLC Fingerprinting[26]
The TLC plate containing the sample spot was placed at 45 ^{0} angle in the development chamber covering the bottom of the plate by the solvent up to nearly 1 cm. The solvent front was marked and the plate was finally allowed to dry. The colored substances were visual on the chromatogram. Colorless components were detected by using visualizing agent, iodine vapors. The qualitative evaluation of the plate was done by determining the migrating behavior of the separated substances given in the form of Rf value.

3.6. Fourier Transformed Infrared Spectroscopy (FTIR)
The physical mixture of drug and polymer were used for compatibility study by FTIR spectroscopy. The samples of pure drug and physical mixture of *Curcuma amada* & *Nyctanthes arbortristis* extract, carbopol 980 were scanned in solid state KBr dispersion medium and the scanning range was kept from 4000 to 400 cm-1.

3.7. Determination of total polyphenolic content[25, 27]
Curcuma amada ethanolic extract and *Nyctanthes arbortristis* methanolic extract in the concentration of 10 mg/10ml was used in the analysis. The reaction mixture was prepared by mixing methanol solution of both the extract and 2.5 ml of 10% Folin-Ciocalteu's reagent followed by addition of 2.5 ml 7.5% NaHCO3. Blank was prepared, containing 0.5 ml water, 2.5 ml 10% Folin- Ciocalteu's reagent and 2.5 ml of 7.5% of $NaHCO_3$. The samples were incubated in a thermostat at 45^{o}C for 45 min. The absorbance of test sample was determined by spectrophotometer at 765 nm. The same procedure was repeated for the standard solution of Gallic acid for calibration curve. Content of phenolic compounds in extracts was expressed in terms of gallic acid equivalent (mg of GAE/g of extract).

3.8. Determination of total flavonoid content[25]
The total flavonoid content of *Curcuma amada* extract and *Nyctanthes arbortristis* was determined by the aluminium chloride colorimetric method. In brief, 1ml of extract or a standard solution quercetin or rutin (20, 40, 60, 80 and 100 ug/ml) were added to a 10 ml volumetric flask, containing 4mL of distilled water. To the flask 0.3 mL of 5% $NaNO_2$ solution, 0.3 mL of 10% $AlCl_3$ solution was added after 5 min of incubation, and the mixture was allowed to stand for 6 min. Then, 2 mL of 1 mol/L NaOH solution were added, and the final volume of the mixture was brought to 10 mL with double-distilled water. The mixture was allowed to stand for 15 min, and absorbance was measured at 510 nm. The total

flavonoid content was calculated from a calibration curve, and the result was expressed as mg Quercetin equivalent per g dry weight.

3.9. Determination of total phytosterol content[26]

The total phytosterol content of *Curcuma amada* and *Nyctanthes arbortristis* extract was determined by the Lieberman-Burchard reagent. Stigmasterol was used as a reference standard. Stock solution was prepared by 10 mg of standard dissolved in 10 ml of chloroform (1000 µg/ml). Then different aliquots from 200-1000 µg/ml were prepared. 2ml Lieberman-Burchard reagent (freshly prepared) was added, Green color was observed. The mixture was allowed to stand for 30 min at dark place. The sample was prepared using different concentration of extract by using same procedure. The calibration curve was plotted by taking absorbance at 698 nm (Stigmasterol). The total phytosterol content was calculated and the results was expressed as mg stigmasterol equivalent per g dry weight

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3.10. Estimation of Curcumin content in *Curcuma amada* rhizome extract[27,28]

Stock solution of test sample was prepared by adding 10 mg of extract in 10 ml of ethanol in volumetric flask (1000 ug/ml). The solution was sonicated for about 10 minutes, filtered and absorbances taken by UV spectrophotometry at 424 nm. The percentage of curcumin content was found out from calibration curve of standard curcumin.

3.11. Formulation of Gel[29]

Weighted quantity of carbopol 980 was dispersed slowly in 50 ml of distilled water in 250 ml beaker. Then the mixture was stirred using mechanical stirrer at high speed to form a gel base. Required quantity of methyl and propyl paraben were dissolved in small quantity of distilled water on water bath then cooled and propylene glycol was added to it. Further, required quantity of *Curcuma amada* extract & *Nyctanthes arbortristis* extract were mixed to the above mixture and volume made up to 100 ml by adding remaining distilled water. All the ingredients were mixed properly with continuous stirring. Tri-ethanolamine was added drop wise to the formulation for the adjustment of skin pH and also to obtain a gel at required consistency (Table 1).

Table 1: Formulation of Gel

Ingredients	F1	F2	F3	F4	F5	F6
Carbopol 980(g)	0.5	1	1.5	0.5	1	1.5
Extracts %W/W	1	1	1	2	2	2
Propylene glycol 400 (ml)	5	5	5	5	5	5
Methyl paraben (g)	0.2	0.2	0.2	0.2	0.2	0.2
Propyl paraben (g)	0.1	0.1	0.1	0.1	0.1	0.1
Triethanolamine	q.s	q.s	q.s	q.s	q.s	q.s
Distilled water	Up to100 ml					

3.12. Evaluation of Gel

3.12.1. pH Determination[30]

The pH of various gel formulations was determined by using digital pH meter. One gram of gel was dissolved in 25 ml distilled water and stored for two hours. The measurement of pH of each formulation was done in triplicate and average values were calculated.

3.12.2. Viscosity[31]

Viscosity of herbal gel was determined by using Brookfield viscometer at 0.5 rpm, 1 rpm and 1.5 rpm using spindle no.6. Each reading was taken after equilibrium of the sample at the end of two minutes.

3.12.3. Spreadability[31]

Spreadability refers to the extent of area to which gel readily spreads on application. Spreadability was measured on the basis of slip and drag characteristics of gels. Two sets of glass slides of standard dimensions were taken. The herbal gel formulation was placed over one of the slides. The other slide was placed on the top of the gel, such that the gel was sandwiched between the two slides in an area occupied by a distance of 7.5 cm along the slides. An excess of gel (about 2 g) under study was placed on this ground slide. The gel was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide and provided. Weight of 1 kg was placed on the top of the slide for 5 minutes to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped off from the edges. The top plate was then subjected to pull and the time (in seconds) required by the top slide to cover a distance of 7.5 cm be noted. A shorter interval indicates better Spreadability.

Spreadability was calculated using the following formula:
$S = M \times L / T$

Where,
S = Spreadability,
M = Weight in the pan (To the upper slide) L = Length moved by the glass slide and
T = Time (in sec.) taken to separate the slide completely each other. The results are tabulated in table No.5.

3.12.4. Drug content[32]
Drug content was determined by dissolving accurately weighed 1 g of gel was taken in 25 ml of volumetric flask and diluted with methanol. The solution was filtered through whatmann filter paper. After suitable dilution, total flavonoid content and total phenol content were determined spectrophotometrically aluminium chloride colorimetric method and Folin-Ciocalteau method. Absorbance was recorded by using UV-visible spectrophotometer at 510 nm and 765 nm.

3.12.5. *In vitro* release study [32-34]
In vitro release study of gel were carried out by using dialysis membrane bag. 1 g of the gel was kept in bag submerged in a beaker containing phosphate buffer saline pH 7.4 at 37±1ºC temperature on a magnetic stirrer with continuous stirring at 100 rpm to maintain the sink condition of the receptor medium. The samples were withdrawn at specific time intervals from the aliquot reservoir and the same volume was replaced with similar pre-warmed aliquot at 0, 15, 30, 45, 60, 90, 180, 210 and 300 min. The samples were assayed spectrophotometrically to quantify the amount of flavonoid at 510 nm and phenol at 765nm released through the membrane.

3.12.6. *Ex-vivo* skin permeation studies[35]
Ex-vivo skin permeation studies were performed on goat skin. Fat and muscle debris of skin were removed by washing with phosphate buffer saline. The skin was placed on modified Franz diffusion cell and gel sample was applied on to the stratum corneum side of the skin facing towards donor compartment. Phosphate buffer (pH 7.4) was used as the permeation media. Topical gel was applied as a thin uniform film on the skin layer. The receptor compartments were maintained at the constant stirring of 100 rpm and 37°C. The samples were withdrawn at specific time intervals from the aliquot reservoir and the same volume was replaced with similar pre-warmed aliquot at 0, 15, 30, 45, 60, 90, 180, 210 and 300 min. The samples were assayed spectrophotometrically to quantify the amount of flavonoid at 510 nm and phenol at 765nm permeated through the membrane.

3.12.7. Stability study[35,36]
The stability study was performed as per ICH guidelines 6. The formulated gel were stored at 40^0 C ± 2^0C/ 75% ± 5% RH for the selected formulation for three months.

3.13. Anti-Inflammatory Activity[37,38]
Carrageenan induced rat paw edema
The carrageenan-induced hind paw edema test was performed according to Winter (Winter et al., 1962). Briefly, rats of either sex (Sprague–Dawley rats) of 150–200 g body weight were used. Animals were allowed to free access to feed and water before the experiment. Animals were divided into control, Standard and test group containing six animals in each group. Animals were injected subcutaneously into the plantar side of the hind paw with 0.1 ml of a freshly prepared 1% carrageenan suspension. Rat paw edema was assessed by volume displacement method (plethysmometer) before and after carrageenan injection each 10 min during the first hour and at 2, 3, 5, 7 and 24 h afterwards. The time course of the increase in paw swelling was determined by comparing it to the initial volume

To the test group required quantity of gel containing both the extracts were applied to the plantar surface of the right hind paw by gently rubbing with the index finger. Rats of the control groups received only the gel base, Diclofenac gel applied, in the same way was used as a standard. One hour after the application of the gel base, topical preparation of test extracts and standard, 0.1ml of 1% carrageenan solution in saline was administered into plantar surface of the right hind paw of rat. Paw volume was measured immediately after carrageenan injection and at 0, 1, 2, 3 and 4 hr up to 6 hr by using a plethysmometer.

The percentage of anti-inflammatory activity was calculated using the following formula:
Percentage inhibition = $(Vc – Vt)/Vc \times 100$.
Where, Vt is the paw volume of the test group,
and Vc is the paw volume of the control group.

4. Results and Discussion

4.1. Percentage Yield and Weight of Extracts

Table 2: Percentage Yield of Extracts

Extract obtained	Weight obtained (g)	Percentage Yield (%)
Ethanolic extract of *Curcuma amada* Roxb.	11.2± 0.02	6.5
Methanolic extract of *Nyctanthes arbortristis* Linn.	12.5± 0.01	8.3

The percent yield of ethanolic extract of *Curcuma amada* Roxb. was found to be 6.5% and methanolic extract of *Nyctanthes arbortristis* Linn. was found to be 8.3%.

4.2. Preliminary Phytochemical Screening

Phytochemical screening of various extracts of *Curcuma amada* & *Nyctanthes arbortristis* are shown in table 3

Table 3: Phytochemical screening of various extract

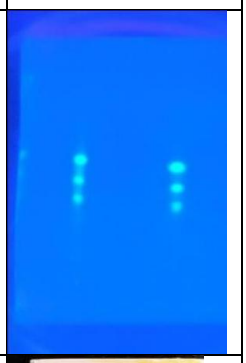
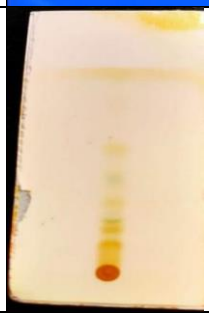
Phytochemicals	Ethanolic extracts Of *Curcuma amada*	Methanolic extracts of *Nyctanthes arbortristis*
A. Alkaloids		
Mayer's test Dragendroff's test	+	+
Wagner's test	+	+
Hager's test	+	+
	+	+
B. Tannins & Phenols		
Ferric chloride	+	+
Lead acetate	+	+
Bromine water	+	+
C. Amino acid		
Ninhydrin test	-	-
D. Flavonoids		
Shinoda test	+	+
Lead acetate test	+	+
E. Proteins		
Biuret test	-	-
Millon's test	-	-
F. Steroids		
Libermann-burchard test	+	+
Solkowski test	+	+
G. Carbohydrates		
Molisch's test	+	+
Benedict's test	+	+
Fehling's test	+	+

+ : Indicates the presence of phytochemical in extract, - : Indicates the absence of phytochemical in extracts. Ethanolic extract of *Curcuma amada* shown Alkaloids, Phenol, Flavonoid, Steroids and Carbohydrates. Methanolic extract of *Nyctanthes arbortristis* shown Alkaloids, Tannin, Phenol, Flavonoid and Steroids.

4.3. TLC Fingerprinting

Assessment of secondary metabolites were observed by TLC and it is shown in table 4.

Table 4: Thin layer chromatography

Sr. no.	SampleExtracts	Samples Developing solvents and visualizing agents	Rf value	TLC plate
1.	Ethanolic extracts of *Curcuma amada*	Chloroform: Ethanol: Glacial acetic acid (13.2 :0.69 :0.1)	0.48	
2.	Methanolic extracts of *Nyctanthes arbortristis*	Toluene: Acetone: Acetic acid (8.9: 0.9: 0.2)	0.4	
Compared TLC of ethanolic extract of *Curcuma amada* with standard Curcumin				
3.	Sample(T) Ethanolic Extractof *Curcuma Amada* Standard (S) Curcumin	Chloroform :Ethanol: Glacial acetic acid (13.2:0.69:0.1) Observed in UV chamber At 360nm	T–0.48 S-0.5	

4.4. Compatibility Study by FTIR Spectrum of Extract, Physical Mixture

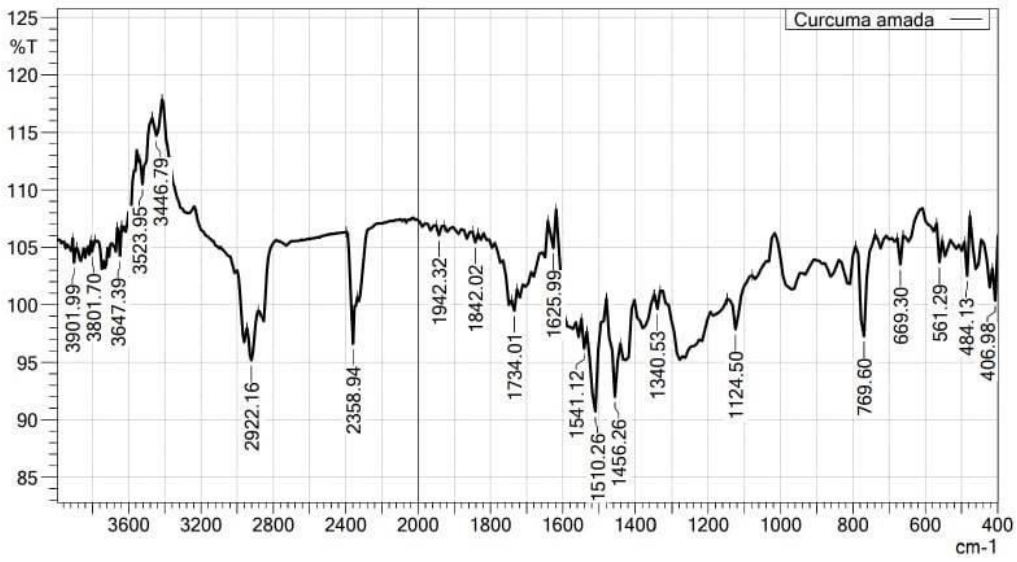


Figure 1: FTIR spectrum of *Curcuma amada*

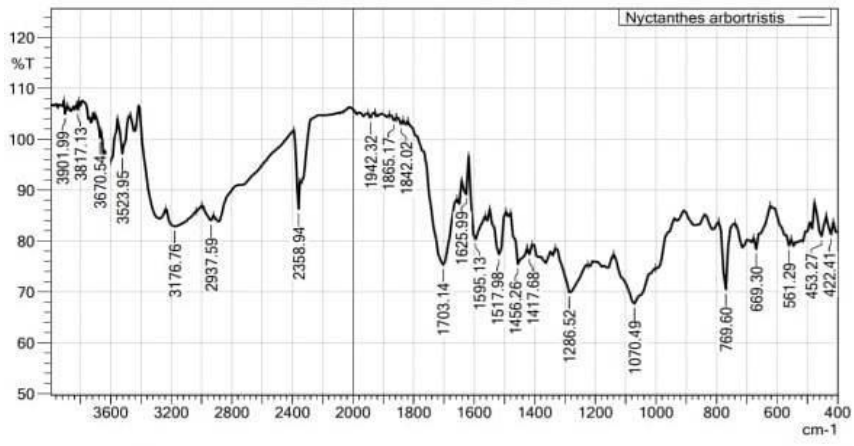


Figure 2: FTIR spectrum of *Nyctanthes arbortristis*

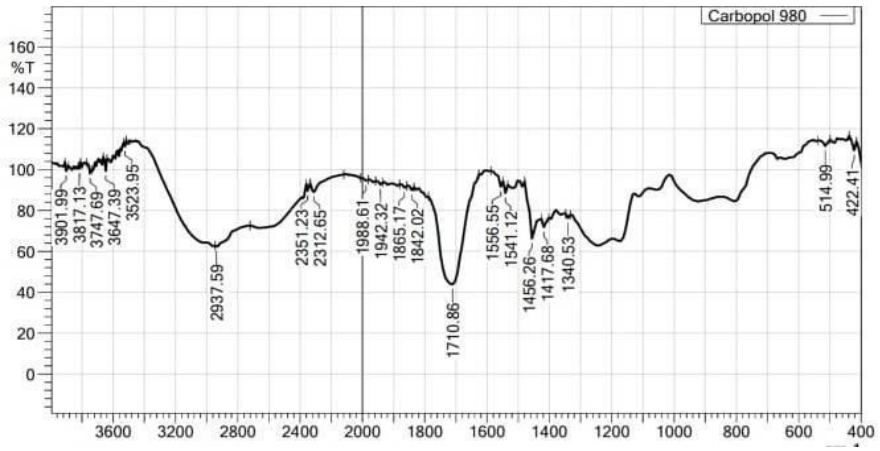


Figure 3: FTIR spectrum of Carbopol 980

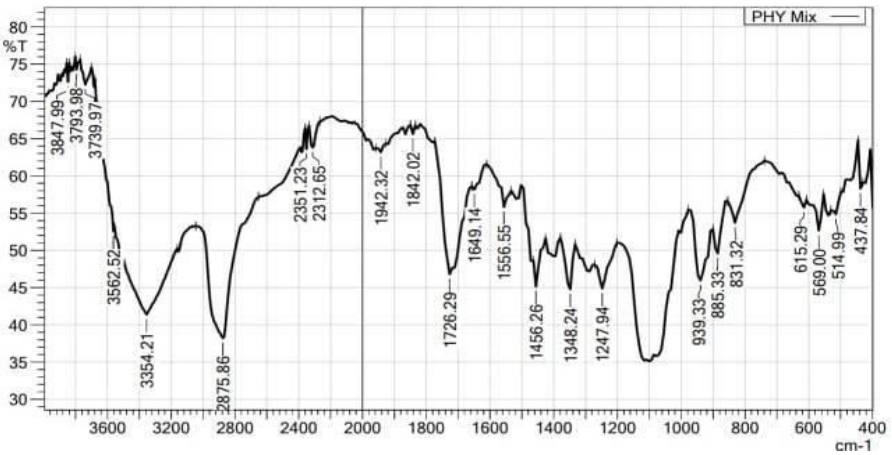


Figure 4: FTIR spectrum of *Curcuma amada* + *Nyctanthes arbortristis* + Carbopol980

FTIR Interpretation:

The FTIR spectrum analysis of the ethanolic extract of the rhizomes of Curcuma amada, methanolic extract of leaves of Nyctanthes arbortristis, polymer carbopol 980 indicates the presence of similar functional groups includes Hydroxyl group(O-H stretching) at 3600-3450cm^{-1}, C-H stretching at 2950-2850cm^{-1}, carbonyl group (C=O stretching) at 1750-1700cm^{-1}, alkene group(C=C stretching) at 1625-1445cm^{-1}. The results of FTIR analysis confirmed the presence of the carbonyl group, Hydroxyl group and alkene group.

Table 5: FTIR Interpretation

Sr.no.	Material	Standard range	Peak (cm^{-1})	Characteristic functional Group
1	Ethanolic extract	3600-3450	3523.95	O-H stretching
		2950-2850	2922.16	C-H stretching
		1625-1445	1510.26	C=C Stretching
		1750-1700	1734.01	C=O stretching
		1170-1050	1124.50	C-O stretching
2	Methanolic extract	1250-1310	1286.52	C-O stretching
		1740-1690	1703.14	C=O stretching
		2950-2850	2937.59	C-H stretching
3	Carbopol 980	2950-2850	2937.59	C-H stretching
		1725-1700	1710.86	C=O stretching
		3650-3450	3523.95	O-H stretching
4	Carbopol 980 + ethanolic extracts of *Curcuma amada* + methanolic extract of *Nyctanthes arbortristis*	3650-3450	3562.52	O-H stretching
		2950-2850	2875.86	C-H stretching
		1750-1700	1726.29	C=O stretching
		1250-1350	1247.94	C=C Stretching

4.5. Determination of total polyphenolic content

Total phenolic content was found to be 36.2 ± 0.213 and 38.2 ± 0.422 mg of Gallic acid /g in ethanolic extract of *Curcuma amada*. Total phenolic content was found to be 31.6 ± 0.156 and 34.2 ± 0.412 mg of Gallic Acid/g in methanolic extract of *Nyctanthes arbortristis*.

4.6. Determination of total flavonoid content

Total flavonoid content was determined by taking Rutin as standard. It was found to be 30.33±0.042 and 32.5± 0.056 mg of Rutin /g in Ethanolic extract of *curcuma amada*. Total flavonoid content were found to be 28.6±0.012 and 30.8± 0.035 mg of Rutin/g in Methanolic extract of *Nyctanthes arbortristis.*

4.7. Determination of total Phytosterols content

Total Phytosterol content were found to be 12.11±0.512 and 13.00± 0.323 mg of Stigmasterol/g in ethanolic extract of *curcuma amada*. Total Phytosterol content were found to be 8.7±0.436 and 9.80± 0.218 mg of Stigmasterol /g in methanolic extract of *Nyctanthes arbortristis*.

4.8. Curcumin content :

Total curcumin content in *Curcuma amada* rhizome was found to be 3.19% by taking curcumin as standard.

4.9. Evaluation of Topical Gel Formulations

Table 6: Evaluation of Topical gel formulation

Formulation batches	Color	Homogeneity	pH	Spreadability g.cm/sec
F1	Light Yellow	Excellent	6.5 ± 0.1	17.20 ± 0.1
F2	Light Yellow	Excellent	6.3 ± 0.1	15.32 ± 0.11
F3	Light Yellow	Excellent	6.73 ± 0.2	13.11 ± 0.1
F4	Light Yellow	Excellent	6.66 ± 0.15	19.32 ± 0.15
F5	Light Yellow	Excellent	7.0 ± 0.1	21.48 ± 0.1
F6	Light Yellow	Excellent	7.1 ± 0.2	20.12 ± 0.12

4.9.1 Viscosity

The viscosity of the prepared gel was determined using Brookfield Viscometer with Spindle no. 63 at 0.5rpm, 1 rpm, 1.5 rpm and the viscosities for all the formulation batches were given in the table 7.

Table 7: Viscosity of formulation

Formulation batches	Viscosity (cps) at rpm		
	0.5	**1.0**	**1.5**
F1	163400	146200	109200
F2	182400	97600	117000
F3	224600	168000	66200
F4	159800	138300	96500
F5	193100	132000	98400
F6	205800	146000	88300

4.9.2 Drug content

The drug content of gel formulation is indicated in following table that uniform amount of drug is present in all gel formulation.

Table 8: Drug content

Formulation batches	% Drug content of Phenol	% Drug content of flavonoid
F1	47.64 ±0.01	40.24 ±0.04
F2	50.35 ±0.04	46.49 ±0.01
F3	46.10 ±0.03	42.71 ±0.01
F4	49.54 ±0.01	45.02 ±0.06
F5	51.21 ±0.02	48.52 ±0.02
F6	48.12 ±0.02	44.40 ±0.03

4.9.3. *In vitro* drug release study

To investigate the effect of gel formulation bases on the release of phenolic and flavonoid compound, the *in vitro* release was conducted using a Franz diffusion cell. The *in vitro* release profile of different gel formulations batches is shown in Table 9 & 10.

Table 9: Percentage Cumulative Drug Release (%CDR) of phenol content

Time (min)	% CDR of F1	% CDR of F2	% CDR of F3	% CDR of F4	% CDR of F5	% CDR of F6
0	0	0	0	0	0	0
30	1.361±0.06	1.574±0.01	1.101±0.02	1.810±0.08	1.889±0.01	1.101±0.02
60	5.924±0.01	3.305±0.01	3.935±0.04	3.817±0.01	4.643±0.02	3.361±0.01
90	9.72±0.07	5.824±0.01	6.729±0.04	4.391±0.01	8.438±0.01	5.942±0.06
120	11.734±0.05	9.051±0.01	8.366±0.01	8.028±0.08	10.641±0.05	9.838±0.06
150	17.716±0.06	12.357±0.02	10.105±0.01	12.71±0.03	15.498±0.08	13.419±0.03
180	23.337±0.03	14.167±0.01	12.994±0.01	16.961±0.02	19.552±0.02	16.922±0.03
210	26.288±0.01	20.031±0.01	15.827±0.07	21.408±0.06	25.448±0.01	19.763±0.07
240	29.909±0.02	25.462±0.07	21.195±0.05	25.305±0.04	31.855±0.01	25.603±0.06
270	31.955±0.02	31.129±0.04	26.249±0.06	31.404±0.04	35.641±0.06	30.988±0.04
300	33.687±0.01	36.521±0.03	32.083±0.01	35.615±0.01	38.252±0.01	34.317±0.04

Gallic acid was taken as standard and the purpose of the release test is to calculate the potential amount of active ingredients that could be released from the gel base for each unit of time.

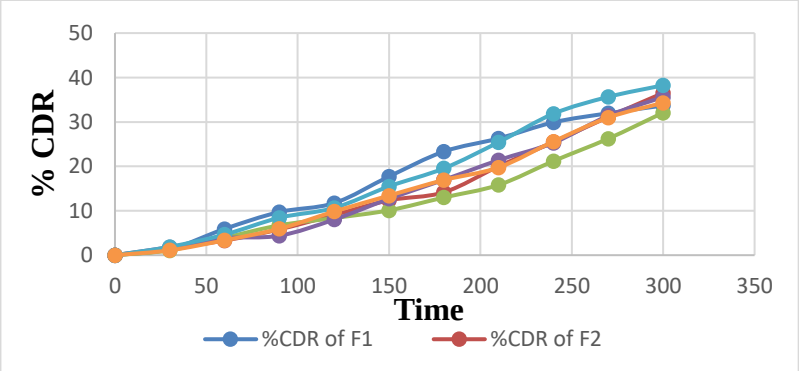


Figure 5 : Percent Cumulative Release of Phenol

It was observed that gel formulations F2 (36.51%) and F5 (38.25%) showed better drug release as compared to other formulations. The graph show that as the time increases, amount of total phenol released increased. As the concentration of polymer increased, the drug release gets decreased. This observation suggest that the entire amount of *Curcuma amada* and *Nyctanthes arbortristis* extracts loaded in the gel would be available for partitioning the stratum corneum, and hence, formulation F2 & F5 was selected for further studies.

Table 10 : Percentage Cumulative Drug Release (%CDR) of flavonoid content

Time (min)	% CDR of F1	% CDR of F2	% CDR of F3	% CDR of F4	% CDR of F5	% CDR of F6
0	0	0	0	0	0	0
30	0	0	0	0	0	0
60	1.085±0.03	1.751±0.01	1.861±0.03	1.396±0.08	1.861±0.02	1.706±0.08
90	2.869±0.02	3.102±0.01	3.412±0.03	2.869±0.08	4.412±0.01	2.180±0.01
120	5.197±0.02	4.731±0.02	4.188±0.01	6.360±0.02	6.670±0.04	4.731±0.01
150	15.358±0.01	13.962±0.07	7.446±0.07	14.349±0.01	12.488±0.01	11.014±0.03
180	21.874±0.04	18.926±0.07	13.463±0.07	17.219±0.06	16.289±0.08	14.815±0.02
210	25.674±0.04	24.821±0.01	19.039±0.04	20.012±0.06	19.779±0.02	20.710±0.06
240	28.312±0.06	30.291±0.01	22.710±0.04	25.054±0.06	27.924±0.03	26.450±0.04
270	31.026±0.02	32.190±0.03	26.364±0.06	31.182±0.07	33.586±0.04	28.328±0.04
300	33.276±0.02	34.138±0.04	31.026±0.07	33.362±0.05	35.844±0.05	30.190±0.08

Rutin was taken as standard for release of flavonoid content.

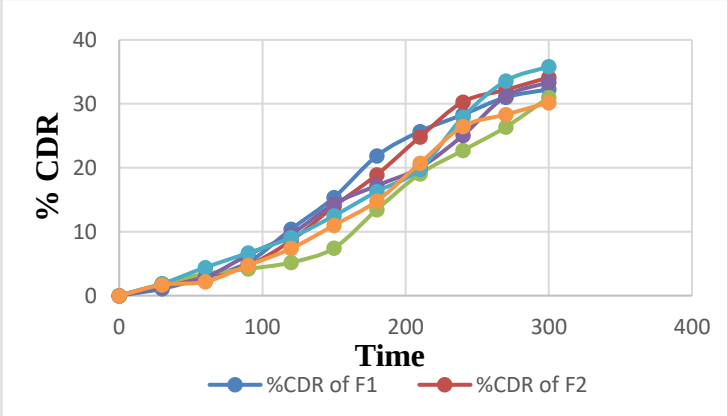


Figure 6 : Percent Cumulative Release of Flavonoid

Figure 6 shows the percentage of the total phenol released. The % drug release studies of patches (F1–F6) were carried out to observe the release of the drug from the patches.F2 (34.13%) and F5 (35.84%) formulations show better drug release when compared to other formulations.

4.10. Ex vivo diffusion study

The permeation parameters for *Curcuma amada* R. and *Nyctanthes arbortristis* L. from the gel formulation are shown in table 11.

According to *in vitro* drug release study Formulation F2 & F5 shows the maximum release and hence *ex vivo* diffusion study was done with this F2 & F5 formulation.

Table 11: Permeation Profile of Gel

Time (min)	Permeation study of phenol		Permeation study of flavonoid	
	F2	F5	F2	F5
0	0	0	0	0
30	1.252±0.1	1.469±0.12	1.351±1.2	1.588±0.12
60	2.053±0.12	3.299±0.11	2.255±0.11	3.596±0.11
90	4.266±0.1	5.372±0.13	4.166±0.12	5.271±0.11
120	6.947±1.13	7.983±0.14	5.842±0.13	7.981±0.1
150	10.021±0.12	11.345±0.12	8.023±0.12	9.663±0.13
180	13.314±0.1	14.767±0.11	10.589±0.12	11.871±0.1
210	16.371±0.12	18.121±0.11	12.926±0.13	13.963±0.12
240	19.285±0.11	20.872±0.1	15.176±0.2	16.776±0.12
270	21.213±0.12	23.561±0.3	17.652±0.13	20.334±0.1
300	23.546±0.1	27.322±0.1	21.361±0.12	25.389±0.24

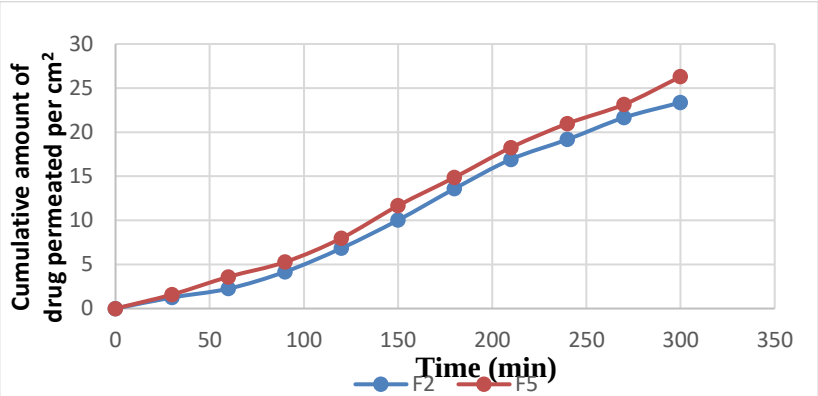


Figure 7: Permeation profile of phenol

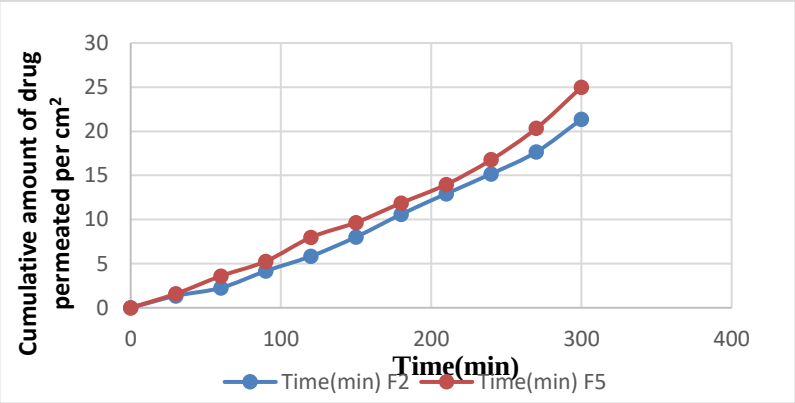


Figure 8: Permeation profile of flavonoid

Figure-7 & 8 shows the curve plotted in between the calculated amount of drug permeated per unit area across the skin and time. It was observed that the amount of gel permeated of phenol after 5 hr. F2 batch was 23.54±0.1 µg cm^{-2}& F5 batch was 27.32±0.1 µgcm^{-2}. The amount of gel permeated of flavonoid after 5 hr. F2 batch was 21.36±0.12 µg cm^{-2}& F5 batch was 25.38±0.24 µgcm^{-2}.

Flux (Js) & Permeability coefficient (Kp) of herbal gel formulation

Table 12: Flux & Permeability Coefficient of Herbal Gel Formulation

Parameters	**F2**	**F5**	**F2**	**F5**
Flux (µg/cm^2/hr)	9.9×10^{-2}	1.1×10^{-1}	9.0×10^{-2}	1.0×10^{-1}
Kp(cm/hr)	1.9×10^{-2}	2.2×10^{-2}	1.8×10^{-2}	2.0×10^{-2}

4.11. Anti inflammatory activity

The subplantar injection of carrageenan produced a local edema that increased progressively to reach its maximum in 3–5 h. At this time, the volume of the injected paw was almost 100% greater than the initial measured before carrageenan, afterwards herbal gel and standard gel formulations showed better reduction in paw volume measurement as compared to control group the edema decreased

Statistical analysis was done by Numen's Kuel method and the results showed that the edema inhibition of preparations containing extracts shows significant result when compared with controlled group. ANOVA analysis show that both the formulation (test and standard) were significantly different from control group.

Table 13: Paw volume of rats (ml)

Groups	Treatment	Paw volume (ml)					
		0h	1h	2h	3h	4h	5h
Group I	Control	1.49±0.009	1.50±0.01	1.52±0.01	1.54±0.008	1.51±0.01	1.50±0.01
Group II	Control + Base	1.47±0.01	1.49±0.01	1.50±0.009	1.53±0.02	1.52±0.03	1.48±0.02
Group III	Dose I	1.43±0.01	1.45±0.03*	1.47±0.01*	1.48±0.02**	1.46±0.02*	1.44±0.03*
Group IV	Dose II	1.41±0.01	1.43±0.02*	1.44±0.03*	1.46±0.02**	1.45±0.03*	1.42±0.04*
Group V	Standard	1.40±0.01	1.42±0.02**	1.43±0.008**	1.45±0.05**	1.44±0.04**	1.41±0.01**

*P value<0.05 compared to control group

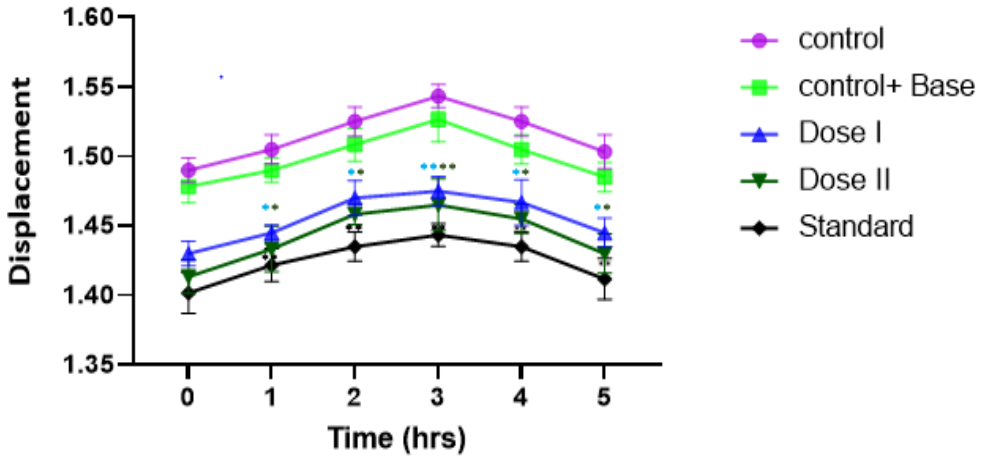


Figure 9: Paw volume of rats (ml)

4.12. Stability Study

The colour and appearance, pH, viscosity and % drug content of optimized batch are shown in table 14

Table 14: Accelerated Stability Study of Optimized Batch F5

Parameter	Storage (in month)			
	Initial	**1st month**	**2nd month**	**3rd month**
Colour	Light yellow	Light yellow	Light yellow	Light yellow
pH	7.0±0.1	7.1±0.1	6.9±0.05	7.1±0.1
Viscosity	193100±26.21	177900±33.29	178500±15.96	167000±11.23
% drug content	51.21±0.02	50.32±0.01	51.19±0.1	50.58±0.02

From table 14 it can be concluded that their was no significant changes in formulation on storage.

5. Conclusion

In the recent years, topical drug delivery has been used extensively to impart better patient compliance. Natural remedies are more acceptable in the belief that they are safer with fewer side effects than the synthetic ones. Hence; Herbal formulations have growing demand in the world market. The present study was an attempt to formulate and evaluate the topical herbal gel using plant extract. Ethanol extracts *of Curcuma amada* rhizome and Methanol extract of *Nyctanthes arbortristis* leaf showed the presence of flavonoid content and phenolic content. Topical herbal gels containing combination of *Curcuma amada* and *Nyctanthes arbortristis* extracts at different concentration (1% and 2%) can be successfully prepared using carbopol-980 as gelling agents. The study revealed that the developed polyherbal gel formulation consisting 2% concentration of extracts comparatively better results than other formulation and it shows the significant anti-inflammatory activity. Therefore it can be inferred that the possible inhibitory effect of aqueous extract of *Curcuma amada* Roxb. and *Nyctanthes arbortristis* Linn.. in carragenan induced inflammation may be due to inhibition of cycloxygenase leading to inhibition of prostaglandin synthesis.

REFRENCES:

1 Saha D, Guite D, Das T. A Complete Review on the Pharmacological Evaluation of Averrhoa Carambola Plant. World J. Pharm. Res. (2018) 12;7:199-210.
2 Lee H. Lazan H, Othman R, Ali. Purification and properties of a ß-from carambola fruit with activity towards cell wall. Phytochemistry, (2005) 66:153.
3 Dasgupta P, Chakraborty P, Bala N. Averrhoa carambola: an updated review. International Journal of Pharma Research & Review. 2013 Jul;2(7):54-63.
4 Chau CF, Chen CH, Lee MH. Characterization and physicochemical properties of some potential fibres derived from Averrhoa carambola. Food/Nahrung. 2004 Jan;48(1):43-46.
5 Palanivelu M, Ramlingam N, Ganesan B. Formulation and Evaluation of Herbal Gel Containing Averrhoa

carambola linn Fruit Extract. Asian Journal of Pharmaceutical and Health Sciences.2019;9(2):2084-2093.
6 Akter, J.; Takara, K.; Islam, M.J.; Hossain, M.A.; Sano, A. and Hou, D. (2019). Isolation and structural elucidation of antifungal compounds from *Curcuma amada*. Asian Pacific Journal of Tropical Medicine, 12(3): 123-129.
7 Tamta, A.; Prakash, O.; Punetha, H. and Pant, A.K. (2016).Chemical composition and in vitro antioxidant potentialof essential oil and rhizome extracts of *Curcuma amada*Roxb. Cogent Chemistry, 2(1): 1168067.
8 Sasikumar, B. (2005). Genetic resources of Curcuma: diversity, characterization and utilization. Plant Genetic Resources, 3: 230-251.
9 Policegoudra RS, Aradhya SM, Singh L. Mango ginger (Curcuma amada Roxb.)–A promising spice for phytochemicals and biological activities. Journal of biosciences. 2011 Sep;36(4):739-748.
10 Meshram MM, Rangari SB, Kshirsagar SB, Gajbhiye S, Trivedi MR, Sahane RS. Nyctanthes arbor-tristis a herbal panacea. International Journal of pharmaceutical sciences and research. 2012 Aug 1;3(8):2432.
11 Sasmal D, Das S, Basu SP. Phytoconstituents and therapeutic potential of *Nyctanthes arbortristis*. Pharmacognosy Reviews 2007; 1: 344-349.
12 Wallander E, Albert VA. Phylogeny and classification of Oleaceae based on RPS16 and TRNL-F sequence data. American Journal of Botany 2000; 87: 1827-1841.
13 Chidi BB, Pandeya S, Gharti KP, Bharati L. Phytochemical Screening and Cytotoxic Activity of Nyctanthes Arbor-Tristis. Indian Research Journal of Pharmacy and Science. 2015;5:205-17.
14 Biswas1 I, Mukherjee A. Pharmacognostic Studies On The Leaf Of Nyctanthes Arbor-Tristis, Acta Botanica Hungarica. 2011; 53(3):225-34.
15 M. W. Whitehouse, Antiinflammatory glucocorticoids drugs:reflections after 60 years. Inflammo Pharmacology.2011;19(1):119.
16. Jyothi D, Koland M. Formulation and evaluation of an herbal anti-inflammatory gel containing Trigonella foenum greacum seed extract. International Journal Pharm Pharm Sci. 2016;8(1):41-.44.
17. Norris DA. Mechanisms of action of topical therapies and the rationale for combination therapy. J Am Acad Dermatol 2005;53:17-25.
18. Yasir EN, Khashab AL, Yasir MK, Hamadi SA, Al-Waiz MM. Formulation and evaluation of ciprofloxacin as a topical gel. Asian J Pharm sci 2010;8:80-95.
19. Anonymous. Indian Pharmacopoeia. Vol. II. Controller of Publications, Edition 4th, New Delhi: Government of India; 1996.
20. Sawant SB, Bihani G, Mohod S, Bodhankar S. Evaluation of analgesic and anti-inflammatory activity of methanolic extract of curcuma caesia roxb. rhizomes in laboratory animals. Int J Pharm Pharm Sci. 2014 Apr;6(2):243-7.
21. Jayaprakasha GK, Selvi T, Sakariah KK. Antibacterial and antioxidant activities of grape (Vitis vinifera) seed extracts. Food research international. 2003 Jan 1;36(2):117-22.
22. Kandelwal KR, Pawar AP, Kokate CK, Gokhale SB. Practical Pharmacognosy Technique and Experiments. Nirali Prakashan. 1996.pp 9.1-9.15
23. Kokate CK, Purohit AP, Gokhale SB. Text book of Pharmacognosy. Pune: Nirali Prakashan. 2003;8(66):1-624.
24. Hossain MA, AL-Raqmi KA, Al-Mijizy ZH, Weli AM, Al-Riyami Q. Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown Thymus vulgaris. Asian Pacific journal of tropical biomedicine. 2013 Sep 1;3(9):705-10.
25. Araujo LB, Silva SL, Galvao MA, Ferreira MR, Araujo EL, Randau KP, Soares LA. Total phytosterol content in drug materials and extracts from roots of Acanthospermum hispidum by UV-VIS spectrophotometry. Revista Brasileira de Farmacognosia. 2013 Sep;23:736-42.
26. Kadam PV, Yadav KN, Bhingare CL, Patil MJ. Standardization and quantification of curcumin from Curcuma longa extract using UV visible spectroscopy and HPLC. J Pharmacogn Phytochem. 2018;7(5):1913-1918.
27. Pawar H, Karde M, Mundle N, Jadhav P, Mehra K. Phytochemical evaluation and curcumin content determination of turmeric rhizomes collected from Bhandara District of Maharashtra (India). Med. Chem. 2014;4(8):588-91.
28. Dev SK, Choudhury PK, Srivastava R, Sharma M. Antimicrobial, anti-inflammatory and wound healing activity of polyherbal formulation. Biomedicine & Pharmacotherapy. 2019 Mar 1;111:555-67.
29. Bhinge SD, Bhutkar MA, Randive DS, Wadkar GH, Todkar SS, Kakade PM, Kadam PM. Formulation development and evaluation of antimicrobial polyherbal gel. InAnnales Pharmaceutiques Françaises 2017 Sep1;75(5):349-358).
30. Jyothi D, Koland M. Formulation and evaluation of an herbal anti-inflammatory gel containing Trigonella foenum greacum seed extract. Int J Pharm Pharm Sci. 2016;8(1):41-.44.
31. KP MH, Saraswathi R, Mohanta GP, Nayar C. Formulation and evaluation of herbal gel of Pothos scandens Linn. Asian Pacific Journal of Tropical Medicine. 2010 Dec 1;3(12):988-92.
32. Partha N, Snigdha P, Laxmidhar M. Formulation development and in vitro evaluation of dental gel containing ethanol extract of Tephrosia purpurea linn. Int J Pharm Pharm Sci. 2016;8(8):132-41.
33. Gunsuang S, Jaipakdee N, Mahakunakorn P, Limpongsa E. Development of Semisolid preparations containing extract of Thai Polyherbal recipe for antiinflammatory effect. Int J App Pharm. 2019 Jul 7;11(4):345-53.

34. Jain P, Taleuzzaman M, Kala C, Kumar Gupta D, Ali A, Aslam M. Quality by design (Qbd) assisted development of phytosomal gel of aloe vera extract for topical delivery. Journal of Liposome Research. 2021 Oct 2;31(4):381-8.
35. ICH guidelines, Stability testing of new drug substances and products, 27th October,
36. Bajaj S, Singla D, Sakhuja N. Stability testing of pharmaceutical products. Journal of applied pharmaceutical science. 2012 Mar 24;2(3):129-138.
37. Dev SK, Choudhury PK, Srivastava R, Sharma M. Antimicrobial, anti-inflammatory and wound healing activity of polyherbal formulation. Biomedicine & Pharmacotherapy. 2019 Mar 1;111:555-567.
38. Winter, C.A., Risley, E.A., Nuss, G.W., 1962. Carrageenin induced edema in hind paw rat as an assay for antiinflammatory drugs. Proc. Soc. Exp. Biol. Med. 111, 544–547.