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RESEARCH ARTICLE

Development and Characterization of Topical Herbal Formulation of *Curcuma Longa* Extracts Using Plastibase Technology

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ABSTRACT

Turmeric (*curcuma longa*) was known for the Remedy as a healing agent for many years. The grinded rhizomes of *Curcuma longa* (L.) were extracted with methanol, methanol-water, and water respectively. These extracts were dried. The ointment formulations containing extracts of the turmeric in above-mentioned solvents were formulated, optimized and evaluated for various parameters and their wound healing activity was studied on experimentally induced excision wounds in Wistar albino rats. The use of various topical bases and its comparative study was performed with plastibase ointment. The plastibase was employed at three different concentrations (5%, 10% and 15% w/v). The amount of liquid paraffin was also optimized based in plastibase granules. The results showed that the ointment prepared using an alcoholic extract of *Curcuma longa* with plastibase granules revealed best-wound healing activity than ointment prepared from other extracts and marketed formulation. The results of short-term stability study showed stable characteristics of the developed formulation.

KEYWORDS

Curcumin, Plastibase, Wound healing action, Stability study

INTRODUCTION

India has a rich history of using plants for medicinal purposes. Turmeric (*Curcuma longa L.)* is a medicinal plant extensively used in Ayurveda, Unani and Siddha medicine as a home remedy for various diseases[2]. It is botanically related to ginger (Zingiberaceae family), is a perennial plant having a short stem with large oblong leaves and bears ovate, pyriform or oblong rhizomes, which are often branched and brownish-yellow in color. Turmeric is used as a food additive (spice), preservative and coloring agent in Asian countries, including China and South East Asia[4].

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It is also considered as auspicious and is a part of religious rituals. In old Hindu medicine, it is extensively used for the treatment of sprains and swelling caused by injury. In recent times, traditional Indian medicine uses turmeric powder for the treatment of biliary disorders, anorexia, coryza, cough, diabetic wounds, hepatic disorders, rheumatism, and sinusitis. The rhizome (root) part of the plant has also been used for centuries in Indian and Chinese traditional medicines and is the most valuable part of the plant for medicinal purposes[23]. The paste of curcumin mixed 68 with lime has been a popular home remedy for the treatment of inflammation and wounds. Curcumin is one of the three curcuminoids present in turmeric, making up 2 to 5% of the spice and approximately 77% of a singular extract [18].

The skin provides a natural barrier against the environment and exerts a variety of essential

protective functions[1]. When the integrity of the skin is compromised, either by acute or chronic injuries, the body initiates a multi-step and dynamic process at the injured site, leading to partial healing of the tissue and restoration of the skin's barrier function. The immediate goal in wound repair is to achieve tissue integrity and homeostasis[7]. The natural process of wound healing is comprised of four overlapping but well-defined phases: hemostasis, inflammation, proliferation and remodeling.

An optimum wound healing dressing or agent protects the wound tissue from bacterial infection, reduces inflammation and induces cell proliferation to aid in the reconstruction of damaged tissue[22]. It would ideally also act as an anti-oxidant as free radicals are considered the major cause of inflammation during wound healing process [21]. The wound healing potential of curcumin is attributed to its biochemical effects such as its anti-inflammatory, anti-infectious and antioxidant activities[13]. Curcumin has also been found to enhance cutaneous wound healing through involvement in tissue remodeling, granulation, tissue formation, and collagen deposition [19]. Various studies have shown that curcumin's application on wound also enhances epithelial regeneration and increases fibroblast proliferation and vascular density [19].

Plastibase is an ointment base made up of liquid hydrocarbons obtained from petroleum. This technology is available for a long time but not more familiar in public domain and thus its unique property remained unemployed. Thus in the present study platibase technology is employed as a semisolid base in the formulation of curcumin to overcome the pitfalls of existing curcumin formulations. [5, 6, 14, 16]

MATERIALS AND METHOD

Standardized *Curcuma Longa* was purchased from local market. Plastibase granules were procured from SUVIK Pharmaceuticals (Gandhinagar) as a gift sample. Methyl Paraben, Stearyl Alcohol, Glycerin, Polyethylene Glycol 400, Polyethylene Glycol 4000 were purchased from Rakesh chemicals. Double distilled water was used wherever required.

Extraction of Curcumin[20]

Three extracts of *Curcuma Longa* was prepared to obtain active constituent curcumin in concentrated form. The powder was prepared by grinding of dried rhizome of curcuma longa. Two hundred fifty (250) gm of dried powder of curcuma longa was weighed and taken into three different conical flasks I (Methanol-100%), flask II (Methanol: Water-70:30) and flask III (water-100%). Extraction solvent or solvent blend was added to the all conical flask. All three flasks kept overnight, covered with aluminum foil. The successive day all flasks content was heated up to 60°C for 1 with reflux arrangement of extraction assembly. The content was filtered and the filtrate was subjected to evaporate the solvent to obtain a solid residue of curcumin.

Phytochemical evaluation of curcumin extract

All extracts of curcuma longa were subjected to confirm the presence of curcumin and to study the presence of other chemicals. Thin layer chromatography and chemical tests were done for an analytical purpose. Alcoholic, hydroalcoholic and aqueous extracts were spotted on prepared aluminum TLC plate having silica G as stationary phase using chloroform: benzene: methanol: formic acid (8:1.5:0.5:0.5) as a mobile phase.

Chemical tests for curcumin[12]

Different chemical tests were performed to detect the presence of various phytoconstituents for all three extracts including with concentrated sulphuric acid, test with solution of sodium or potassium hydroxide, Dragendroff's test, Foam test (saponin), Molish test (carbohydrate), Fehling test, Liberman buchard test, Reaction with lead acetate (tannin),Phenolics, Test with NH3 (cumarin) and Borntragers test (anthraquinone).

Solubility study of curcumin

All extracts were studied for its solubility in different liquids used for the preparation of ointment base. One percent (1% w/v) of all three extracts was taken in different test tubes containing alcohol, water, PG, PEG 400 and liquid paraffin.

Formulation of curcuma extracts[15, 17]

Different topical formulations were prepared using variable topical bases. Emphasizing was given for plastibase formulations. The plastibase granules were dispersed in liquid paraffin. The above mixture was heated at more than 120°C with continuous stirring. The heating was continued till the granules were dissolved completely. The polymeric mass was immediately cooled down. The detail compositions of developed formulations are shown in Table 1.

Table 1: Formulation of ointment (Batch F1 – F20)

Batch	CA (gm)	LP (ml)	HP (gm)	PBG (%)	PEG 400 (ml)	PEG 4000 (gm)	CO (ml)	PG (ml)	WATER (ml)	ALC (gm)	AQU (gm)	HA (gm)
F1	1	25	5	-	-	-	-	-	15	-	0.5	-
F2	1	25	5	-	-	-	-	-	15	0.5	-	-
F3	1	25	5	-	-	-	-	-	15	-	-	0.5
F4	1	25	5	-	-	-	-	3	15	0.5	-	-
F5	1	25	5	-	-	-	-	3	15	-	-	0.5
F6	-	3	-	-	12.5	5	1.5	-	-	0.2	-	-
F7	-	3	-	-	12.5	5	1.5	-	-	-	0.2	-
F8	-	3	-	-	12.5	5	1.5	-	-	-	-	0.2
F9	-	3	-	-	12.5	5	1.5	-	1.5	-	0.2	-
F10	-	3	-	-	12.5	5	1.5	-	2.5	-	0.2	-
F11	-	3	-	-	12.5	5	-	-	2.5	-	0.2	-
F12	-	q.s. to make 100 ml	-	5	-	-	-	-	-	0.5	-	-
F13	-		-	10	-	-	-	-	-	0.5	-	-
F14	-		-	15	-	-	-	-	-	0.5	-	-
F15	-		-	5	-	-	-	-	-	-	0.5	-
F16	-		-	10	-	-	-	-	-	-	0.5	-
F17	-		-	15	-	-	-	-	-	-	0.5	-
F18	-		-	5	-	-	-	-	-	-	-	0.5
F19	-		-	10	-	-	-	-	-	-	-	0.5
F20	-		-	15	-	-	-	-	-	-	-	0.5

(In all formulation, Methylparaben was added at 1% CA=Cetyl alcohol, LP= Liquid paraffin, PBG: Plastibase granules, HP= Hard paraffin, PEG=Polyethylene glycol, CO= Castor oil, ALC=Alcoholic, HA=Hydro alcoholic, AQU=Aqueous, PG=Propylene glycol).

Characterization of curcumin loaded plastibase ointment

The developed curcumin plastibase ointment was evaluated for following parameters.

1. ***Organoleptic properties***

   The developed formulation was evaluated for color, texture, odor, water washability, skin irritation and ease of application.

2. ***Spreadability*** [3, 9]

   Spreadability of the formulation was determined by an apparatus suggested by Multimer et al., which was suitably modified in the laboratory and used for the study. It consists of a wooden block, which was provided by a pulley at one end. A rectangular ground glass plate was fixed on this block. An excess of ointment (about 3 gm.) under study was placed on this ground plate. The ointment was then sandwiched between this plate and another glass plate having the dimension of fixed ground plate and provided with the hook. One kilogram weight was placed on the top of the two plates for 5 minutes to expel air and to provide a uniform film of the ointment between the plates. Excess of the ointment was scrapped off from the edges. The top plate was then subjected to pull of weight with the help of string attached to the hook.

   The spreadability was calculated from the following formula:

   $S = (mx\ l)/t$

   Where, S = Spreadability, m = weight tied to the upper slid, l = length of the glass slid, t = time

3. ***Extrudability***[10]

   In the present study, the method adopted for evaluating ointment formulation for extrudability was based upon the quantity in the percentage of ointment and ointment extruded from the tube on the application of finger pressure. More quantity extruded better was extrudability. The formulation under study was filled in a clean, lacquered aluminum collapsible tube with a nozzle tip of 5 mm opening and applies the pressure on the tube by the help of finger. Tube extrudability was then determined by measuring the amount of ointment extruded through the tip when a pressure was applied to the tube.

4. ***pH***

   The pH of the formulation was measured by preparing 10% W/V aqueous solution of it and measured by digital p^H meter.

5. ***Microbial limit test***

   Optimized formulations were studied for the presence of *E.coli* and *B.subtilis.* The formulation (0.1 gm) was inoculated to nutrient broth aseptically in the aseptic hood. It was incubated for 48 hrs in an incubator at 35±2°C temperature.

6. ***Viscosity***

   The viscosity of the gel is enough that it can flow easily and easily extrude from the container. The viscosity of optimized formulation and the market product was measured by Brookfield viscometer.

7. ***Stability study***[8]

   The optimized formulation was checked for its stability at an ambient condition of pressure and temperature for three months. After that, it was checked for physical degradation (phase separation, particle agglomeration, color etc.)

8. ***Pharmacodynamics study (excision wound method)***[11, 24]

   As per the best physical, organoleptic properties and stability criteria, the most stable formulation was selected and it was extended to the animal study. The animal experiments were performed according to CPCSEA guidelines and after the approval from Institutional Animal Ethics Committee (I.A.E.C.) K. B. Institute of pharmaceutical education and research, Gandhinagar, Gujarat; experiments were conducted in accordance with the standard guidelines.

8.1 Animal used

Albino rats (Wistar strain; 150- 180 g) were obtained from the animal house of K. B. Institute of pharmaceutical education and research, Gandhinagar, Gujarat. Animals were kept in the animal caging system (four rats per cage on beds of sawdust) under the laboratory conditions (25 ± 2°C, 12 h light). They were provided with animal feed pellets manufactured by Hindustan Lever (India) Ltd. Mumbai. Animals were randomly selected for different experimental groups (3 animal/ group) and used for the *in vivo* determination of wound healing activity. During the course of the experiment, the animal behavior was normal.

8.2 Excision wound model

An excision wound model was used for studying wound healing activity. Female Wistar albino rats weighing 150-180 gm were randomly divided into 4 groups of 3 animals each. The back of the each animal was shaved and prepared after washing with spirit. An area of about 100 mm^2 was defined with a marker on the shaven back of the animals. The circular marked area was excised with its full thickness using a surgical sterile blade and scissors under ketamine anesthesia. The formulations were applied to the wounded rats of the respective groups, two times a day. The wound contraction was measured as the percentage of wound reduction in the wound area for every two days. (Charde et al, 2003; Sunilkumar et al, 1998).

The reduction in the wound size was calculated by the formula:

Wound contraction (%) = (A/B) * 100

A = Difference in the area of the wound in mm^2 between the initial and on a particular post-operative day, B = Area of the wound in mm^2 immediately after the wound excision.

RESULT AND DISCUSSION

Yield of curcumin

The yield of curcuma extracts is shown in Table.2.

Table.2: % Yield of extracts

S.N	Extracts	Yield	% yield
1	Alcoholic	14.5 gm	5.80 %
2	Hydro-alcoholic	12.1 gm	4.84 %
3	Aqueous	10.0 gm	4.00 %

The highest yield was obtained by extracting the powder in an only alcoholic solvent. Use of a mixture of water with alcohol decreased the yield. Only water as extracting solvent gave the lowest yield.

Phytochemical evaluation of curcumin extract

The spots of curcumin in TLC plate are shown in fig.1. The figure indicates the clear visible spots in TLC plates.

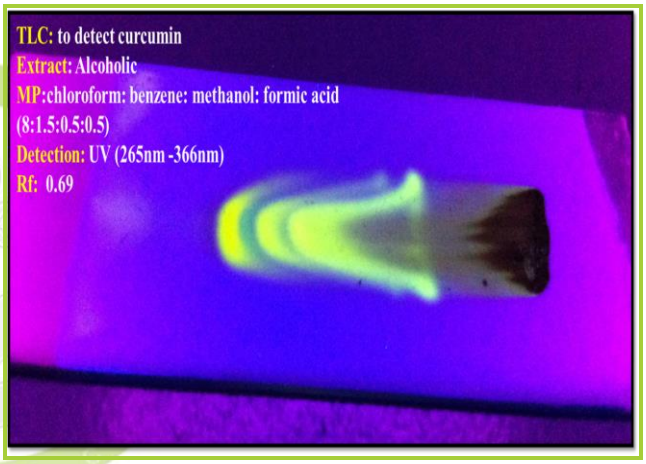

Figure.1: Curcumin spots in TLC plate

The retention factor (Rf) values of various spots of different extracts are given in Table 3.

Table 3: Rf values of different TLC spots

S. N	Extract	Standard Rf value	Experimental Rf value (nearest to Standard)
1	Alcoholic	0.68	0.69
2	Hydro-alcoholic		0.68
3	Aqueous		0.70

The Rf value of at least one solute front matched with the standard Rf value of curcumin for used mobile and stationary phase for all three extracts

of C*urcuma longa*. Thus TLC of all three extracts showed the presence of curcumin in UV light (265nm-366nm).

Chemical tests for curcumin

The observation and inference of chemical tests of all three extracts are revealed in Table 4. The results indicated the presence of curcumin in the all three extracts.

Table.4: Results of chemical tests of Curcumin

S N	Extract	Test	Observation	Inference
1	Alcoholic	Test with concentrated sulphuric acid.	Red color	Curcumin present in the extract
		Test with a solution of sodium or potassium hydroxide.	Red-violet color	Curcumin present in the extract
2	Hydro-alcoholic	Test with concentrated sulphuric acid.	Red color	Curcumin present in the extract
		Test with a solution of sodium or potassium hydroxide.	Red-violet color	Curcumin present in the extract
3	Aqueous	Test with concentrated sulphuric acid.	Red color	Curcumin present in the extract
		Test with a solution of sodium or potassium hydroxide.	Red-violet color	Curcumin present in the extract

Chemical tests for phytochemicals present in extracts

The results of the presence of various phytochemical constituents present in extracts are shown in Table.5.

Table.5: Presence of various phytoconstituents in extracts

Sr. No	Chemical test	Alcoholic extract	Hydro-alcoholic extract	Aqueous extract
1	Alkaloids	-	-	-
2	Saponin	-	+	+
3	Carbohydrates	-	+	+
4	Steroids & Triterpenoids	+	+	+
5	Tannins	-	-	-
6	Phenolics	+	+	+
7	Coumarins	+	+	+

(+: Present, -: Absent)

Solubility study of curcumin extracts

The results of the solubility study of all three extracts are shown in Table 6. From the results, it can be inferred that PG and PEG 400 can be used to improve solubility of active in the liquids used for the formulation because all extracts having solubilizing capacity in it.

Table.6: Results of solubility study of extracts

Sr. No	Extract	Alcohol	Water	PG	PEG 400	Liquid paraffin
1	Alcoholic	Soluble	Insoluble	Soluble	Soluble	Insoluble

2	Hydro-alcoholic	Soluble	Insoluble	Soluble	Soluble	Insoluble
3	Aqueous	Insoluble	Soluble	soluble	Soluble	insoluble

Physical characterization

Organoleptic characterization

The results of organoleptic evaluation of developed formulations are given in Table 7. The results indicate that as the amount of plastibase is increased in the formulation (15%), the appearance and quality of final product is decreased. More amount of plastibase in the product revealed oily appearance.

Table 7: Results of organoleptic evaluation of developed formulations

Batch	Colour	Texture	Odour	Irritability	Washability	Ease of application
F1	Yellow	Smooth	Characteristic Turmeric	No	Poor	Good
F2	Base incompatibility with active					-
F3						-
F4	Yellow	Smooth	Characteristic Turmeric	No	Poor	Good
F5	Yellow	Smooth	Characteristic Turmeric	No	Poor	Good
F6	Yellow	Smooth	Characteristic Turmeric	No	Easy	Good
F7	Base incompatibility with active					
F8	Yellow	Smooth	Characteristic Turmeric	No	Easy	Good
F9	Yellow	Moderately Hard	Characteristic Turmeric	No	Easy	Good
F10	Yellow	Moderately Hard	Characteristic Turmeric	No	Easy	Good
F11	Yellow	Moderately Hard	Characteristic Turmeric	No	Easy	Good
F12	Yellow	Smooth	Characteristic Turmeric	No	Easy	Good
F13	Yellow	Smooth	Characteristic Turmeric	No	Easy	Good
F14	Yellow	Smooth	Characteristics oily	No	Difficult	Good
F15	Yellow	Smooth	Characteristic Turmeric	No	Easy	Good
F16	Yellow	Smooth	Characteristic Turmeric	No	Easy	Good
F17	Yellow	Smooth	Characteristics oily	No	Difficult	Good
F18	Yellow	Smooth	Characteristic Turmeric	No	Easy	Good

F19	Yellow	Smooth	Characteristic Turmeric	No	Easy	Good
F20	Yellow	Smooth	Characteristics oily	No	Difficult	Good

Physicochemical characterization

The results of the physicochemical characterization of developed formulations are given in Table 8.

Table No 8: Results of physicochemical characterization of developed formulations

Batch	Spreadability (gm)	Film Formation	Extrudability	pH	Stability (Extemporaneous)
F1	20	Uniform, Thin	Good	7	Stable
F2	Base incompatible with active				Stable
F3					Stable
F4	20	Uniform, Thin	Good	7	Stable
F5	20	Uniform, Thin	Good	7	Stable
F6	80	Uniform, Thin	Good	7	Stable
F7	Base incompatible with active				Stable
F8	180	Uniform, Thin	Good	7	Stable
F9	173	Difficult to apply	Good	7	Stable
F10	189	Difficult to apply	Good	7	Stable
F11	170	Difficult to apply	Good	7	Stable
F12	21	Uniform, Thin	Poor	7	Stable
F13	25	Uniform, Thin	Poor	7	Stable
F14	26	Uniform, Thin	Poor	7	Stable
F15	24	Uniform, Thin	Good	7	Stable
F16	28	Uniform, Thin	Good	7	Stable
F17	25	Uniform, Thin	Good	7	Stable
F18	100	Difficult to apply	Poor	7	Stable
F19	120	Difficult to apply	Poor	7	Stable
F20	115	Difficult to apply	Poor	7	Stable

Microbial limit test

No microbial growth was detected in any of studied formulation.

Stability study

The results of stability study of the optimized formulation are depicted in Table 9. This study indicates the stable behavior of developed formulation for three months.

Table No 9: Results of stability study for optimized batch

Characteristics	Optimized batch (F13)			
	0 month	1 month	2 month	3 month
Spreadability	25	26	25	24
Film formation	Thin, Uniform	Thin, Uniform	Thin, Uniform	Thin, Uniform

Extrudability	Good	Good	Good	Good
pH	7	7	7	7
Sign of instability	No	No	No	No
Microbial growth	No	No	No	No

Pharmacodynamics study (Wound healing activity)

The diameter of wound developed in animals was measured every two days up to 14 days. The wound healing was expressed in % contraction in the wound and the results are depicted in fig.2.

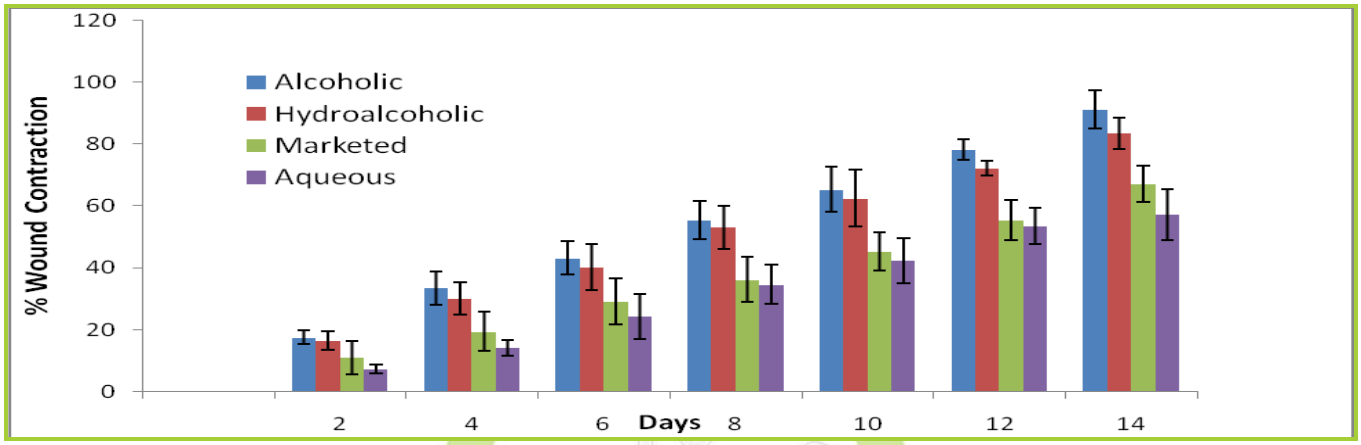

Figure.2: % Wound Contraction

Moreover, the fig.3 reveals the visual confirmation of wound healing. The wound healing effect can be ordered in the form of Alcoholic>Hydro alcoholic>Marketed>Aqueous. This confirms the capacity of alcoholic extract of *Curcuma longa* to heal wound and excision to a great extent.

Figure.3: Visual image of wound contraction at 14th day (A: Alcoholic, B: Hydro-alcoholic, C: Marketed, D: Aqueous

CONCLUSION

In a nutshell, results of chemical tests of extracts and TLC spotting confirmed the presence of curcumin. Extracts showed significant solubility in PG and PEG 400. Bioburden in all formulation was under the limit. Plastibase revealed better organoleptic properties compared to other common semisolid bases. Particularly, thin film formulation and better spreadability were attributed due to plastibase. Amongst three extracts alcoholic extract exhibited remarkable wound contraction compared to another extract at the end of 14th day of study.

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