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Formulation and evaluation of herbal cream containing extracts of *Murraya Koenigii* and *Cajanus Cajan*

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ABSTRACT

Herbal medicines are being used by about 80% of the world population primarily in the developing countries for primary health care. Herbal Plants such as *Murraya Koenigii* and *Cajanus Cajan* traditionally used for the treatment of wound healing activity. In the present investigation an attempt was made to prepare and evaluate the herbal cream comprising extracts of *Murraya Koenigii* and *Cajanus Cajan*. The herbal cream namely F1 to F6 were formulated from the ethanol extract of *Murraya Koenigii* and *Cajanus Cajan*. The extraction was done by the Soxhlation process. Formulation of Herbal Skin Cream for wound healing was successfully developed that met the relevant pharmaceutical characteristics. The prepared formulations are then evaluated for parameters like physical properties, pH, viscosity, Spreadability and stability of the formulated cream. The prepared formulations showed good Spreadability, no evidence of phase separation and good consistency during the study period. Stability parameters like visual appearance, nature, viscosity and pH of the formulations showed that there was no significant variation during the study period. The prepared formulations showed proper pH range that is approximately pH 6; it confirms the compatibility of the formulations with skin secretions. The creams were found to be stable during stability to ICH guidelines (40±2 °C/ 75±5 % RH) for 3 months. In-vitro Diffusion studied conducted on all the 6 formulations and F5 and F6 has shown good diffusion when compared to other formulations. Now it can be possible to develop creams containing herbal extracts and can be used as a barrier to protect skin.

Keywords: *Murraya Koenigii*, *Cajanus Cajan*, Beeswax, Liquid Paraffin, Tween 80, Stearic Acid.

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INTRODUCTION

Creams are the semisolid dosage forms and intended for topical application to the skin, placed on the surface of eye, or used nasally, vaginally or rectally for therapeutic or protective action or cosmetic function. These preparations are used for the localized effects produced at the site of their application by drug penetration in to the underlying layer of skin or mucous membrane. These products are designed to deliver drug into the skin in treating dermal disorders, with the skin as the target organ. [1]

Creams are semi-solid emulsions of oil and water. They are divided into two types: oil- in-water (O/W) creams which are composed of small droplets of oil dispersed in a continuous phase, and water-in-oil (W/O) creams which are composed of small droplets of water dispersed in a continuous oily phase. [2] Oil-in-water creams are more comfortable and cosmetically acceptable as they are less greasy and more easily washed off using water. Water-in-oil creams are more difficult to handle but many drugs which are incorporated into creams are hydrophobic and will be released more readily from a water-in-oil cream than an oil-in-water cream. Water-in-oil creams are also more moisturising as they provide an oily barrier which reduces water loss from the stratum corneum, the outermost layer of the skin. [3]

World Health Organization (WHO) as well our country has been promoting traditional medicine because they are less expensive, easily available and comprehensive, especially in developing countries. It is also true that eight percent of the world's population relies on medicinal plants for their primary health care. [4] Whole world including the developed country rec-

ognized the importance of traditional medicine and has treatment strategies, guidelines and standard for ethno medicine. [5]

Figure 1: ***Murraya Koenigii and Cajanus Cajan***

Wound is defined simply as the disruption of the cellular and anatomic continuity of a tissue. Wound may be produced by physical, chemical, thermal, microbial or immunological insult to the tissue. [6,7] Wound healing is the process by which skin or other body tissue repairs itself after trauma. The process of wound healing consists of integrated cellular and biochemical events leading to reestablishment of structural and functional integrity with regain of strength of injured tissue.

Clinically, one often encounters non-healing, underhealing or over healing. Therefore the aim of treating a wound is to either shorten the time required for healing or to minimize the undesired consequences. [8]

MATERIALS AND METHODS

Murraya Koenigii, *Cajanus Cajan* leaves were collected from the local place of Telangana. Stearic acid, Liquid paraffin, Bees wax, Stearyl alcohol, Methyl paraben, Potassium hydroxide, Tween 80, Sorbitol were purchased from Merck Life Science Private Limited, Mumbai.

METHODOLOGY

Collection of plant material: The whole plant of *Cajanus Cajan* and *Murraya Koenigii* was collected at sirisinagandla village, Siddipet district, Telangana state. The fresh leaves of *Cajanus Cajan* were collected and the leaves were cleaned and shade dried. Then the leaves were mixed to course powder. Then the powder was collected to extraction.

Extraction process- Soxhlet apparatus: The extraction of *Murraya Koenigii & Cajanus Cajan* powder was done individually by the soxhlation Process by using ethanol as a solvent. After the completion of the Soxhlation process the solution was filtered twice to get particles free solution. Then the solution was heated to suitable temperature to get the product. Then the product was kept in desiccator with vacuum to observe the moisture from product. That product was kept aside for four to five days to get the extract.

Phytochemical Screening of *Cajanus Cajan*[9]

The extract of each powdered parts of plants were used for phytochemical tests and to identify the constituents, standard procedures were carried out for Tannins, saponins, reducing sugars, alkaloids, terpenoides, flavonoids, cardiac glycosides and anthraquinones were estimated following standard methods.

Tannins: 0.5 g of the extract was dissolved in 10 ml of distilled water, then a few drops of 1% ferric chloride solution was added to obtain a brownish green or blue black precipitate, which confirms the presence of tannin.

Saponins: 0.5 g of the extract was dissolved in 5 ml distilled water. The mixture was shaken vigorously. Formation of stable persistent froth shows the presence of saponins. A further addition of 6 drops of olive oil while shaking forms an emulsion, confirming the presence of saponins.

Reducing sugars: 1 gm of the extract was dissolved in 10 ml of distilled water. This extract was boiled with Fehling solution A and B in test tube and colour changes were observed. Presence of brick red colour indicated the presence of reducing sugar.

Alkaloids: 6 ml of extract was mixed with 6 ml of 1% HCl in steam bath, then it was filtered. 1 ml of Mayer's reagent was added. Presence of turbidity shows presence of alkaloids. Further addition of a few drops of olive oil to form an emulsion confirmed the presence of alkaloids.

Terpenoids: 0.5 gm extract was dissolved in 2 ml of chloroform then 3 ml concentrated sulfuric acid was added, a reddish brown colour in interphase indicates the presence of terpenoids.

Flavonoids: 5 ml dilute ammonia was added to 5 ml extract and then 5 ml concentrated sulfuric acid was added. Formation of yellow colour shows the presence of flavonoids.

Cardiac glycosides: 2.5 g of extract was added to 2.5 ml distilled water. 1 ml glacial acetic acid containing a few drops of ferric chloride was added then 0.5 ml of concentrated sulfuric acid was added. Presence of brown ring at the interphase indicates the presence of deoxy sugar. A violet ring below the brown ring was observed, while a greenish ring also appears above the brown ring, confirming the presence of Cardiac Glycosides.

Anthraquinones: 2.5 g extract was dissolved in 5 ml of conc. Sulfuric acid and filtered. The filtrate was dissolved in 2.5 ml of chloroform. Chloroform layer was pipetted into a tube and 0.5 ml of 10% diluted ammonia was added. Formation of pink red or violet color shows the presence of anthraquinones.

Phenols: 2 ml of extract was dissolved in 4 ml of distilled water and added few drops of 10% $FeCl_3$. Appearance of blue or green colour indicates presence of phenols.

Phytochemical Analysis of *Murraya Koenigii*[10]

Crude extracts (Aqueous, Methanol and Ethanol) of *M. koenigii* Linn were subjected to different phyto-

chemical screening for alkaloid, carbohydrate, tannins, terepnoids, cardiac glycosides, flavonoids, phenols, phylobatannins, quinons, amino acids and protein.

Out of ten tested phytochemicals, alkaloid, carbohydrate, tannins, terepnoids were present in all extracted samples, however cardiac glycosides and phylobatannins were extracted only in methanolic and ethanolic extracts of plant leaves sample. Test for presence of phenolic compounds showed positive results in aqueous and ethanolic extracts, while quinons only extracted in aqueous medium. Flavonoids and Amino acids and protein were showed negative results in all crude extracts.

Test for Alkaloid (Wagner's test): 3-5 drops of wagner's reagent were added to 5 ml extract The formation of red/brown precipitate indicated the positive result.

Test for Carbohydrate (Molisch's test): Few drops of molisch's reagent were added to 2 ml extract and 2 ml conc. H_2SO_4 was also added. Allowed to stand for 2-3 min. The formation of red/dull violet color at the interphase of the two layers indicated the positive result.

Test for Cardiac glycosides (Keller kiliani test): 2 ml of glacial acetic acid was added to 5 ml extract then few drops of ferric chloride were also added with 1 ml conc. H_2SO_4. The formation of brown/violet/green ring indicated the positive result.

Test for Flavonoids (Alkaline reagent test): Few drops of 20% NaOH solution were added to 2 ml extract which showed yellow color within a second and became colorless on addition of dilute HCl which indicated the positive result.

Test for Phenols (Ferric Chloride test): Aqueous 5% ferric chloride was added to 2 ml extract. The formation of deep blue/black color indicated the positive result.

Test for Phylobatannins (Precipitate test): 1 ml of aq. HCl was added to the 2 ml extract which was boiled and marked the volume of 1 ml. The formation of red precipitate indicated the positive result.

Test for Amino acids and Proteins (Ninhydrin test): 2-5 drops of ninhydrin solution were added to 2 ml extract and boiled in a water bath for 1-2 minutes. The formation of purple color indicated the positive result.

Test for Tannins ($FeCl_3$ solution test): 10% alcoholic ferric chloride solution was added to 2 ml extract. The formation of blue/green color indicated the positive result.

Test for Terpenoids (Salkowski test): In 1 ml of chloroform, 2 ml extract was added and few drops of conc. H_2SO_4 were also added. The formation of reddish brown precipitate indicated the positive result.

Test for Quinons: Few drops of conc. HCl were added to 2 ml extract. The formation of yellow precipitate indicated the positive result.

Preparation of Herbal Cream[11]

The Herbal Cream was prepared by the 2 extracts (*Murraya koeniggi* & *Cajanus Cajan*). The ingredients for the herbal cream preparation were weighed accurately. The formulation trails were done as per formula given in (Table 2). The formulation containing *Murraya Koenigii* and *Cajanus Cajan* extract was formulated by taking aqueous and oil phases into beakers and heated to 75ºC over a water bath. The oil phase was comprised of extracts of *Murraya Koenigii, Cajanus Cajan*, liquid paraffin, bees wax, stearyl alcohol, Tween-80 and stearic acid while the aqueous phase was composed of methyl parabens, sorbitol solution and potassium hydroxide. Drop wise addition of the aqueous phase to the oil phase was done with constant stirring at 2000 rpm in a homogenizer for a period of 15 min. The homogenizer speed was then reduced to 1000 rpm and homogenization was continued for another 5 min. The speed was further reduced to 500 rpm and the homogenization extended for 5 min. Herbal skin cream containing *Murraya koeniggi & Cajanus Cajan* extract was formulated.

Table 1: Phytochemical analysis of leaves of plants extracts

Murraya Koenigii		*Cajanus Cajan*	
Phytochemicals	**Result**	**Phytochemicals**	**Result**
Alkaloids	+	Alkaloids	+
Carbohydrate	+	Flavonoids	+
Cardiac glycosides	+	Terpenes	+
Flavonoids	-	Steroids	-
Phenols	+	Saponins	+
Phylobatannins	+	Tannins	+
Amino acids and protein	-	Anthraquinones	-
Tannins	+	Phlobatannin	-
Terpenoids	+		
Quinons	-		

+ = Present; - = Absent

Evaluation tests [12, 13]

Physical evaluation of the formulation

The formulations were inspected visually for their appearance, colour and odour.

Measurement of pH: The pH was measured using a pH meter, which was calibrated before each use with standard buffer solutions at pH 4, 7, 9. The electrode was inserted into the sample 10 minutes prior to taking the reading at room temperature.

Viscosity: The viscosity of the formulations was checked using a Brookfield Viscometer (DV-I PRIME, USA). The gels were rotated at 0.3, 0.6, 1.5 rotations per minute. The viscosity of the gel was obtained by

Table 2: Formulation ingredients of Herbal Cream

S.No	Ingredient	Formula 1 (%)	Formula 2 (%)	Formula 3 (%)	Formula 4 (%)	Formula 5 (%)	Formula 6 (%)
1	*Cajanus cajan* Extract	5.0	5.0	5.0	5.0	5.0	5.0
2	*Moringa koenigii*.	5.0	5.0	5.0	5.0	5.0	5.0
3	Liquid Paraffin	5.0	5.0	5.0	5.0	5.0	5.0
4	Stearic Acid	3.0	3.0	5.0	5.0	4.0	5.0
5	Bees Wax	5.0	6.0	5.0	4.0	6.0	5.0
6	Stearyl Alcohol	10.0	10.0	10.0	8.0	8.0	7.0
7	Tween-80	8.0	5.0	5.0	5.0	5.0	6.0
8	Methyl Paraben	0.12	0.12	0.12	0.12	0.12	0.12
9	Sorbitol Solution	6.0	6.0	5.0	5.0	5.0	5.0
10	Pot. Hydroxide	5.0	5.0	5.0	5.0	5.0	5.0
11	De-ionized Water	33.0	36.0	37.0	40.0	39.0	38.0

Table 3: Physical Properties of Cream

S.No	Properties	Formula 1	Formula 2	Formula 3	Formula 4	Formula 5	Formula 6
1	Appearance	Semi- solid					
2	Odour	Characteristic					
3	Colour	Dark brown					
4	Thermal stability of cream	Slightly oily separation					
5	pH	5.25	5.89	5.72	6.0	6.08	6.12
6	Spreadability	14.2	14.1	14.4	13.8	13.7	14.0

Table 4: Comparative *in- vitro* diffusion studies of all formulations

Time	%CDR					
	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
30	14	26.4	21.6	26.12	18.01	14.02
60	23	29.55	27.48	34.10	37.70	28.10
90	26.78	34.21	29.52	35.24	43.54	31.67
120	28.08	36.44	34.21	39.28	45.68	37.15
150	33.54	38.74	39.21	40.68	54.98	44.63
180	37.20	39.45	41.28	43.74	57.44	50.40
210	43.38	40.68	49.00	53.49	59.74	56.96
240	45.28	44.54	50.56	54.83	60.85	64.54
270	46.56	46.93	55.41	59%	65.84	71.38
300	51.82	54.64	57.45	60.94	78.89	82.43

Table 5: pH and viscosity of the cream Stability after 3 Months

Properties		Formula 1	Formula 2	Formula 3	Formula 4	Formula 5	Formula 6
pH	Initial	6.05	5.89	6.11	6.02	5.97	5.94
	After 3 Months	6.02	5.91	6.11	5.98	5.97	5.91
Viscosity	Initial	1876	1893	1956	1785	1863	1816
	After 3 Months	1789	1810	1914	1721	1803	1765

multiplying the corresponding dial reading with the factor given in the Brookfield Viscometer catalogue.

Spreadability: Spread ability is measured in terms of time in seconds taken by two slides to slip off from the gel when placed in between the slides under the direction of a certain load. The excess amount of sample was placed between the two glass slides and a definite amount of weight was placed on these glass slides to compress the glass slides of uniform thickness. A weight of 70 g was added and the time re

quired to separate the two slides was noted. Spreabability was calculated using the formula $S = M\frac{L}{T}$ where, M = wt tied to upper slide, L = length of glass slides, T = time taken to separate the slides.

Stability: Stability testing of drug products begins as a part of drug discovery and ends with the demise of the compound or commercial product. To assess the drug and formulation stability, stability studies were done according to ICH guidelines. The stability studies were carried out as per ICH guidelines. The cream filled in bottle and kept in humidity chamber maintained at 40±2°C / 75±5% RH for three months. At the end of studies, samples were analyzed for the physical properties, pH and viscosity.

***In-vitro* diffusion studies[14]:** *In-vitro* diffusion was carried out on Franz diffusion cell having 57ml capacity and whatman filter paper no.41 used as diffusion membrane. Pieces of whatman filter paper no.41 were soaked in phosphate buffer pH 9.0 for 24 hrs prior to experiment. Diffusion cell was filled with phosphate buffer pH 6.0 Whatman filter paper no.41 was mounted on cell. The temperature was maintained at 37±0.5 °c then the formulation was spread on the filter to form thin layer. The time point for cream, gel and cream were different. A sample of 1ml was withdrawn at predetermined time intervals, the solution was filter with 0.45 micron filter paper and make up the volume with 5ml of PB pH 6.0 and equivalent amount of fresh dissolution fluid equilibrated at same temperature was replaced. The sample was diluted to 5ml of PB pH 6.0 for determination the standard was prepared which was of same concentration of that sample. The amount of permeated drug was determined using a UV-Spectrophotometer at 265 nm.

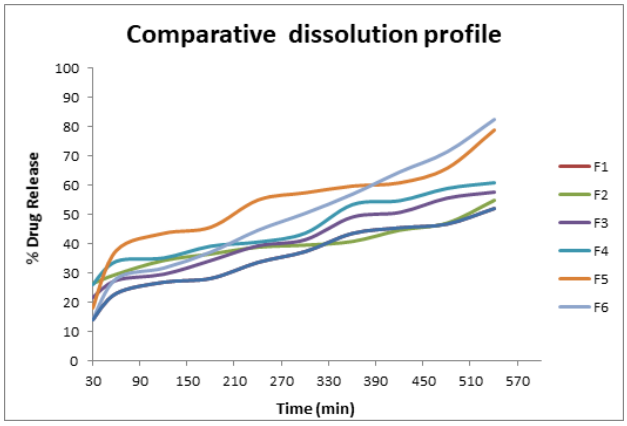

Figure 2: Comparative diffusion studies of F1-F6 formulations

Skin cream was prepared using herbal plants such as *Murraya Koenigii* and *Cajanus Cajan*. Various formulations were prepared by varying the amount of excipients such as stearic acid, bees wax, stearyl alcohol, tween-80, methyl paraben, sorbitol solution, potassium hydroxide and deionised water. Formulation of herbal skin cream for wound healing was successfully developed that met the relevant pharmaceutical characteristics. The prepared formulations are then evaluated for parameters like physical properties, pH, viscosity, spreadability and stability of the formulated cream. The prepared formulations showed good spreadability, no evidence of phase separation and good consistency during the study period. Stability parameters like visual appearance, nature, viscosity and pH of the formulations showed that there was no significant variation during the study period. The prepared formulations showed proper pH range that is approximately pH 6 and it confirms the compatibility of the formulations with skin secretions. The creams were found to be stable during stability to ICH guidelines (40±2 °C/ 75±5 % RH) for 3 months. it is possible to develop creams containing herbal extracts and can be used as a barrier to protect skin. *In-vitro* diffusion studies carried out for all the formulations and the formulation F6 has shown the best release when compared to the other formulations.

CONCLUSION

Formulation of Herbal Skin Cream for wound healing was successfully developed that met the relevant pharmaceutical characteristics. The prepared formulations showed good physico chemical characteristics and the pH is compatible with the skin secretions and release was the best for formulation F6. The creams were found to be stable during stability study conducted for 3 months. From the present study it can be concluded that it is possible to develop creams containing herbal extracts and can be used as the provision of a barrier to protect skin. Plants are more potent healers because they promote the repair mechanism in the natural way. The wound healing property of the formulated herbal skin cream has yet to be experimented and will be done in future.

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