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Formulation and evaluation of herbal ointment containing *Cajanus Cajan* extract

R. Navitha*[1], Shaik. Harun Rasheed[1], SY. Manjunath[2]

[1]*Department of Pharmaceutics, Srikrupa institute of Pharmaceutical Sciences, Siddipet - 502277, Telangana, India.*
[2]*Department of Pharmaceutical Chemistry, Srikrupa institute of Pharmaceutical Sciences, Siddipet- 502277, Telangana, India.*

ABSTRACT

From the ancient times being a rich source of protein and a most important forage crop, *Cajanus Cajan* is the most widely used and cultivated crop. It has also been used traditionally in many parts of the world for its innumerable medicinal properties but still its identity as a medicinal plant is not established. To date, several flavonoids, isoflavonoids, tannins and protein fractions have been isolated from its different parts and their medicinal uses have been established, but many bioactive constituents and pure compounds have so far been neglected by phytochemists and pharmacologists and a large amount of work has been done only on extracts and not the isolated fractions which shows scope for further study in this direction. Traditional knowledge of the past and present folk is of massive value to the development of newer drug compounds. In the present work an attempt has been made in the development of four formulations of herbal ointment of *Cajanus Cajan*. All the formulations were studied for physicochemical properties like spreadability, extrudability, washability, solubility, loss on drying and showed satisfactory results. The prepared formulations showed proper pH range that is approximately pH 6; it confirms the compatibility of the formulations with skin secretions. The ointment formulations were found to be stable during stability study according to ICH guidelines (40 ± 2°C/ 75 ± 5% RH) for 3 months. F4 was found to be the best formulation as it shows 97.36% drug release within 5 hours, drug content 97.68% as compared to other three formulations.

Keywords: Inserts; Suppositories; Ocular Inserts; Lipid; Polymers; Drug Delivery.

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Corresponding Author

Name: R. Navitha
Email: navithareddy970@gmail.com

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INTRODUCTION

Plants had been used for medicinal purposes long before recorded history. Ancient Chinese and Egyptian papyrus writings describe medicinal uses for plants as early as 3,000 BC. Indigenous cultures (such as African and Native American) used herbs in their healing rituals, while others developed traditional medical systems (such as Siddha, Ayurveda, Unani and TCM) in which herbal therapies were used. [1] Herbal medicines are being used by about 80% of the world population primarily in the developing countries for primary health care. They have stood the test of time for their safety, efficacy, cultural acceptability and lesser side effects. Ancient literature also mentions herbal medicines for age-related diseases namely memory loss, osteoporosis, osteoarthritis, diabetes, immune and liver disorders, etc. for which no modern medicine or only palliative therapy is available.[2] The chemical constituents present in them are a part of the physiological functions of living flora and hence they are believed to have better compatibility with the human body.[3] The use of herbal drugs due to toxicity and side effects of allopathic medicines, has led to rapid increase in the number of herbal drug manufacturers. For the past few decades, herbal drugs have been more and more consumed by the people with no prescription. These drugs have survived real world testing and thousands of years of human testing. Some drugs have been discontinued due to their toxicity, while others have been modified or combined with additional herbs to counterbalance side effects.[4]

The present investigation was therefore proposed to develop a herbal ointment of *Cajanus Cajan* plant which possess various pharmacological activities and

evaluate the formulated ointment to various parameters.

Figure 1: *Cajanus Cajan* Plant

MATERIALS AND METHODS

Erythromycin was obtained as gift sample from FDC Pharmaceuticals Pvt. Ltd., Mumbai. Wool fat, hard paraffin cetostearyl alcohol, white soft paraffin were purchased from Merck Life Science Private Limited, Mumbai.

METHODOLOGY

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

Phytochemical Screening: [5,6] The extract of each powdered parts of plants were used for phytochemical tests and to identify the constituents, standard procedures were carried out. Tannins, saponins, reducing sugars, alkaloids, terpenoides, flavonoids, cardiac glycosides and anthraquinones were estimated following standard. methods.

Qualitative Analysis

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 colour 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.

Extraction process- Soxhlet apparatus[7]

Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent (water, ethanol, benzene), and the impurity is insoluble in that solvent. If the desired compound has a high solubility in a solvent then a simple filtration can be used to separate the compound from the insoluble substance. Normally a solid material containing some of the desired compound is placed inside a thimble made from thick filter paper, which is loaded into the main chamber of the Soxhlet extractor. The Soxhlet extractor is placed onto a flask containing the extraction solvent. The Soxhlet is then equipped with a condenser. The solvent is heated (55^0C) to reflux. The solvent vapour travels up a distillation arm and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapour cools, and drips back down into the chamber housing the solid material. The chamber containing the solid material slowly fills with warm solvent. Some of the desired compound will then dissolve in the warm solvent. When the Soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm, with the solvent running back down the distillation flask. This cycle may be allowed to repeat many times two days during each cycle; a portion of the non-volatile compound dissolves in the solvent. After many cycles the desired compound is concentrated in the distillation flask. The advantage of this system is

that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled. After extraction the solvent is removed, typically by means of a rotary evaporator, yielding the extract compound. The non soluble portion of the extracted solid remains in the thimble, and is usually discarded.

Analytical Method Development for *Cajanus Cajan* extract[8]

Preparation of Phosphate buffer pH7.4: Dissolve 2.38 g of disodium hydrogen phosphate, 0.19 g of potassium dihydrogen phosphate and 8 gm of Nacl in sufficient distilled water to produce 1000ml buffer.

Preparation of stock solutions of the *Cajanus Cajan* extract

Stock solution A: Accurately weighed 100mg of *Cajanus Cajan* extract was taken in 100ml volumetric flask and volume was made up to 100 ml with phosphate buffer pH 7.4 to get a concentration of 1000µg/ml.

Stock solution B: From stock solution A, 10 ml was taken in 100ml volumetric flask and volume was made up to 100 ml with phosphate buffer pH 7.4 to get a concentration of 100µg/ml.

Determination of absorption maxima (λmax) for *Cajanus Cajan* extract: [9] 1ml of stock solution B was taken in 10ml volumetric flask and volume was made up with solutions of pH 7.4. Concentration of 10µg/ml solution in PBS was prepared and scanned on a double beam spectrophotometer against Phosphate buffer pH7.4 as a blank in the absorbance range from 200-400nm. An absorption maxima (λmax) of 220 nm was obtained. This λmax was selected for preparation of standard curve of *Cajanus Cajan* extract in Phosphate buffer pH 7.4.

Preparation of Standard Curve for *Cajanus Cajan* extract in Phosphate Buffer pH 7.4: [10] From the stock solution B-100µg/ml, take aliquots of 1,2,3,4 and 5ml solution and dilute up to 10ml to obtain concentrations from 10 to 50µg/ml with Phosphate Buffer pH 7.4. Then determine the absorbance at λmax 220 nm against phosphate buffer pH 7.4 as blank. Repeat the experiment three times and plot a calibration curve from the mean value.

Procedure for preparation of herbal ointment[13]

a) Initially prepare the ointment base by weighing accurately grated hard paraffin which is to be place in evaporating dish on water bath. After melting of hard paraffin add remaining ingredients and stir gently to aid melting and mixing homogeneously followed by cooling of ointment base.

b) Prepare the herbal ointment by mixing accurately weighed *Cajanus Cajan* extract to the ointment base by levigation method to prepare a smooth paste with 2 or 3 times its weight of base, gradually incorporating more base until to form homogeneous ointment, finally transferred in a suitable container.

Table 1: Composition of ointment base and Formulation of ointment base

S. No.	Name of Ingredient	Quantity to be taken
1.	Wool fat	0.5g
2.	Cetostearyl alcohol	0.5g
3.	Hard paraffin	0.5g
4.	White soft paraffin	8.5g

Table 2: Composition of herbal ointment

Formulation code	Prepared *Cajanus Cajan extract* (g)	Ointment base q.s. (g)
F1	0.5	10
F2	1	10
F3	1.5	10
F4	1.75	10

Evaluation of Herbal Ointment [14]

Colour and Odour: Physical parameters like colour and odour were examined by visual examination.

Consistency: Smooth and no greediness was observed.

pH: pH of prepared herbal ointment was measured by using digital pH meter. The solution of ointment was prepared by using 100ml of distilled water and set aside for 2hrs. pH was determined in triplicate for the solution and average value was calculated. It was found to be 7.2.

Spreadability: The spreadability was determined by placing excess of sample in between two slides which was compressed to uniform thickness by placing a definite weight for definite time. The time required to separate the two slides was measured as spreadability. Lesser the time taken for separation of two slides results better spreadability. Spreadability was calculated by following formula: $S = M \times \frac{L}{T}$ Where S= Spreadability, M= Weight tide to the upper slide L= Length of glass slide, T= Time taken to separate the slides It was found to be 5 seconds.

Extrudability: The formulation was filled in collapsible tube container. The extrudability was determined in terms of weight of ointment required to extrude 0.5cm of ribbon of ointment in 10 seconds.

Drug Content: 10mg of the ointment was taken and dissolved in distilled water. Then absorbance was measured at 220nm using UV-Visible spectrophotometer. Drug content of F4 was found to be 98%.

LOD: LOD was determined by placing the formulation in petri- dish on water bath and dried for the temperature 105^0C. It was found to be 20%.

Solubility: Soluble in water, alcohol and chloroform.

Washability: Formulation was applied on the skin and then ease extend of washing with water was checked.

Non irritancy Test: Prepared herbal ointment was applied to the skin of human being and observed for the effect.

Stability study:[15] Physical stability test of the herbal ointment was carried out for four weeks at various temperature conditions like 2^0C, 25^0C. The herbal ointment was found to be physically stable at different temperature i.e. 2^0C, 25^0C and 35^0C within four weeks.

RESULTS AND DISCUSSION

Phytochemical analysis of the leaves of *Cajanus Cajan*

Table 3: Phytochemical Analysis of *Cajanus Cajan*

S.No	Phytochemicals	Result
1	Alkaloids	+
2	Flavonoids	+
3	Terpenes	+
4	Steroids	_
5	Saponins	+
6	Tannins	+
7	Anthraquinones	-
8	Phlobatannin	-

Determination of absorption maxima (λmax) for *Cajanus Cajan* extract

Maximum absorbance of the *Cajanus Cajan* extract was found to be 0.63 at 220 nm

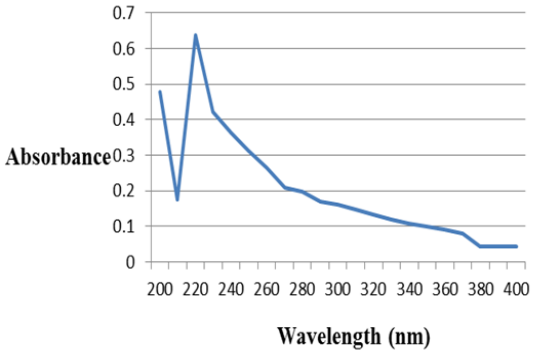

Figure 2: Absorption maxima of *Cajanus Cajan*

Table 4: Absorption maxima values

Sl.No	Wave length (nm)	Absorbance
1	200	0.4772
2	210	0.1762
3	220	0.6369
4	230	0.4205
5	240	0.3621
6	250	0.3113
7	260	0.2659
8	270	0.2092
9	280	0.1969
10	290	0.1700
11	300	0.1606
12	310	0.1457
13	320	0.1328
14	330	0.1179
15	340	0.1090
16	350	0.0985
17	360	0.0901
18	370	0.0796
19	380	0.0426
20	390	0.0425
21	400	0.0434

Preparation of Standard Curve for *Cajanus Cajan* extract in Phosphate Buffer pH 7.4

Table 5: Standard curve in Phosphate Buffer pH 7.4

Conc. (µg/ml)	Absorbance (nm)
0	0
10	0.19
20	0.42
30	0.63
40	0.83
50	0.98

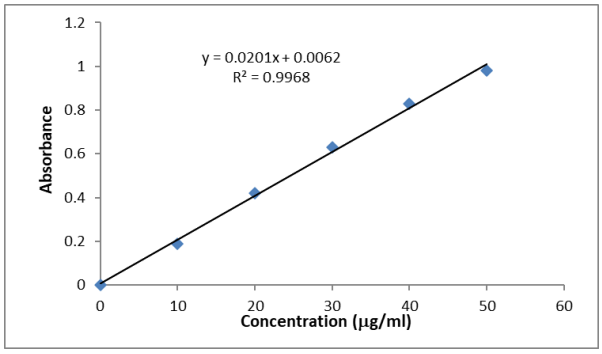

Figure 3: Standard curve in Phosphate Buffer pH 7.4

Table 6: Physicochemical parameters - Evaluation

S.NO	Physicochemical parameter	Observation
1	Colour	Pale white
2	Odour	Characteristic
3	Consistency	Smooth
4	pH	7.2
5	Spreadability (seconds)	5 seconds
6	Extrudability	0.5 g
7	Loss on drying	20%
8	Solubility	Soluble in water, alcohol and chloroform.
9	Washability	Good
10	Non irritancy	Non irritant
11	Stability study	Stable at 2^0C, 25^0C and 35^0C

Table 7: Drug Content

Formulation Code	% Drug Content
F1	90.31
F2	93.52
F3	95.84
F4	98.86

Diffusion study: [16] *In vitro* drug release studies of samples were carried out by using Modified Franz diffusion cell. Dialysis membrane previously soaked in pH 7.4 phosphate buffer was taken and placed in between donor and receptor compartments. In the donor compartment 10mg of formulation was added.

Volume of the diffusion medium was maintained 25 ml in receptor compartment and temperature maintained at 34 ± 0.5°C, and rpm was maintained at 25 by using hot plate magnetic stirrer. Aliquots were withdrawn at intervals of 15, 30, 45, 1hr. up to 6hours and replaced by equal volumes of diffusion medium. Aliquots were suitably diluted with pH 7.4 and analyzed by UV Spectrophotometer at 220 nm. F4 shows 98% of drug release with in 6 hours. [17, 18]

Table 8: Comparative in - vitro diffusion studies of all formulations

Time	%CDR			
	F1	F2	F3	F4
0	0	0	0	0
30	22.6	24.4	23.4	28.0
60	25.13	26.42	35.97	37.60
90	28.40	29.52	41.34	49.41
120	36.33	36.21	46.18	57.18
150	40.49	44.94	54.61	64.54
180	46.77	55.16	65.02	75.29
210	51.67	58.85	71.32	80.61
240	54.05	66.21	81.61	87.80
270	61.6	77.04	85.51	91.40
300	69.2	87.92	90.48	97.36

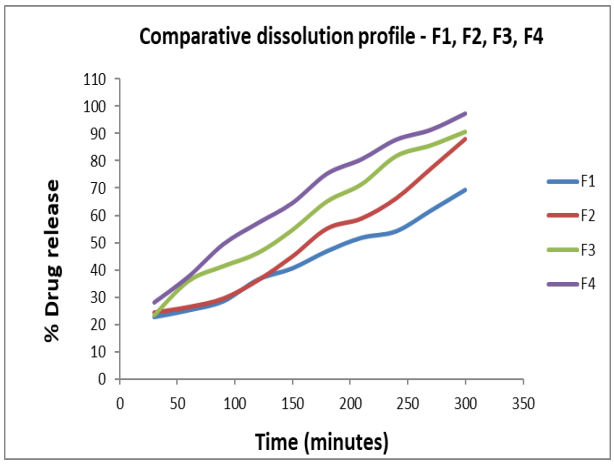

Figure 4: Comparative Diffusion Profile of F1, F2, F3, F4.

In-vitro release studies

In vitro drug release studies of samples were carried out by using modified Franz diffusion cell and results are tabulated.

The present study was done to prepare and evaluate the herbal ointment. For this the herbal extracts were prepared by using Soxhlet extraction process to obtain a good yield of extract and there was no harm to the chemical constituents and their activity. The levigation method was used to prepare ointment so that uniform mixing of the herbal extract with the ointment base was occurred which was stable during the storage. The physicochemical properties were studied which shows satisfactory results for spreadability, extrudability, washability, solubility, loss on drying and others. 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 Invitro diffusion studies were carried out for all the formulations using Franz diffusion cell and the results were analyzed and F1, F2, F3 and F4 formulations has shown 69.20, 87.92, 90.48 and 97.36 % respectively. From the above results, F4 was found to be the best formulation as it shows 97.36% drug release within 5 hours, drug content 97.68% as compared to other three formulations. The ointment formulations were found to be stable during stability study according to ICH guidelines (40 ± 2 °C/ 75 ± 5 % RH) for 3 months.

CONCLUSION

From the present study it can be concluded that it is possible to develop ointments 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 ointment has yet to be experimented and will be done in future.

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