



E-ISSN: 2707-2835

P-ISSN: 2707-2827

www.pharmacognosyjournal.com

IJPLS 2024; 5(2): 05-07

Received: 04-04-2024

Accepted: 09-05-2024

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Formulation and evaluation of blood clotting ointment using: *Tagetes erecta*, *Cynodon dactylon*, aloe barbadense

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DOI: <https://doi.org/10.33545/27072827.2024.v5.i2a.119>

Abstract

Homeostasis is the process of maintaining proper bodily function, in bleeding-related terms homeostasis is the process of forming clots in the blood vessels while an injury causes bleeding due to damaged vessels to prevent abnormal bleeding and to maintain intramuscular blood in a liquid state. The modern trend is to shift towards herbal sources as a chemical alternative to drugs. The present study comprises the possible coagulation effect of a combination of marigold leaves, doob grass, and Aloe-vera extract in an ointment formulation. The study comprises an extensive parameter study and an in-vivo study of coagulation and ointment prepared by the above-stated extract and its effects on coagulation. This is also a subject for further studies on efficacy and safety, it also can be used further as a supplementary coagulation agent in blood vessels.

Keywords: Homeostasis, in-vivo coagulation, efficacy, herbal

Introduction

Herbal medicine use is going worldwide. Single herb preparations and ethnic and modern herbal medicine formulations are widely used as adjunct therapies or to improve consumer well-being^[1].

A hydrophilic ointment was prepared so that there was no washing of the ointment from the skin during application. The ointment is prepared with the extract of Marigold leaves, Doob grass, and Aloe-vera extract.

The formulation has been tested on various factors such as its organoleptic, physical, and chemical characteristics, along with the specialized study of clotting factors. The coagulation mechanism is a complex cascade involving the conversion of precursor enzymes into active enzymes. Mostly, substances that are necessary for coagulation are inert, and cascade when activated^[2].



Herbs used and their uses

- 1) Marigold leaf {*Tagetes erecta*}
 - i) Antibacterial activity.
 - ii) Wound healing.

iii) Blood clotting^[3].

2) Doob grass {*Cynodon dactylon*}

i) Wound healing]

ii) Anti-microbial

3) Aloe-vera {*Aloe barbadense*}

i) Anti-allergic

ii) Anti-microbial

iii) Healing^[4].

Materials and Methods

Collection of plant material

1. Collection of Mari gold leaves

The leaves of *Tagetes erecta* (Marigold) were collected from Siliguri, West Bengal. After that, the leaves were shade-dried and coarsely powdered using mortar and pestle.

Collection of doob grass

The grass (*Cynodon dactylon*) was collected from Majhitar, East Sikkim. After that, the grass was shade-dried and coarsely powdered in the mixer grinder.

Collection of Alovera

The whole plant of (*Aloe barbadense*.) was collected from Siliguri, West Bengal. After that, the fresh extract of the plant was extracted.

Preparation of plant extract

The dried leaf of Marigold and Doob grass was collected and shade dried for 3 days and then a coarse powder was prepared using mortar pastel and mixer grinder. And then it was macerated for 3 days.

The marigold leaves were macerated in water and the Doob grass was macerated in a hydroalcoholic solution of 70:30 (ethanol: water).

Then the solution was dried in a water bath till a dry extract form was prepared. And the Alovera was taken as a fresh extract.

0.10 gm of extract was taken (as a reference to formulation).^[5].

Formulation of hydrophilic ointment

Prepared Extract

Ingredient	Quantity
Marigold extract	0.10gm
Doob grass extract	0.10gm
Aloe-vera extract	Q. S

Chemical required

Ingredient	Quantity
Sodium lauryl sulphate	1%
Propylene glycol	12%
Cetyl alcohol	25%
White Petrolatum	25%
Distilled water	37%

Melt the cetyl alcohol & white petrolatum on a hot plate. Heat the mixture to 70 °C. Dissolve remaining ingredient in water & heat the solution to 70 °C. Add the oleaginous phase slowly to the aqueous phase stirring continuously. Add the extracts to the water phase and stir properly. Remove from heat and stir the mixture until it congeals.

Evaluation parameter for Ointment

Colour, Odour, and consistency

Physical parameters like colour and Odor were examined by visual examination. Consistency Smooth and no greediness is observed.

pH

pH of prepared herbal ointment was measured by using pH paper.

Spreadability

The spreadability was tested by placing an excess sample in between two slides which was compressed to uniform thickness by placing a definite weight for a definite time. The time required to separate the two slides was measured as spreadability. Lesser time is taken for separation of two slides results in better spreadability. Spreadability was calculated by following the formula: -

$$S=M*L/T$$

Were,

S= spreadability

M=weight tide to the upper slide

L= Length of glass slide

T= Time taken to separate the slides

Extrudability

The extrudability test is the measure of the force required to extrude the material from a collapsible tube when a certain amount of force has been applied to it. In the present study, the quantity in percentage of ointment extruded from the tube on application of a certain load was determined. The formulations were calculated by using the following formula:

$$\text{Extrudability} = \frac{\text{Amount of ointment extruded from the tube}}{\text{Total amount of ointment filled in the tube}} \times 100$$

LOD

LOD was determined by placing the formulation in a Petri dish in a hot air oven and dried for a temperature of 105C.

Solubility: Solubility was checked with boiling water, alcohol, ether, chloroform.

Washability

It is tested by applying the skin in the glass slide and checking the ability of it to be removed easily after washing.

Stability

The ointment was kept container and allowed to settle and then it was kept in different temperature parameters (20 °C, 10 °C, 30 °C).

Solubility

The sample solution was prepared by taking a test tube and dissolving the ointment in various solutions such as Ethanol, Chloroform, Diethyl ether, and Warm Water.

Squeezability

The ease with which the drug can be extracted from the tube is known as the squeezability^[5-7].

Clotting factor

Collection of blood and separation of plasma

About 5 ml of blood was collected from healthy poultry birds (having no medicine consumption history) by intravenous injection. To the 9 μ l volume of blood, 1 μ l volume of 3.8% trisodium citrate solution is added to avoid the natural coagulation process. Immediately centrifugation was carried out for 15 min at a rate of 3000 rpm to separate the blood cells from plasma for the prothrombin time (PT) test.

The plasma sample was divided into 5 groups

Blank: 0.2 ml plasma + 0.1ml Normal saline solution + 0.3ml CaCl₂

Sample 1: plasma 0.2 ml + extract 0.1ml +0.3ml CaCl₂

Sample 2: plasma 0.4 ml + extract 0.2ml +0.6ml CaCl₂

Sample 3: plasma 0.6 ml + extract 0.4ml +0.9ml CaCl₂

Sample 4: plasma 0.8 ml + extract 0.8ml +1.2ml CaCl₂

All the tubes are shaken vigorously and tilted at an angle of 45° for every 30 seconds to measure the frothing time. A

Group	Test	Sample	Result
Blank: 0.2 ml plasma + 0.1ml saline solution + 0.3mlCaCl ₂	-	-	No froth formation had been seen
Sample 1: plasma 0.2 ml + extract 0.1ml +0.3ml CaCl ₂	1 st test group	Plant extract	Small froth observed and sustained in the duration of the period
Sample 2: plasma 0.4 ml + extract 0.2ml +0.6ml CaCl ₂	2 nd test group	Plant extract	Froth observed and sustained in the duration of the period
Sample 3: plasma 0.6 ml + extract 0.4ml +0.9ml CaCl ₂	3 rd test group	Plant extract	Slightly higher froth was observed and sustained in the duration of the period
Sample 4: plasma 0.8 ml + extract 0.8ml +1.2ml CaCl ₂	4 th test group	Plant extract	High froth was observed and sustained for the duration of the period.

Discussion

The present study was done to prepare and evaluate the herbal ointment. For this the herbal extracts were prepared by using a simple maceration process to obtain a good yield of extract. The normal method was used to prepare ointment so that uniform mixing of the herbal extract with the ointment base 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.

Coagulation is a process that occurs mainly due to the complex interaction of cellular and molecular components. Initially, clotting involves common pathways both intrinsic and extrinsic pathways but lately, it is found to be due to a balance between procoagulants and anticoagulants. The present study signifies that samples 1, 2, 3 & 4 promised to show the coagulant activity under the heading of a naturally occurring source of drug that may be less toxic concerning synthetic coagulant drugs that are subjected to future experimentation.

Conclusion

Blood clotting ointment using Marigold, Doob grass, and Alovera extract was prepared and evaluation were carried out for the above stated parameter. The formulated ointment was not only safer than the chemical agents but also helped clotting. This ointment helps clotting and is a very good alternative to wound healing but due to restriction we were not able to perform the animal trials, just the clotting factor was demonstrated and still, it had a promising effect on the clotting factor. The evaluation parameter data was shown in the acceptance range. Further studies are appreciated for the

stopwatch was used for measuring the froth formation. (2)

Results and Discussion

Parameter	Observation
Colour	Off white
Odour	Characteristic
Consistency	Smooth
Ph	5.8
Non-irritancy	Non-irritant
Washability	Good
Stability (20, 10, 30° c)	Stable
Spreadability	3 sec
Solubility (ethanol)	Insoluble
Solubility (chloroform)	Partially soluble
Solubility (di-ethyl ether)	Partially soluble
Solubility (warm water)	Insoluble
L. O. D	0.388
Extrudability	Easily removable
Squiseability	Easily extracted

Clotting Time

advancement in the formulation for establishing better effects.

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