Formulation and Assessment of a Therapeutic Herbal ointment from Veld Grape Plant

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Abstract:

The creation and assessment of an ointment made from the Cissus quadrangularis plant also referred to as Devil's Backbone or Veld Grape are the main objectives of the study. Because of their lubricating, therapeutic, and protective qualities, ointments which are semi-solid formulations applied topically to the skin or mucous membranes are frequently employed. Ointments' greasy foundation helps hold onto moisture and distribute active substances over time. Because of its ability to cure bones, Cissus quadrangularis, a member of the Vitaceae family, has long been employed in Ayurvedic medicine. It increases bone regeneration, accelerates fracture healing, and activates osteoblasts. This study intends to promote natural remedies in topical treatments by harnessing the therapeutic potential of Cissus quadrangularis to create an efficacious herbal ointment.

Keywords: Cissus quadrangularis, Veld Grape, Herbal ointment, Bone healing, Ayurveda, Topical formulation, Osteoblast stimulation, Traditional medicine.

Introduction:

The tropical world is home to the perennial herb Cissus quadrangularisis, which has therapeutic qualities. In India, it is among the most widely utilised medicinal plants. According to some accounts, the plant is native to India, Sri Lanka, Java, Malaysia, and West Africa. The phytochemical composition, pharmacological properties, and toxicity assessment of this plant are being investigated. Bone repair is one of its uses [1,2,3]. This plant is recommended by Ayurveda for a number of medical conditions. Cissus quadrangularis synonym Cissus succulent, also known as pirandai in Tamil and horjora in Hindi, belongs to the Vitaceae family. The plant is common in Asiatic and African tropical forest regions [2,4,5].

Figure: 1 Veld Grape Plan

Plant Profile:

Subkingdom	Tracheobionta
Super division	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Vitales
Family	Vitaceae
Genus	Cissus
Species	Quadrangularis

Formulation of Ointment: Materials:(2)

1. Herbal Drug extract
2. Wool fat (Emulent)
3. Cetostearyl alcohol (Emulsifying agent)
4. Hard paraffin (Stiffen ointment bases)
5. White soft paraffin (Emulent and retain moisture)

Methods:

PREPARATION OF HERBAL DRUG EXTRACT:(7,2)

1. Take the required amountof the Veld Grape (Cissus quadrangularis) plant material.
2. Cut it into small pieces for easier drying and processing.
3. Air-dry the plant material thoroughly.
4. After the material has dried, grind it into a coarse powder.
5. Sieve the powder to obtain a uniform particle size.

6. Use a Soxhlet apparatus to extract the powdered material using ethanol as the solvent.
7. Transfer the extract into a Petri plate once it has been extracted.
8. Place the Petri dish in a hot air oven at 40–50°C for 1 hour to evaporate the solvent.
9. Collect the dried extract, which constitutes the herbal drug.

Table 1: Formulation of Ointment Base:(2,7)

S.NO	Ingredients	Amount
1.	Yellow Soft Paraffin	8.5gm
2.	Hard Paraffin	0.5gm
3.	Wool Fat	0.5gm
4.	Cetostearyl Alcohol	0.5gm

Table 2: Formulation of Herbal Ointment:(2,7)

Amount to be Taken (Herbal Extract)		
F1	F2	F3
0.2	0.4	0.6

Procedure for Preparation of Herbal Ointment: (7,2)

a) To create the ointment base, grated hard paraffin was first carefully weighed and placed in an evaporating dish on a water bath. Once the hard paraffin had melted, the additional ingredients were added and gently agitated to facilitate uniform melting and mixing. After then, the ointment base was left to cool.

b) To make the herbal ointment, precisely weighed veld grape extract was mixed with the ointment base using the levigation method to create a smooth paste that was two or three times the weight of the base. More base was then added gradually until the ointment was homogenous, and it was finally transferred to an appropriate container.

Evaluation:

Colour and Odour:

Physical Parameters like colour and odour were examined by Visual Examination

Consistency:

Smooth and no greediness is observed.

PH: (7)

A digital pH meter was used to measure the produced herbal ointment's pH. 100 millilitres of distilled water were used to make the ointment solution, which was then left for two hours. The solution's pH was measured three times, and the average was computed.

Spreadability: (7)

Spreadability was assessed by sandwiching extra sample between two slides and compressing it to a consistent thickness over a predetermined amount of time with a predetermined weight. Spreadability was calculated as the amount of time needed to separate the two slides. Better spreadability is achieved when two slides are separated in less time. Spreadability was computed using the formula below. One

S = M×L/T

S stands for spreadability.

M is the weight of the upper slide.

L is the glass slide's length.

T is the amount of time needed to split the slides

Extrudability: (8)

When a specific amount of force is provided to a collapsable tube in the form of weight, the extrudability test measures the force needed to extrude the material. The amount of ointment that extruded out the tube as a percentage when a specific load was applied was calculated in this investigation. The following formula was used to determine the extrudability of produced ointment Extrudability is calculated as follows: ointment extruded from the tube x 100/ointment filled in tube

LOD: (2)

LOD was calculated by drying the formulation at 105 °C in a Petri plate set over a water bath Take 1gm in sample in watch glass. Weight the watch glass. Keep in hot air oven for 1hour. Take the Weight

Formula:

$$LOD = \frac{\text{Intital Weight} - \text{Final Weight}}{\text{Weight of the sample}} \times 100$$

Figure 3: LOD

Solubility: (2)

Miscible with alcohol, ether, and chloroform, and soluble in boiling water.

Figure 4: Solubility

Washability: (2)

After applying the formulation to the skin, the ease of washing with water was assessed

Test for non-irritability: (2,6,9)

A prepared herbal ointment was applied to a person's skin, and the results were monitored. To screen for effects like redness, erythema, inflammation, etc., a little amount of the sample is applied to the hand and monitored for 24 hours. Because of this, no such impact was noticed, and the skin is not irritated.

Result and discussion:

Qualitative Phytochemical Analysis

A qualitative phytochemical screening of the ointment extract was performed using standard protocols as described previously. The ointment was extracted using etoh, and the extract subjected to the following tests:

Test for Carbohydrates:
Molisch's Test: 1mL extract + 1mL α-naphthol solution + a few drops concentrated sulfuric acid along the side of the test tube. Formation of a purplish-red ring at the interface indicates the presence of carbohydrates.
Fehling's Test: 1mL extract + equal parts Fehling's A and B solutions; boil. Brick-red precipitate indicates the presence of reducing sugars.
Test for Alkaloids:
Mayer's Test: 1mL extract + 2–3 drops of Mayer's reagent. Creamy white precipitate indicates alkaloids.
Wagner's Test: 1mL extract + 2–3 drops Wagner's reagent. Reddish-brown precipitate confirms alkaloids.
Test for Glycosides:
Baljet Test: 1mL extract + sodium picrate solution. Presence of glycosides is indicated by yellow to orange coloration.
Keller-Killiani Test: 1mL extract + acetic acid containing a trace of $FeCl_3$, then carefully add concentrated H_2SO_4. Reddish-brown ring turning blue suggests cardiac glycosides.
Test for Saponins:
Foam Test: 1mL extract + 10mL distilled water; shake vigorously. Persistent foam indicates saponins.
Test for Flavonoids:
Alkaline reagent tests: The extract was treated with a few drops of sodium hydroxide solution. Formation of an intense yellow colour, which becomes colourless on further addition of dilute acid, indicated the presence of flavonoids.
Lead Acetate Test: 1mL extract + few drops of lead acetate solution. Yellow precipitate denotes flavonoids.

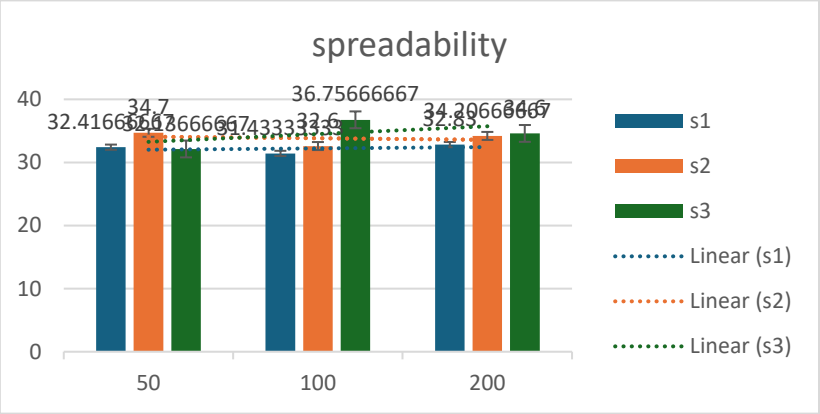
Test for Tannins:
Ferric Chloride Test: 1mL extract + 2–3 drops of $FeCl_3$. Formation of blue-black or greenish-black coloration confirms tannins.
Test for Proteins and Amino Acids:
Biuret Test: 1mL extract + 1mL 40% NaOH + 2 drops 1% copper sulfate. Violet color denotes proteins.
Test for Terpenoids: 1mL extract + 2mL chloroform; add 2mL concentrated H_2SO_4. Reddish-brown interface indicates terpenoids.

Alkaloids	**Mayer's test**	**-**
	Wagner's test	-
Carbohydrate	Molisches test	-
Reducing sugar	Fehling's test	-
	Benedict's test	-
saponin	Foam test	++
	Froth test	++
Phenolic compounds	Ferric chloride test	+

	Lead acetate test	+
Proteins and amino acids	Millon test	-
	Biuret test	+
	Ninhydrin test	+
Terpenoids	Salkowski's test	+

Spreadability:

		T1	T2	T3	Average	Sd	Sem
sample 1	50	19.25	38.5	39.5	32.41667	11.41362	6.559555
sample 1	100	27.5	33.4	33.4	31.43333	3.406367	1.957682
sample 1	200	45.29	27.2	26	32.83	3.646592	2.095743
sample 2	50	27	38	39.1	34.7	6.691039	3.845425
sample 2	100	30.8	33.1	33.9	32.6	1.609348	0.924912
sample 2	200	48.12	26.8	27.7	34.20667	12.0577	6.929713
sample 3	50	29.61	28.5	38.3	32.13667	5.36638	3.084126
sample 3	100	42.77	33.8	33.7	36.75667	5.207939	2.993069
sample 3	200	40	38.2	25.6	34.6	7.846018	4.509206



UV-Visible Spectrophotometric Analysis:

The active constituent in the veld grape ethanolic extract was quantitatively analyzed using UV- Visible spectrophotometry. Standard solutions with concentrations ranging from 20 to 100 µg/ml exhibited a linear increase in absorbance values, conforming to Beer-Lambert's law within this range.

Conc	Std	Sample 1	Sample2	Sample3
20	0.186	0.159	0.167	0.19
40	0.382	0.276	0.276	0.327
60	0.579	0.401	0.539	0.59
80	0.763	0.593	0.611	0.602

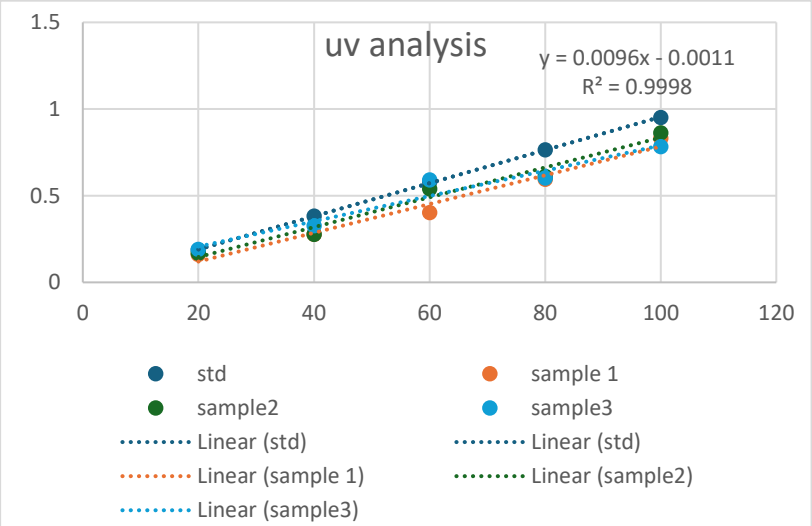
100	0.951	0.83	0.861	0.782

A calibration curve constructed from the standard data yielded the regression equation: y=0.00956x−0.00110(R2=0.9998)y=0.00956x−0.00110(R2=0.998)

where y represents absorbance and x denotes concentration in µg/ml. The high coefficient of determination (R^2 = 0.9998) confirms excellent linearity and accuracy of the method.

Using this calibration curve, the concentrations of the active constituent in the Veld grape extract samples were calculated. Sample concentrations varied across dilutions, with averages ranging approximately from 18.12 µg/ml to 86.39 µg/ml among the three replicates.

These findings demonstrate that the UV-Visible spectrophotometric method is reliable and suitable for quantifying the active compound in the veld grape ethanolic extract with high precision and reproducibility



Excrudability test:

Sample	Replicate 1	Replicate 2	Replicate 3	Mean	SD	SEM
A	0.85	0.88	0.82	0.85	0.03	0.017321
B	1.02	0.97	1	0.996667	0.025166	0.01453
C	0.75	0.78	0.76	0.763333	0.015275	0.008819

Physical properties:

S.NO	Specification	Limit
1.	State	Semisolid
2.	Colour	Yellow
3.	Odour	Characteristic
4.	Texture	Smooth

Determination of PH:

The normal PH range of ointment is 5-6.5

S.NO	Formulation	PH
1.	F1	6
2.	F2	6.5
3.	F3	6.9

Loss on Drying:

Sample	Intital weight	Final weight	%LOD
F1	28.71	28.70	1
F2	31.92	31.83	9
F3	33.4	33.10	30

Conclusion:

The present study successfully formulated and evaluated a herbal ointment using veld grape plant extract. The prepared formulation showed desirable physical characteristic. Phytochemical analysis confirmed the presence of bioactive constituents which is known for its anti-inflammatory, antimicrobial properties etc. It is conclued that this plant extract ointment can serve as an effective and natural alternative for therapeutic application, promoting the use of traditional medicinal plants in modern formulation.

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