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Formulation and Evaluation of a Herbal Topical Anti-Inflammatory Gel Containing Methanolic Leaf Extract Of Scleropyrum Pentandrum (Dennst.) Mabb

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ABSTRACT: Scleropyrum pentandrum (Dennst.) Mabb belonging to Santalaceae family is a small tree of a maximum height of 6 to 7 meters, commonly found in the evergreen forests of Peninsular India, Western Ghats, South and Central Sahyadris and on sandy soil. It is traditionally used for its anti-inflammatory activity. It is used for various activities by tribal people in different parts of the world. The present research has been undertaken with the aim to formulate and evaluate the herbal gel containing Scleropyrum pentandrum extract. The gel formulation was designed by using methanolic extract of S. pentandrum leaves in Soxhlet apparatus. The gel was prepared by using Carbopol 934, S. pentandrum leaves extract, Propylene glycol, Methyl paraban, Propyl paraben and required amount of distilled water. Then skin pH (6.8- 7) was maintained by drop wise addition of tri-ethanolamine. The physiochemical parameters of formulations (pH, viscosity, spreadability, extrudability etc.) were determined. Accelerated stability testing was carried out as per ICH guidelines for 1 month at different temperatures and humidity.

KEYWORDS: Scleropyrum pentandrum (Dennst.) Mabb, anti-inflammatory activity, physiochemical parameters.

I. INTRODUCTION

Herbal medicines are still the mainstay of about 75-80% of the world's population, mainly in developing countries, for primary health care because of better cultural acceptability, better compatibility with human body and lesser side effects. Herbal medicines consist of plant or its part to treat injuries, disease or illnesses and are used to prevent and treat diseases and ailments or to promote health and healing. It is a drug or preparation made from a plant(s) and used for any such purpose. Herbal medicines are the oldest form of health care known to mankind. [1]

World Health Organization (WHO) has defined herbal medicines as finished, labelled medicinal products that contain active ingredients, aerial or underground parts of the plants or other plant material or combination. Herbal formulations have reached widespread acceptability as therapeutic agents like anti-microbial, anti-diabetic, anti-ageing, anti- arthritic, anti-depressant, anti-anxiety, anti-inflammatory, anti-HIV, in treatment of cirrhosis, asthma, migraine, Alzheimer's disease and memory enhancing activities. [1]

Herbal medicines constitute a major part in all traditional systems of medicine. Indian Materia Medica includes 2000 natural products of therapeutic importance of which 400 are of mineral and animal origin. There are approximately 1250 Indian medicinal plants which are used in formulative therapeutic preparations according to Ayurveda and other medicinal plants are being used in medicine from time immemorial as they are accessible and inexpensive. [2]

One such herb is Scleropyrum pentandrum (Dennst.) Mabb,. belonging to Santalaceae family, a small tree of a maximum height of 6 to 7m, commonly found in the evergreen forests of Peninsular India, Western Ghats, South and Central Sahyadris and on sandy soil. It is traditionally used for its anti-inflammatory, anticariogenic, anthelmintic, antibacterial, contraceptive and cytotoxic activities. [3]

Extraction is the separation of medicinally active portion of plant tissue using selective solvent through standard procedure. The product so obtained from plants are relatively complex mixture of metabolites in liquid or semisolid state or in dry powder form and are intended for oral or external use. During extraction, solvent diffuse into the solid plant material and solubilize the

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compound with similar polarity. The successful determination of biologically active compound from plant material is largely dependent on the type of solvent used in the extraction procedure. A solid sample with limited solubility in a solvent in the presence of insoluble contaminants has traditionally been extracted using the Soxhlet method. In the main chamber of the Soxhlet extractor, a porous thimble filled with a solid sample is put. The extraction cycle is often repeated several times by refluxing the solvent through the thimble using a condenser and a syphon side arm.[4]

The present study is aimed at the formulation and evaluation of topical anti-inflammatory gel containing methanolic extract of Scleropyrum pentandrum(Dennst) Mabb,.

TOPICAL DRUG DELIVERY SYSTEM1

The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body to promptly achieve and then maintain the desired drug concentrations. The route of administration has a significant impact on the therapeutic outcome of a drug. Skin is one of the most readily accessible organs in the human body for topical administration and is the main route of topical drug delivery system. Topical delivery can be defined as the application of a drug- containing formulation to the skin, to directly treat cutaneous disorders (e.g. acne) or the cutaneous manifestations of a general disease (e.g. psoriasis) with the intent of containing the pharmacological or other effect of the drug to the surface of the skin or within the skin. Semi- solid formulation in all their diversity dominate the system for topical delivery, but foams, spray, medicated powders, solutions, as well as medicated adhesive systems are also in use.

ANTI-INFLAMMATORY ACTIVITY

Inflammation is a complex process, which is frequently associated with pain and involves occurrences such as: the increase of vascular permeability, increase of protein denaturation and membrane alteration. When cells in the body are damaged by microbes, physical agents or chemical agents, the injury is in the form of stress. Inflammation of tissue is due to response to stress. It is defensive response that is characterized by redness, pain, heat, and swelling and loss of function in the injured area. The most common causes of inflammation are infections, burns and trauma, and many types of immune reactions. [1]

Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process.[5]

Protein Denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. Denaturation of proteins is a well-documented cause of inflammation.

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PLANT PROFILE[3]

Figure No.1: *Scleropyrum pentandrum*

Figure No.3: *Scleropyrum pentandrum* leaf

Figure No.2: *Scleropyrum pentandrum* male and female flowers

Figure No.4: *Scleropyrum pentandrum* fruits

Scleropyrum pentandrum (Dennst.) Mabb (Synonym: Scleropyrum wallichianum Arn.) belongs to the family Santalaceae and grows along the margin of evergreen to semi-evergreen forests between 600 and 1600 m.

DISTRIBUTION

S. pentandrum is distributed in Cambodia, China, Thailand, Sri Lanka and Laos. In India, it is distributed in Peninsular India, Western Ghats, South and Central Sahyadris and generally found on sandy soil, as well found in semi and dry evergreen forests, in open forest near stream and in lowland Dipterocarps forest, from 500 to 1000m. Flowering occurs in January to March, fruiting in August to October. The whole plant parts are applied externally to treat skin irritation in Kani tribal settlement, Agasthiayamalai biosphere reserve, Tirunelveli, South India. It is commonly called Naaikuli in Kasargod, Kerala and is used as a mechanical barrier (fencing) in dried or live condition. The crushed roots are given for curing stomach ailments in Kurichyas tribal community in Kannur district of Kerala.

BOTANICAL NAME: Scleropyrum pentandrum (Dennst.)Mabb, Scleropyrum wallichianum Arn.

GENUS: Scleropyrum
FAMILY: Santalaceae

COMMON NAME:Kannada- Naikkuli, Benduga Malayalam- Irumulli, Vathamparatti
CHEMICAL CONSTITUENTS: alkaloids, glycosides, flavonoids, saponin, tannin, sterols, fatty acid, carboxylic acids etc.

II. MATERIALS AND METHODS

PLANT MATERIAL[6]

The leaves of Scleropyrum pentandrum were collected from sacred grooves of Poyilkavu Durgadevi temple, Kozhikode district, Kerala in the month of November. The plant material was

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taxonomically identified at the centre for Medicinal Plant Research, Kottakkal. It's authenticated by Dr.A.K.Pradeep, Asst.professor Department of Botany (NO:107864) and herbarium is deposited at Botany department, Calicut University, Kerala, India

Preparation of methanolic extract of scleropyrum pentandrum

The leaves are dried and powdered and is subjected to successive extraction with methanol using Soxhlet apparatus. This extract and the powdered crude drug were used for anti- inflammatory activity study and gel formulation.

Figure No.5: Soxhlet apparatus

PHARMACOGNOSTIC STUDIES

Determination of moisture content[8]

Five grams of the powdered whole plant were placed in tared evaporating dish. Drying was carried out at 105 C for five hours. The drying was continued with intermittent weighing at one hour interval until difference between two successive weighing was not more than 0.25%. Constant weight was reached when the two consecutive weighing after drying for 30minutes and cooling for 30 minutes in desiccator, showed not more than 0.01 gm difference.

Loss on drying=initial weight-final weight

Determination of ash value [8,9]

The ash value is an important parameter for the evaluation of crude drugs, due to variation of values within fairly wide limit. The ash value of any organic material is composed of inorganic material like metallic salt and silica. The following three methods were developed;

- Total ash
- Acid insoluble ash
- Water soluble ash

Ashing involves an oxidation of the component of the product and a high ash value involves the contamination, substitution, adulteration or carelessness in the preparation of crude for marketing.

- Total ash

An air-dried sample weighing two grams was weighed out in a crucible previously ignited for 30 minutes, spread evenly, and ignited at a temperature of more than 450 degrees Celsius until it showed no signs of carbon, then cooled in the desiccator and weighed. Calculated the content of total ash per gram of air-dried material.

$$\% \text{ Total ash} = \frac{weight\ of\ ash}{weight\ of\ sample} \times 100$$

- Acid insoluble ash

The crucible containing the total ash was filled with 25 ml of 2N HCI, covered with watch glass, and boiled gently for 5 minutes. The watch glass was rinsed with 5 ml of hot water and then added into the crucible. Collected the insoluble matter on an ash-less filter paper and washed with hot water until the filtrate was neutral. Filter paper containing the insoluble matter was transferred to the original crucible, dried on a hot plate and ignited to constant weight. The residue was allowed to cool in a desiccator for 30 minutes; then weighed, calculated the content of acid insoluble ash per gram of air-dried material.

$$\% \text{ Acid insoluble ash} = \frac{weight\ of\ acid\ insoluble\ ash}{weight\ of\ sample} \times 100$$

- Water soluble ash

To the crucible containing the total ash, 25 ml each of water was added and boiled for 5 minutes. The insoluble matter was collected in sintered glass crucibles. Washed with hot water and ignited in a crucible for minutes at a temperature not exceeding 450 C. The weight of those residue in mg was subtracted from the weight of total ash. The content of water- soluble ash was calculated per gram of air-dried material.

Weight of water-soluble ash=weight of total ash - weight of water insoluble ash

$$\% \text{ Water soluble ash} = \frac{weight\ of\ water\ soluble\ ash}{weight\ of\ sample} \times 100$$

Determination of extractive values[9]

This method determines the amount of active constituents in a given amount of plant material when extracted with solvent. The extractive value is used as a means of evaluating

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crude drug which are not readily estimated by other means.

- Alcohol soluble extractive value

Macerated 5 grams of coarsely powdered air-dried whole plants of S.pentandrum with 100 ml ethanol in a stoppered flask for 24 hours with occasional shaking during the 1' 6 hours and allowed to stand undisturbed for another 18 hours. Filtered rapidly, by taking precautions against loss of alcohols. The 25 ml of the filtrate was evaporated to dryness in a tared Nat bottomed shallow dish, dried at 105 C and weighed. Calculated %w/w ethanol soluble extractive with reference to air dried material.

- Water soluble extractive value

Macerated 5 grams of coarsely powdered air-dried whole plants of S.pentandrum with 100 ml water in a stoppered flask for 24 hours, with occasional shaking during the first 6 hours and allowed to stand undisturbed for another 18 hours. Filtered rapidly, then 25 ml of the filtrate was evaporated to dryness in a tared flat bottomed shallow dish, dried at 105°C and weighed. Calculated %w/w water soluble extractive with reference to air dried material.

PREFORMULATION STUDIES FTIR SPECTROSCOPY

The compatibility studies were carried out at room temperature using FTIR spectroscopy to determine the interaction of drugs with the excipients used in the formulation.

PREPARATION OF GEL FORMULATION[7]

Various gel formulations were prepared from S.pentandrum leaf extract using carbopol-934 as gelling agent. Gels were prepared by dispersion method. Required quantity of polymer (carbapol-934), propylene glycol 400 was weighed individually, and sufficient amount of distilled water were mixed in a separate beaker, after which it was continuously stirred till the polymer is soaked in the water and kept for 24hr at room temperature.

With continuous stirring, now the appropriate quantity of methyl paraben and propyl paraben was added which acts as a preservative. Small quantities of triethanolamine were added with continuous stirring to achieve neutral pH. Finally, the extract was added to gel with continuous stirring till drug get dispersed completely.

Table 1: Composition of gel formulation

Ingredients (%w/w)	Gel formulation
Scleropyrum pentandrum extract	0.5g
Carbopol – 934	0.1g
Triethanolamine	q.s
Propylene glycol	0.1ml
Methyl paraben	0.15 ml
Propyl paraben	0.05 ml
Purified water	10 ml

EVALUATION OF GEL FORMULATION[7]

Prepared formulations were evaluated for various physicochemical parameters such as color, homogeneity, pH, spreadability, viscosity, and diffusion study.

Measurement of Ph[7]

5 gm of gel formulation was dispersed separately in 45 ml of water, and the pH of the suspension was determined using digital pH meter. Measurements of pH were carried out in triplicate and the averages of three readings were noted.

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Homogeneity[7]

Formulations were tested for homogeneity by visual inspection after the formulations have been set in the container. They were tested for their appearance and presence of any aggregates.

Measurement of viscosity

The viscosity of gel was determined by using a Brookfield viscometer DVII model with a T- Bar spindle in combination with a helipath stand. 50 g of gel was filled in a 100 ml beaker. T-bar spindle (T95) was used for the measurement of viscosity of all the gels. The helipath T- bar spindle was moved up and down and viscosity was measured at 2.5, 4, 5 and 10 rpm.

Spreadability[10]

0.5 g of the gel on a circle of 2 cm diameter pre-marked on a glass plate and then a second glass plate is placed over it. 500g weight placed on the upper glass plate for 5 min. Diameter of the circle after spreading of the gel determined.

ANTI-INFLAMMATORY ACTIVITY[10]

- Inhibition of Protein Denaturation

The reaction mixture (0.5ml) consisted of 0.45ml bovine serum albumin (5% aqueous solution) and 0.05ml plant extract of 50,100,150,200 µg/ml concentrations and pH was adjusted to 6.3 using 1N HCl. The sample were incubated for 37°C for 20 min and then heated at 57°C for 30 min. Ibuprofen used as standard drug (50, 100, 150, 200 C). After cooling the samples, 2.5ml phosphate buffer saline (pH 6.3) was added to each tube.

Absorbance was measured spectrophotometrically at 660nm. For control tests 0.05ml distilled water was used instead of extracts while product control lacked bovine serum albumin. The percentage inhibition of protein denaturation was calculated as follows.

Percentage inhibition = $\frac{Abs\ control - Abs\ sample}{Abs\ control} \times 100$

PHARMACOGNOSTIC STUDIES

Determination of moisture content

The moisture content of whole plant was determined and the average loss of moisture content of the leaves was determined. The results are shown in the table no 2.

Table No. 2: Results showing moisture content of leaves of S. pentandrum

Sl. No	Weight of Drug+dish before drying (g)	Weight of Drug+dish after drying (g)	Loss on drying (g)	Percentage loss on drying (%w/w)	Average(%w/w)
1	22.01	21.86	0.15	7.5	
2	23.12	22.96	0.16	8	8.16
3	22.87	22.69	0.18	9	

Determination of Ash value

The ash value of leaves was determined. The percentage content of acid insoluble ash was found to be less than that of water-soluble ash. The results are shown in the table no:

Table No. 3: Results showing total cash value of leaves of S. pentandrum

Sl. No	Weight of empty crucible (g)	Weight of crucible +sample (g)	Weight of crucible +ash (g)	Weight of ash (g)	Percentage yield (%w/w)	Average(%w/w)

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1	42.70	44.70	43.05	0.35	17.50	
2	39.57	41.57	39.19	0.38	19.70	18.56
3	38.02	40.02	38.39	0.37	18.50	

Table No. 4: Results showing acid insoluble ash value of leaves of S. pentandrum

Sl.No	Weight of empty crucible (g)	Weight of crucible+ insoluble ash (g)	Weight of Acid(g)	Weight of ash (%w/w)	Percentage yield	Average(%w/w)
1	42.70	42.79	0.09	4.50		
2	39.57	39.64	0.07	3.50	3.84	
3	38.02	38.09	0.07	3.50		

Table No 5: Results showing water soluble ash value of leaves of S. pentandrum

Sl.No	Weight of empty crucible (g)	Weight of crucible+ Water insoluble ash (g)	Weight of ash (g)	Percentage (%w/w)	yield	Average(%w/w)
1	42.70	42.91	0.21	10.5		
2	39.57	39.76	0.19	9.5	10.34	
3	38.02	38.24	0.22	11		

Determination of extractive value

Table No. 6: Results of water-soluble extractive value of leaves of S. pentandrum

Sl. No	Weight of dry powder (g)	Weight of empty dish(g)	Weight of dish + extract (g)	Percentage yield (%w/w)	Average(%w/w)
1	5	55.50	55.682	3.64	
2	5	56.65	56.828	3.56	2.40
3	5	51.45	51.640	4.00	

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Table No 7: Results showing alcohol soluble extractive values of leaves of S.pentandrum

Sl. No	Weight of drypowder (g)	Weight of empty dish (g)	Weight ofdish + extract (g)	Percentageyield (%w/w)	Average(%w/w)
1	5	51.62	51.751	2.62	
2	5	52.25	52.37	2.40	1.54
3	5	45.56	45.67	2.20	

FORMULATION AND EVALUATION OF HERBAL GELS FROM
Scleropyrum pentandrum (DENNST) Mabb EXTRACT

Gel formulations were prepared using the polymer carbopol-934 as gelling agent. Propylene glycol was used in the formulation as permeation enhancer, triethanolamine was used to neutralize the pH and methyl paraben; propyl paraben was used as preservatives.

Gel formulations showed brown color, good homogeneity and spreadability. The pH of gel formulations was in the range of 7.0-7.1 which lies in the normal pH range of the skin. Theviscosity of gel formulation was found to be 3.9cP.

Table 8: Evaluations of topical formulations of Scleropyrum pentandrum (Dennst) Mabb

FORMULATION CODE	COLOUR	HOMOGENITY	pH	SPREADABILITY (cm)
G1	Brown	Good	7.1	4.0
G2	Brown	Good	7.0	4.2
G3	Brown	Good	7.0	4.5

PHARMACOGNOSTIC EVALUATION
Test for identification

Table No. 9: Results showing test for identification

Test for S.pentandrum	Observation	Inference
Ferric chloride test	Green color	+
Lead acetate solution test	Yellow precipitate	+

Microbial limit

The plate was observed for the presence of microorganisms and the formulation was found to remain clear after the incubation period with no colonies detected. Thus the formulation passes the test for microbial limit.

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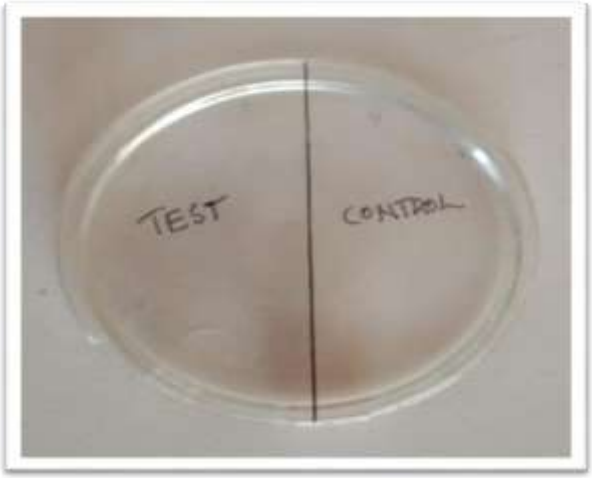

Figure No.6: Microbial limit

EVALUATION OF ANTI- INFLAMMATORY ACTIVITY OFFORMULATED GEL
Inhibition of Protein Denaturation

Table No. 10: Results showing percentage inhibition of protein denaturation

Sl. No	Sample	Concentration (µg/ml)	Absorbance at 660nm	% inhibition
1.	Control	-	0.812	-
2.	Standard	50	0.480	40.90
		100	0.342	57.88
		150	0.236	70.94
		200	0.150	81.53
3.	Drug extract	50	0.497	38.79
		100	0.356	56.16
		150	0.263	67.61
		200	0.187	76.97

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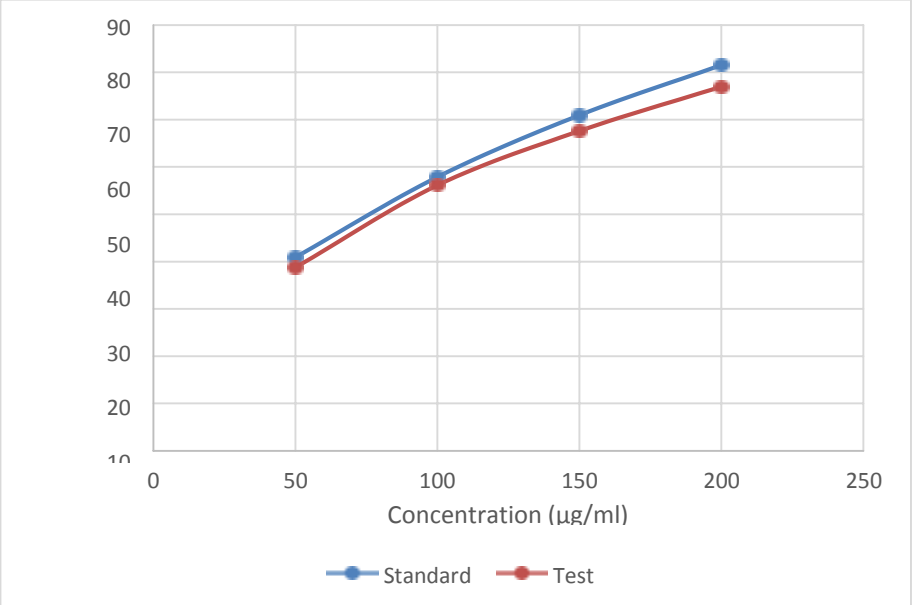
Figure No: 7 Percentage inhibition v/s concentration graph



III. SUMMARY

S. pentandrum is a herb belonging to the family Santalaceae. It was collected from the sacred groves of Poyilkavu Durga Devi temple situated at the coastal area of Calicut, Kerala. The whole plant parts are applied externally to treat skin irritation in Kani tribal settlement, Agasthiayamalai biosphere reserve, Tirunelveli, South India. Paste of stem bark and leaf is applied externally to treat skin diseases. When searched for literatures, it revealed that a proper investigation was not done with this plant. Hence an attempt to formulate and evaluate the effect of this herb as gel for anti-inflammatory activity is made herein.

Phytochemical screening was already conducted and the presence of alkaloids, carbohydrates, steroids, tannins, flavonoids, phenols and terpenoids was reported.

The methanolic extract was prepared using Soxhlet extraction technique. Pharmacognostic studies such as determination of moisture content, ash value and extractive value of the extract were carried out. The extract was found to be water-soluble in nature. Thus, water was used as vehicle for the preparation of the gel, carbopol-934 as gelling agent, propylene glycol as permeation enhancer, triethanolamine to attain neutral pH and methyl paraben and propyl paraben as preservatives. Three batches of formulations were prepared with different composition and among those, one was found to have better physical characteristics.

REFERENCES

[1]. Jamadar MJ, Shaikh RH. Preparation and evaluation of herbal gel formulation. Journal of Pharmaceutical Research and Education. 2017;1(2):201-24.
[2]. Kokate C.K., Practical Pharmacognosy, 4th edition, Delhi, Nirali Prakashan, 2008, Pg No. 186-188.
[3]. Ajithbabu TK, Ganesan V, Sajith P. Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry.
[4]. Blench R. Archaeology, language, and the African past. Rowman Altamira; 2006.
[5]. Leelaprakash G, Dass SM. Invitro anti-inflammatory activity of methanol extract of Enicostemma axillare. International journal of drug development and research. 2011 Jul;3(3):189-96.
[6]. Ajithbabu TK, Ganesan V, Sajith P. Phytochemical constituents and anti inflammatory activity of Leaf extracts of Scleropyrum pentandrum (Dennst) Mabb. Int Journal of Research in Pharmaceutical and Nano Sciences. 2013;2(3).
[7]. Jyothi D, Koland M. Formulation and evaluation of an herbal anti-inflammatory

gel containing Trigonella foenum greacum seed extract. Int J Pharm Pharm Sci. 2016;8(1):41-4.

[8]. Dr. Pulok K Mukherjee:Quality control of herbal drugs, an approach to evaluation of botanicals:1 st edition/Delhi: Business horizon;2002.P.529-534 and 554-55

[9]. Sass J E. Elements of Botanical Microtechnique. New York: Mc Grow Hill Book Co; 1940

[10]. Sakat S, Juvekar AR, Gambhire MN. In vitro antioxidant and anti-inflammatory activity of methanol extract of Oxalis corniculata Linn. Int J Pharm Pharm Sci. 2010;2(1):146-55.